Section 2. Call: Multi-topic 2019

Topic 2.3.1 Extending shelf-life of perishable Mediterranean food products by sustainable technologies and logistics and by optimized pest and microbial control

Type of action: RIA

Bio-protective cultures and bioactive extracts as sustainable combined strategies to improve the shelf-life of perishable Mediterranean food

Document Information

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Project partners/consortium:

P1 – Cukurova University – CUNI (coordinator)

P2 – Alma Mater Studiorum Università di Bologna – UNIBO

P3 – Universita' Cattolica del Sacro Cuore – UCSC

P4 – C.L.A.I. ScA – CLAI

P5 – University of Split – UNIST (vice-coordinator)

P6 – Croatian Veterinary Institute, Regional Veterinary Institute Split – CROVET

P7 – Centaurus d.o.o. – CROSME

P8 – DOMCA SAU – DOMCA

P9 – University of Ljubljana – UNILJUB

P10 – University of Maribor (Faculty of Mechanical Engineering) – UNIMB

Deliverable D2.1. Report of the bioactive component composition in brown algae and agro-food by-products

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1. AIM OF THE WORK

The aim of the work was to characterize and identify the chemical profile of the brown algae and agro-food by-products extracts to determine the compounds in the extracts that might carry the bioactive properties and could be selected for potential for application in the next phases of the project.

2. REPORT OF THE BIOACTIVE COMPONENT COMPOSITION IN BROWN ALGAE

2.1. Material and methods

2.1.1. Sample collection (UNIST)

UNIST collected five species of brown algae off the coast of the island Čiovo in the Adriatic Sea from May to September 2020 (Table 1). During sampling the sea temperature and salinity was measured using a YSI Pro2030 probe (Yellow Springs, OH, USA). A voucher specimen of tested species is deposited in the herbarium at the University Department of Marine Studies in Split. Harvested algae were washed thoroughly with tap water to remove epiphytes, and freeze-dried by FreeZone 2.5, Labconco (Kansas City, MO, USA) (freeze drying method was selected as optimal drying method based on the preliminary work described in section 2.2.2.). All samples were then grounded (1 min in a high-speed grinder) and stored for analyses.

Table 2.1. The five species of brown algae and their markings

	Sea temperature	*Cystoseira compressa*	*Padina pavonica*	*Cystoseira amentacea*	*Dictyopteris polypodioides*	*Sargassum vulgare*
May	18.3	CCOM5	PPAV5	CAME5	DPOL5	SVUL5
June	22.4	CCOM6	PPAV6	CAME6	DPOL6	SVUL6
July	23.8	CCOM7	PPAV7	CAME7	DPOL7	SVUL7

August	26.9	CCOM8	PPAV8	CAME8	DPOL8	SVUL8
September	24.7	CCOM9	PPAV9	CAME9	DPOL9	SVUL9

2.1.2. Extraction (UNIST)

The dry algal material was mixed with 50% ethanol and extracted by microwave assisted extraction (MAE) in advanced microwave extraction system (ETHOS X, Milestone Srl, Sorisole, Italy). Extraction conditions were as follows: power and temperature were kept constant at 200 W and 60°C over 15 minutes. The extracts were than centrifuged at 5000 rpm for 8 min at room temperature and filtered, the EtOH evaporated and the extracts freeze dried.

2.1.3. Total phenolic content (UNIST)

The TPC of extracts was determined by the Folin–Ciocalteu method. Briefly, 25 µL of the extract was mixed with 1.5 mL distilled water and 125 µL Folin–Ciocalteu reagent. The solution was mixed and after one minute 375 µL 20% sodium carbonate solution and 475 µL distilled water was added. The mixture was left in the dark for 2 h at room temperature. The absorbance was read at 765 nm using a spectrophotometer (SPECORD 200 Plus, Edition 2010, Analytik Jena AG, Jena, Germany). The standard calibration (0–500 mg/L) curve was plotted using gallic acid (y = 0.001x, R 2 = 0.9998). The TPC was expressed as gallic acid equivalents in mg/g of dried algae (mg GAE/g).

2.1.4. Compounds Analysis by UPLC-PDA-ESI-QTOF (UNIBO/UNIST)

The analysis of compounds from algae was carried out with the use of an ACQUITY Ultra Performance LC system equipped with photodiode array detector with a binary solvent manager (Waters Corporation, Milford, MA, United States) series with a mass detector Q/TOF micro mass spectrometer (Waters) equipped with an electrospray ionization (ESI) source operating in negative mode at the following conditions: capillary voltage, 2300 kV; source temperature, 100°C; cone gas flow, 40 L/Hr; desolvatation temperature, 500°C; desolvatation gas flow, 11,000 L/h; and scan range, m/z 50–1500. Separations of individual compounds were carried out using an ACQUITY UPLC BEH Shield RP18 column (1.7 µm, 2.1 mm × 100 mm; Waters

Corporation, Milford, MA, United States) at 40°C. The elution gradient was carried out using water containing 1% acetic acid (A) and acetonitrile (B), and applied as follows: 0 min, 1% B; 2.3 min, 1% B; 4.4 min, 7% B; 8.1 min, 14% B; 12.2 min, 24% B; 16 min, 40% B; 18.3 min, 100% B, 21 min, 100% B; 22.4 min, 1% B; 25 min, 1% B. The sample volume injected was 2 µL and the flow rate used was 0.6 mL/min. The compounds were monitored at 280 nm. Integration and data elaboration were performed using MassLynx 4.1 software (Waters Corporation, United States).

2.1.5. Characterization of *C. compressa* essential oils by gas chromatography (UNIBO/UNIST)

2.1.5.1. Extraction of essential oils

C. compressa essential oils were obtained by hydrodistillation of dried algal material (100 g) that was immersed in a flask with distilled water (1000 mL). The extraction process was performed in the Clevenger apparatus for 3 h. Pentane and diethyl ether (1:1, v/v) in the inner tube of the apparatus were used for trapping the volatile compounds carried through the system by vapour. Finally, after hydrodistillation, distillate was dried over anhydrous sodium sulfate while nitrogen was used to evaporate organic solvent. The samples of essential oils were stored at 4°C in the dark until analysis.

2.1.5.2. GC-MS Analysis of Volatiles

The seaweed VOCs were analyzed by GC-MS (Shimadzu QP2010, Shimadzu, Kyoto, JP) equipped with an autosampler and a Zebron ZB-WAX 52 30 m × 0.25 µm column (Phenomenex, Torrance, CA). The VOCs fractions were resuspended in hexane and 1 µl was injected in the following gas chromatographic conditions: injection temperature 260°C; interface temperature 280°C; ion source 220°C; carrier gas (He) flow rate 30 cm/sec; splitting ratio 1:10. The oven temperature was programmed as follows: 40°C for 4 min; from 40°C to 175°C with a 3°C/min rate of increase; from 175°C to 300°C with a 7°C/min increase, then holding for 10 min. VOCs were identified by referencing NIST 8.0 (US National Institute of Standards and Technology). For each sample, the volatile profile composition was expressed as relative percentage of each single peak area with respect to the total peak area. Data reported

are the means of three repetitions (the results of this task have been published in: Generalić Mekinić et al. 2021).

2.2. Results

Figure 2.1. shows the total phenolic content of the extracts over five months of sampling.
The highest TPC was found for *C. compressa* in June, followed by the *C. amentacea* in June. Change in TPC through the months is evident in all algae. All algae had the highest TPC in June, except *S. vulgare*. We could not correlate rising sea temperatures during the summer with TPC rise.
Identification of the compounds from 25 algae extracts was done primary to identify phenolic content, however the profile reviled that the phenolic content is very low or that the phenolics from algae have much complex structure (large tannins) which due to their large mass cannot be identified by means of HPLC.
The characterization of the compounds from the algae extracts is shown in the Figures 2-6, and the list of compounds in the Tables 2-6.
The results of the characterization of C. compressa essential oils by gas chromatography have been published in: Generalić Mekinić et al. 2021.

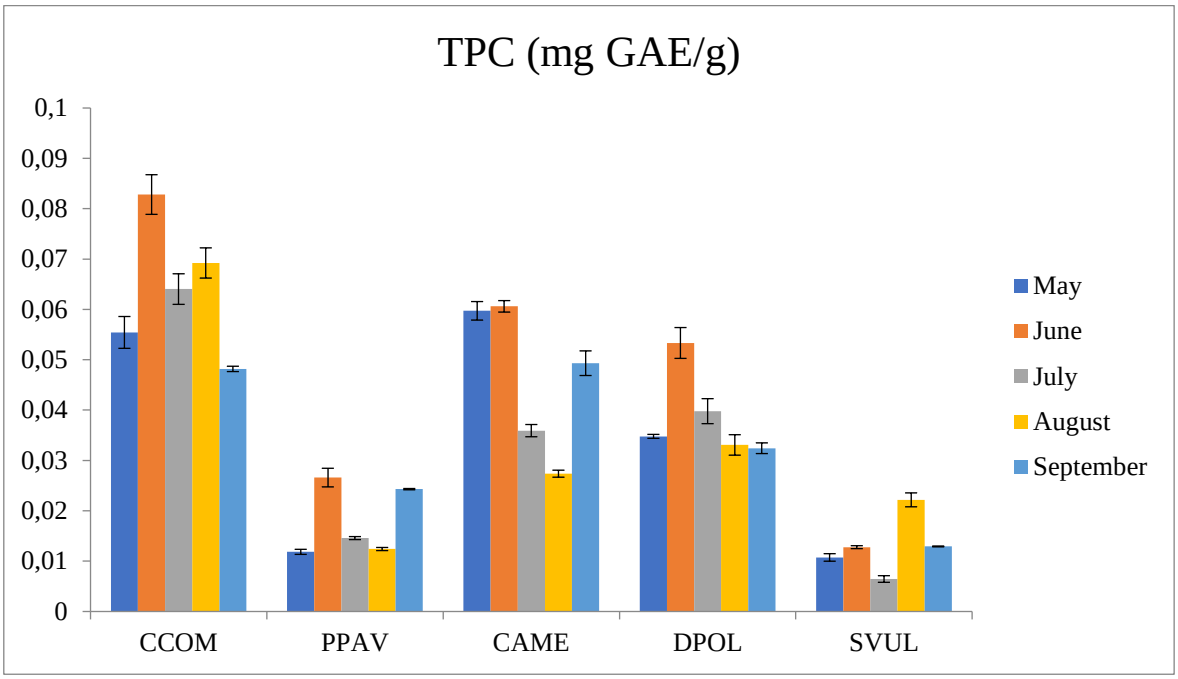

Figure 2.1. The total phenolics content of *Cystoseira compressa* (CCOM), *Padina pavonica* (PPAV), *Cystoseira amentacea* (CAME), Dictyopteris polypodioides (DPOL) and *Sargassum vulgare* (SVUL) extracts in the period from May till September.

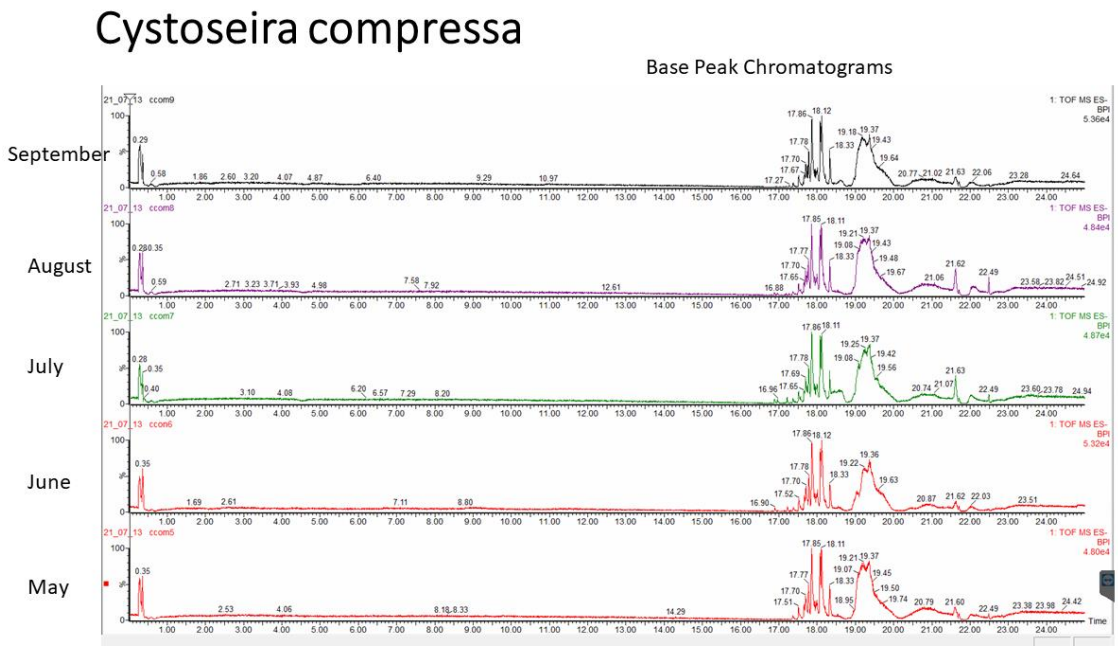

Figure 2.2. Chromatograms of the HPLC-qTOF-MS analyses of *C. compressa*.

Table 2.2. Identification of the compounds from *C. compressa* extracts.

	RT (min)	Mass	Formula (-)	Name	May	June	July	August	September
1	0,28	343,03670	C20 H3 N6 O	1a,9b-Dihydrophenanthro[9,10-b]oxirene-2,3,4,7,8,9-hexacarbonitrile	+	+	+	+	+
2	0,29	201,02360	C4 H9 O9	2-(1,2,2,2-Tetrahydroxyethoxy)ethane-1,1,1,2-tetrol	+	+	+	+	+
3	0,32	141,01550	C2 H N6 O2	Diazidoacetic acid	+	+	+	+	+
4	0,35	181,06990	C6 H13 O6	D-Sorbitol	+	+	+	+	+
5	0,40	317,05300	C12 H13 O10	D-glucaric acid derivate	+	+	-	-	-
6	16,90	275,20060	C18 H27 O2	Stearidonic acid (C18:4n-3) isomer a	-	+	+	+	-
7	16,97	275,20080	C18 H27 O2	Stearidonic acid (C18:4n-3) isomer b	-	+	+	+	-
8	16,97	293,21180	C18 H29 O3	13-ketooctadecadienoic acid isomer a	-	-	-	+	-
9	17,18	295,22680	C18 H31 O3	9,10-Epoxyoctadecenoic acid (vernolic acid)	-	-	-	+	-
10	17,22	429,30090	C27 H41 O4	24-Keto-1,25-dihydroxyvitamin D3	-	+	+	-	-
11	17,26	247,16850	C16 H23 O2	2,4,6-Triisopropyl benzoic acid	-	-	-	+	+
12	17,31	515,32490	C27 H47 O9	Octyl-decyl-triglyceride	-	+	+	-	-
13	17,37	199,16820	C12 H23 O2	Lauric acid	+	+	+	+	+
14	17,42	293,21120	C18 H29 O3	13-ketooctadecadienoic acid isomer b	-	-	-	+	-
15	17,43	427,28270	C27 H39 O4	Hydroxyprogesterone caproate	-	-	+	-	-
16	17,51	225,18480	C14 H25 O2	Myristoleic acid	+	+	-	+	+
17	17,57	275,20160	C18 H27 O2	Stearidonic acid (C18:4n-3) isomer c	-	-	+	+	-
18	17,59	213,18450	C13 H25 O2	Tridecanoic acid	+	+	+	+	+
19	17,63	251,20050	C16 H27 O2	7-cis,10-cis-hexadecadienoic acid	-	-	+	+	+
20	17,66	239,20030	C15 H27 O2	Myristoleic acid methyl ester	+	+	+	+	+
21	17,74	277,21590	C18 H29 O2	gamma-Linolenic acid (C18:3n-6)	-	-	+	+	+
22	17,77	227,20040	C14 H27 O2	Tetradecanoic acid (C14:0)	+	+	+	+	+
23	17,85	253,21570	C16 H29 O2	Palmitoleic acid isomer a (C16:1n-7)	+	+	+	+	+
24	17,94	241,21700	C15 H29 O2	Pentadecanoic acid (C15:0)	+	+	+	+	+

25	17,97	279,23140	C18 H31 O2	Octadeca-10,12-dienoic acid (C18:2n-6)	+	+	+	+	+
26	18,01	267,23180	C17 H31 O2	9-Heptadecenoic acid (C17:1n-8)	+	+	+	+	+
27	18,08	255,23110	C16 H31 O2	Hexadecanoic acid (palmitic acid)(C16:0)	+	+	+	+	+
28	18,12	281,24660	C18 H33 O2	Oleic acid (C18:1n-9)	+	+	+	+	+
29	18,22	269,24660	C17 H33 O2	Heptadecanoic acid (C17:0)	+	+	+	+	+
30	18,33	283,26180	C18 H35 O2	Octadecanoic acid (stearic acid) C18:0	+	+	+	+	+
31	18,41	253,21640	C16 H29 O2	Palmitoleic acid isomer b (C16:1n-7)	+	+	-	+	-
32	18,54	311,29340	C20 H39 O2	Arachidic acid	+	+	-	+	-

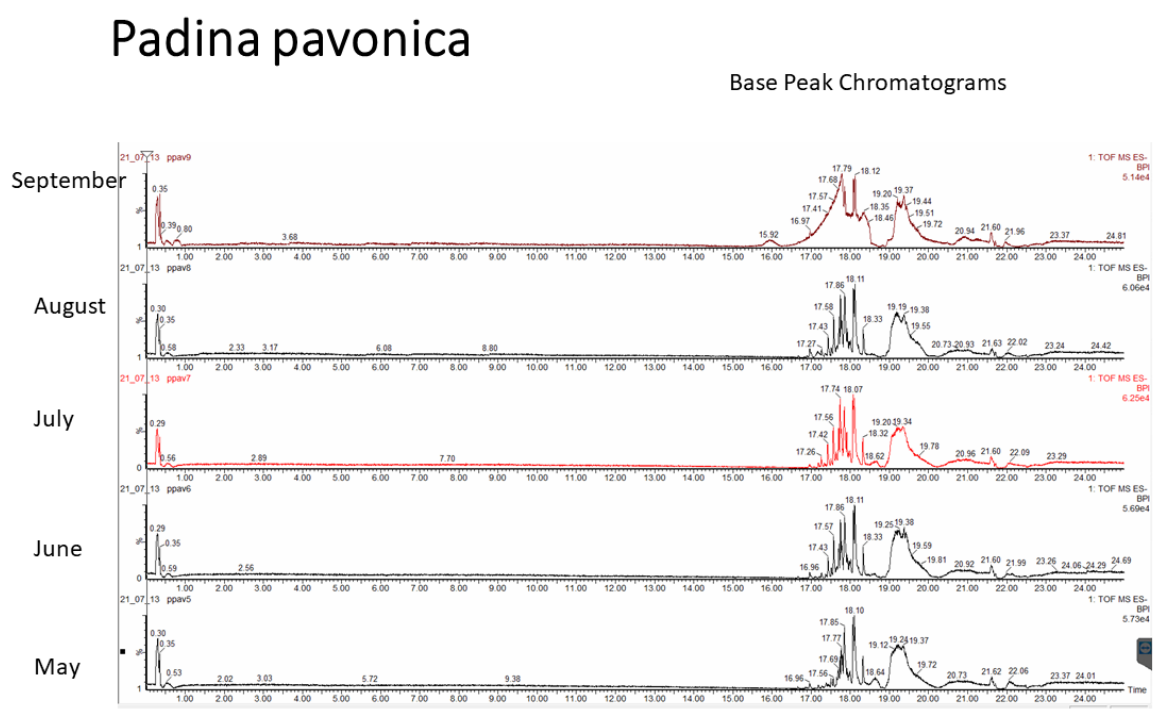

Figure 2.3. Chromatograms of the HPLC-qTOF-MS analyses of *P. pavonica.*

Table 2.3. Identification of the compounds from *P. pavonica* extracts.

	RT (min)	Mass	Formula (-)	Name	May	June	July	August	September
1	0,30	343,0367	C20 H3 N6 O	1a,9b-Dihydrophenanthro[9,10-b]oxirene-2,3,4,7,8,9-hexacarbonitrile	+	+	+	+	-
2	0,30	201,0234	C4 H9 O9	2-(1,2,2,2-Tetrahydroxyethoxy)ethane-1,1,1,2-tetrol	+	+	+	+	+
3	0,34	141,0152	C2 H N6 O2	Diazidoacetic acid	-	+	+	+	-
4	0,35	181,0695	C6 H13 O6	D-Sorbitol	+	+	+	+	+
5	0,39	317,0536	C12 H13 O10	D-glucaric acid derivate	-	-	-	-	+
6	16,60	343,2122	C18 H31 O6	10,11-Dihydroxy-9,12-dioxooctadecanoic acid	+	+	+	+	-
7	16,84	487,3426	C30 H47 O5	Esculentic acid	+	-	-	-	-
8	16,96	275,2012	C18 H27 O2	Stearidonic acid isomer a	+	+	+	+	+
9	17,10	271,2254	C16 H31 O3	Hydroxy-palmitic acid	+	+	+	+	-
10	17,10	309,2056	C18 H29 O4	6,9-Octadecadienedioic acid	+	+	+	+	-
11	17,16	285,2066	C16 H29 O4	Hexadecanedioic acid	+	+	+	+	-
12	17,19	295,2276	C18 H31 O3	9,10-Epoxyoctadecenoic acid (vernolic acid)	+	+	+	+	-
13	17,22	291,1953	C18 H27 O3	12-Oxophytodienoic acid	-	-	+	+	-
14	17,25	293,2108	C18 H29 O3	Oxooctadecadienoic acid derivate	+	+	+	+	-
15	17,27	247,1712	C16 H23 O2	2,4,6-Triisopropyl benzoic acid	-	+	+	+	-
16	17,30	297,2426	C18 H33 O3	10-Oxooctadecanoic acid	+	+	-	-	-
17	17,35	287,2212	C16 H31 O4	10,16-Dihydroxyhexadecanoic acid	-	+	+	+	-
18	17,38	243,1952	C14 H27 O3	3-hydroxymyristic acid	+	+	-	-	-
19	17,43	293,2117	C18 H29 O3	13-ketooctadecadienoic acid	-	+	+	+	-
20	17,44	295,2276	C18 H31 O3	9,10-Epoxyoctadecenoic acid (vernolic acid)	+	+	+	-	-
21	17,51	225,1837	C14 H25 O2	Myristoleic acid	+	+	-	+	-
22	17,51	269,2110	C16 H29 O3	3-Oxohexadecanoic acid	+	+	+	+	-

23	17,56	275,2007	C18 H27 O2	Stearidonic acid	+	+	+	+	+
24	17,61	257,2108	C15 H29 O3	11-Hydroxypentadecanoic acid	+	-	-	-	-
25	17,63	251,2010	C16 H27 O2	7-cis,10-cis-hexadecadienoic acid	+	+	+	+	-
26	17,63	297,2429	C18 H33 O3	10-Oxooctadecanoic acid	+	-	-	-	-
27	17,65	295,2257	C18 H31 O3	9,10-Epoxyoctadecenoic acid (vernolic acid)	-	+	+	+	-
28	17,66	239,2004	C15 H27 O2	Myristoleic acid methyl ester	+	+	+	+	-
29	17,70	301,2156	C20 H29 O2	Eicosapentanoic acid (C20:5n-3)	-	-	+	+	+
30	17,77	227,2005	C14 H27 O2	Tetradecanoic acid (C14:0)	+	+	+	+	+
31	17,85	253,2159	C16 H29 O2	Palmitoleic acid (C16:1n-7)	+	+	+	+	+
32	17,91	279,2319	C18 H31 O2	Octadeca-10,12-dienoic acid (C18:2n-6)	+	+	+	+	+
33	17,93	241,2168	C15 H29 O2	Pentadecanoic acid (C15:0)	+	+	+	+	+
34	18,00	267,2329	C17 H31 O2	9-Heptadecenoic acid (C17:1n-8)	+	+	+	+	-
35	18,07	255,2318	C16 H31 O2	Hexadecanoic acid (palmitic acid) (C16:0)	+	+	+	+	+
36	18,10	281,2472	C18 H33 O2	Oleic acid (C18:1n-9)	+	+	+	+	+
37	18,21	269,2474	C17 H33 O2	Heptadecanoic acid (C17:0)	+	+	-	-	-
38	18,25	339,2000	C15 H31 O8	Hexanedioic acid derivate	-	-	-	-	+
39	18,33	283,2629	C18 H35 O2	Octadecanoic acid (stearic acid) C18:0	+	+	+	+	+

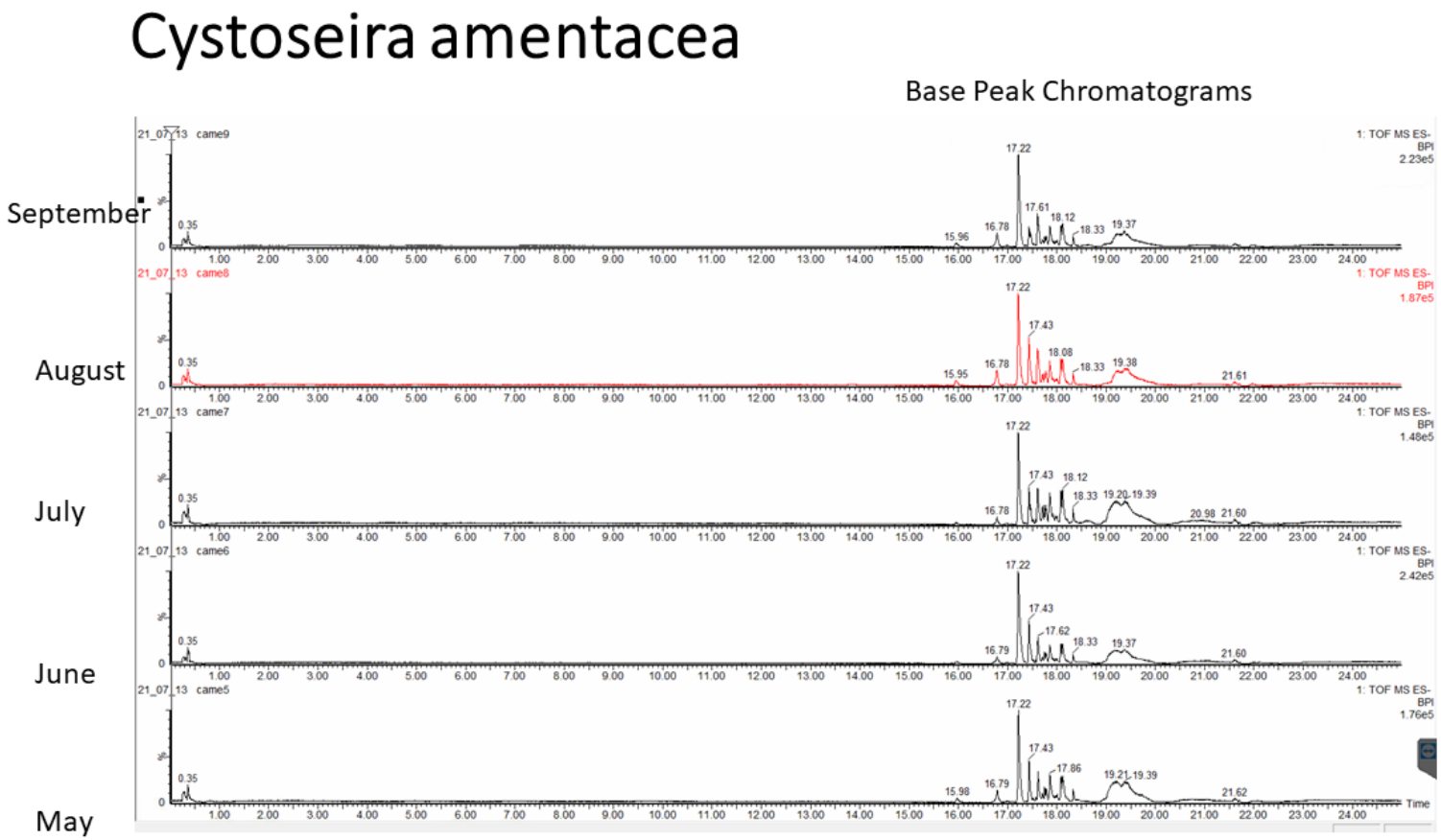

Figure 2.4. Chromatograms of the HPLC-qTOF-MS analyses of *C. amentacea*.

Table 2.4. Identification of the compounds from *C. amentacea* extracts.

	RT (min)	Mass	Formula (-)	Name	May	June	July	August	September
1	0,28	343,0367	C20 H3 N6 O	1a,9b-Dihydrophenanthro[9,10-b]oxirene-2,3,4,7,8,9-hexacarbonitrile	+	+	+	+	+
2	0,29	201,0236	C4 H9 O9	2-(1,2,2,2-Tetrahydroxyethoxy)ethane-1,1,1,2-tetrol	+	+	+	+	+
3	0,32	141,0155	C2 H N6 O2	Diazidoacetic acid	+	+	+	+	+
4	0,35	181,0699	C6 H13 O6	D-Sorbitol	+	+	+	+	+
5	0,40	317,0530	C12 H13 O10	D-glucaric acid derivate	+	+	+	+	+
6	0,49	384,1510	C15 H22 N5 O7	Threonyl-histidyl-glutamic acid	+	+	+	+	+
7	15,97	445,2946	C27 H41 O5	23-Acetoxy-12-O-deacetyl-12-epi-deoxoscalarin isomer A	+	+	+	+	+
8	16,79	445,2947	C27 H41 O5	23-Acetoxy-12-O-deacetyl-12-epi-deoxoscalarin isomer B	+	+	+	+	+
9	16,99	293,2112	C18 H29 O3	13-ketooctadecadienoic acid	+	+	+	+	+
10	17,00	275,1994	C18 H27 O2	Stearidonic acid (C18:4n-3) isomer a	+	+	-	-	-
11	17,06	443,3127	C28 H43 O4	1,24,25-Trihydroxyergocalciferol isomer a	+	+	-	+	+
12	17,09	287,2211	C16 H31 O4	10,16-Dihydroxyhexadecanoic acid isomer a	+	+	+	-	-
13	17,22	429,3004	C27 H41 O4	24-Keto-1,25-dihydroxyvitamin D3	+	+	+	+	+
14	17,35	287,2216	C16 H31 O4	10,16-Dihydroxyhexadecanoic acid isomer b	-	+	+	-	-
15	17,38	199,1689	C12 H23 O2	Lauric acid	+	+	+	+	+
16	17,43	427,2847	C27 H39 O4	Hydroxyprogesterone caproate	+	+	+	+	+
17	17,46	429,3000	C27 H41 O4	24-Keto-1,25-dihydroxyvitamin D3	+	+	+	+	+
18	17,52	225,1848	C14 H25 O2	Myristoleic acid	+	+	+	+	+
19	17,53	255,2319	C16 H31 O2	Hexadecanoic acid (palmitic acid) isomer a (C16:0)	+	+	+	+	+
20	17,58	275,2007	C18 H27 O2	Stearidonic acid (C18:4n-3) isomer b	+	+	+	+	+
21	17,61	443,3150	C28 H43 O4	1,24,25-Trihydroxyergocalciferol isomer b	+	+	+	+	+
22	17,62	427,2839	C27 H39 O4	Hydroxyprogesterone caproate	+	+	+	+	+
23	17,70	239,1998	C15 H27 O2	Myristoleic acid methyl ester	+	+	+	+	+
24	17,71	301,2148	C20 H29 O2	Eicosapentanoic acid (C20:5n-3)	+	+	+	+	+

25	17,75	277,2152	C18 H29 O2	gamma-Linolenic acid (C18:3n-6)	+	+	+	+	+
26	17,78	227,1999	C14 H27 O2	Tetradecanoic acid (C14:0)	+	+	+	+	+
27	17,86	253,2156	C16 H29 O2	Palmitoleic acid (C16:1n-7)	+	+	+	+	+
28	17,92	279,2311	C18 H31 O2	Octadeca-10,12-dienoic acid (C18:2n-6) isomer a	+	+	+	+	+
29	17,94	241,2170	C15 H29 O2	Pentadecanoic acid (C15:0)	+	+	+	+	+
30	17,97	279,2314	C18 H31 O2	Octadeca-10,12-dienoic acid (C18:2n-6) isomer b	+	+	+	+	+
31	18,01	267,2318	C17 H31 O2	9-Heptadecenoic acid (C17:1n-8)	+	+	+	+	+
32	18,08	255,2311	C16 H31 O2	Hexadecanoic acid (palmitic acid) isomer b (C16:0)	+	+	+	+	+
33	18,12	281,2466	C18 H33 O2	Oleic acid (C18:1n-9)	+	+	+	+	+
34	18,22	269,2466	C17 H33 O2	Heptadecanoic acid (C17:0)	+	+	+	+	+
35	18,33	283,2618	C18 H35 O2	Octadecanoic acid (stearic acid) C18:0	+	+	+	+	+
36	19,97	279,2304	C18 H31 O2	Octadeca-10,12-dienoic acid (C18:2n-6) isomer c	+	+	-	-	-

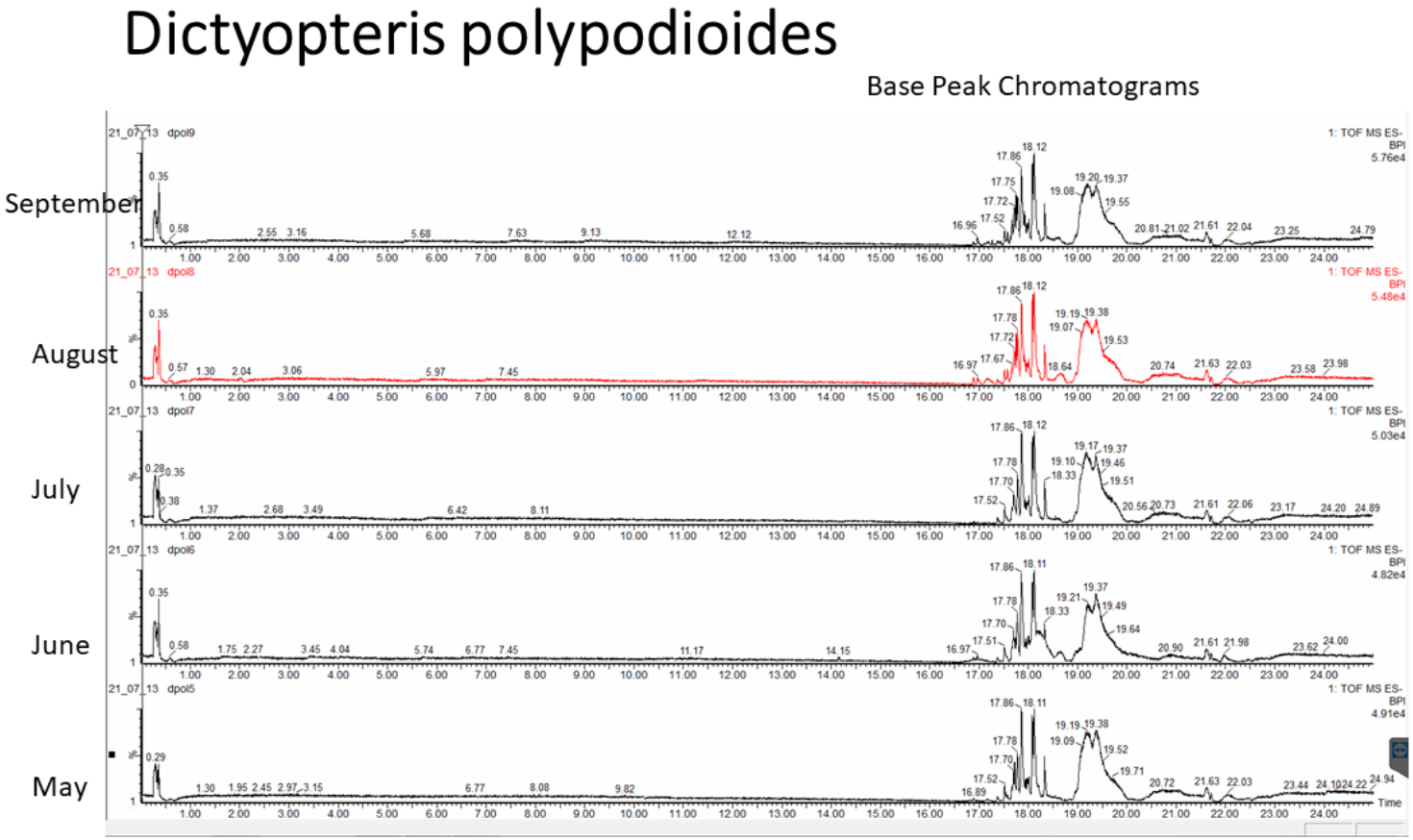

Figure 2.5. Chromatograms of the HPLC-qTOF-MS analyses of *D. polypodioides.*

Table 2.5. Identification of the compounds from *D. polypodioides* extracts.

	RT (min)	Mass	Formula (-)	Name	May	June	July	August	September
1	0,29	343,0363	C20 H3 N6 O	1a,9b-Dihydrophenanthro[9,10-b]oxirene-2,3,4,7,8,9-hexacarbonitrile	+	+	+	+	+
2	0,30	201,0250	C4 H9 O9	2-(1,2,2,2-Tetrahydroxyethoxy)ethane-1,1,1,2-tetrol	+	+	+	+	+
3	0,34	141,0160	C2 H N6 O2	Diazidoacetic acid	+	+	+	+	+
4	0,35	181,0706	C6 H13 O6	D-Sorbitol	+	+	+	+	+
5	16,89	275,2023	C18 H27 O2	Stearidonic acid (C18:4n-3) isomer a	+	+	+	+	+
6	16,96	275,1997	C18 H27 O2	Stearidonic acid (C18:4n-3) isomer b	+	+	+	+	+
7	17,17	277,2168	C18 H29 O2	gamma-Linolenic acid isomer a (C18:3n-6)	-	-	-	-	+
8	17,27	247,1709	C16 H23 O2	2,4,6-Triisopropyl benzoic acid	-	-	-	-	+
9	17,37	199,1684	C12 H23 O2	Lauric acid	+	+	+	+	+
10	17,40	243,1963	C14 H27 O3	3-hydroxymyristic acid	-	-	-	-	+
11	17,43	293,2140	C18 H29 O3	13-ketooctadecadienoic acid	-	-	-	+	+
12	17,47	295,2252	C18 H31 O3	9,10-Epoxyoctadecenoic acid (vernolic acid)	+	-	+	-	+
13	17,52	225,1837	C14 H25 O2	Myristoleic acid	+	+	+	+	+
14	17,56	301,2150	C20 H29 O2	Eicosapentanoic acid isomer a (C20:5n-3)	+	-	-	+	+
15	17,59	277,2159	C18 H29 O2	gamma-Linolenic acid isomer b (C18:3n-6)	+	-	-	-	-
16	17,58	275,2010	C18 H27 O2	Stearidonic acid (C18:4n-3) isomer c	-	-	-	+	+
17	17,63	251,1999	C16 H27 O2	7-cis,10-cis-hexadecadienoic acid	+	+	+	+	+
18	17,70	239,2004	C15 H27 O2	Myristoleic acid methyl ester	+	+	+	-	-
19	17,71	301,2162	C20 H29 O2	Eicosapentanoic acid isomer b (C20:5n-3)	-	-	-	+	+
20	17,72	363,2534	C22 H35 O4	6-O-Acetylaustroinulin / 16,16-Dimethylprostaglandin	+	+	+	+	+
21	17,75	277,2171	C18 H29 O2	gamma-Linolenic acid isomer c (C18:3n-6)	+	+	+	+	+
22	17,78	227,2006	C14 H27 O2	Tetradecanoic acid (C14:0)	+	+	+	+	+
23	17,86	253,2164	C16 H29 O2	Palmitoleic acid (C16:1n-7)	+	+	+	+	+
24	17,93	241,2169	C15 H29 O2	Pentadecanoic acid (C15:0)	+	+	+	+	+
25	17,97	279,2324	C18 H31 O2	Octadeca-10,12-dienoic acid (C18:2n-6)	+	+	+	+	+

26	18,01	267,2324	C17 H31 O2	9-Heptadecenoic acid (C17:1n-8)	+	+	+	+	+
27	18,08	255,2317	C16 H31 O2	Hexadecanoic acid (palmitic acid) isomer a (C16:0)	+	+	+	+	+
28	18,11	281,2473	C18 H33 O2	Oleic acid (C18:1n-9)	+	+	+	+	+
29	18,21	269,2477	C17 H33 O2	Heptadecanoic acid (C17:0)	+	+	+	+	+
30	18,33	283,2631	C18 H35 O2	Octadecanoic acid (stearic acid) C18:0	+	+	+	+	+
31	18,42	255,2321	C16 H31 O2	Hexadecanoic acid (palmitic acid) isomer b (C16:0)	+	-	-	-	-
32	18,54	311,2944	C20 H39 O2	Arachidic acid	+	-	+	-	-

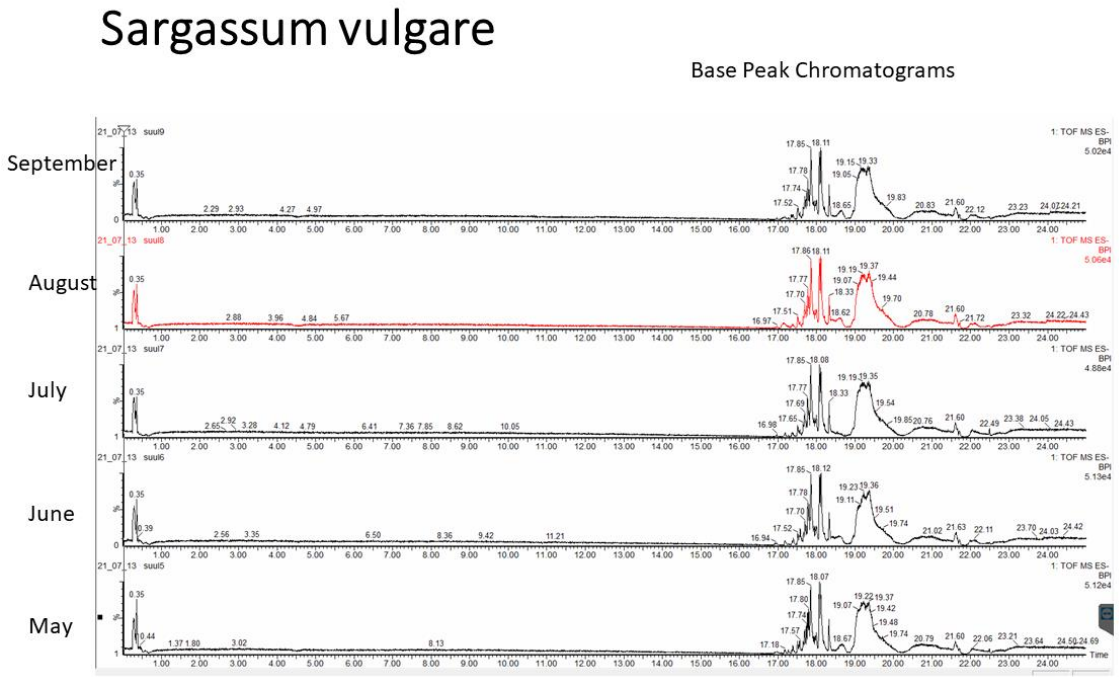

Figure 2.6. Chromatograms of the HPLC-qTOF-MS analyses of *S. vulgare*.

Table 2.6. Identification of the compounds from *S. vulgare* extracts.

	RT (min)	Mass	Formula (-)	Name	May	June	July	August	September
1	0,35	181,0702	C6H13O6	D-Sorbitol	+	+	+	+	+
2	0,44	317,0533	C12H13O10	D-glucaric acid derivative	+	+	+	+	+
3	16,96	293,2104	C18H29O3	13-ketooctadecadienoic acid	+	+	+	+	+
4	16,97	275,2004	C18H27O2	Stearidonic acid isomer a	+	+	+	+	+
5	17,19	285,2052	C11H29N2O6	Bis[tris(hydroxyMethyl)MethylaMino]propane	+	+	+	+	+
6	17,36	199,1689	C12H23O2	Lauric acid	+	+	+	+	+
7	17,38	243,1950	C14H27O3	3-hydroxymyristic acid	+	+	+	-	-
8	17,50	269,2117	C16H29O3	3-Oxohexadecanoic acid	+	+	+	+	+
9	17,56	275,2011	C18H27O2	Stearidonic acid isomer b	+	-	+	+	+
10	17,65	239,2011	C15H27O2	Myristoleic acid methyl ester isomer a	+	+	+	+	+
11	17,69	239,2012	C15H27O2	Myristoleic acid methyl ester isomer b	+	+	+	+	+
12	17,74	277,2168	C18H29O2	(11E)-Octadec-11-en-9-ynoic acid (santalbic acid)	+	+	+	+	+
13	17,77	227,2002	C14H27O2	Tetradecanoic acid (C14:0)	+	+	+	+	+
14	17,80	271,2266	C16H31O3	Hydroxy-palmitic acid	+	+	+	+	+
15	17,85	253,2161	C16H29O2	Palmitoleic acid	+	+	+	+	+
16	17,93	241,2161	C15H29O2	Pentadecanoic acid (C15:0)	+	+	+	+	+
17	17,96	279,2320	C18H31O2	Linoleic acid derivative (Octadeca-10,12-dienoic acid (C18:2n-6))	+	+	+	+	+
18	17,99	267,2309	C17H31O2	9-Heptadecenoic acid (C17:1n-8)	+	+	+	+	+
19	18,07	255,2313	C16H31O2	Palmitic acid (C16:0)	+	+	+	+	+
20	18,11	281,2475	C18H33O2	Oleic acid isomer a (C18:1n-9)	+	+	+	+	+
21	18,16	281,2471	C18 H33 O2	Oleic acid isomer b (C18:1n-9)	+	+	+	+	+
22	18,32	283,2631	C18H35O2	Octadecanoic acid (stearic acid) C18:0	+	+	+	+	+
23	18,54	311,2946	C20 H39 O2	Arachidic acid	+	+	+	+	+

2. REPORT OF THE BIOACTIVE COMPONENT COMPOSITION IN AGRO-FOOD BY-PRODUCTS

3.1. Material and methods

2.1.1. Sample collection (UNIST, CROSME, UNILJUB, UNIBO, CUNI)

The agro-food by-products were collected by different partners according to Table 3.1 from traditional agro-productions (berry wine production, cherry and aronia juice production, wine and olive oil production, rosehip extract production, juniperus extract production) and wastes from these industries. All samples were shade-dried for approximately four to six days before being pulverized.

Table 3.1. List of the agro-food by-products collected by different partners

	Matrix	Collecting partner	Extraction method*	Mark
1.	Blackberry whole 2020	UNIST	MAE	PRIMA_01
2.	Blackberry leaves 2020	UNIST	MAE	PRIMA_02
3.	Blackberry juice by-product 2020	UNIST	MAE	PRIMA_03
4.	Blackberry juice by-product 2020	UNILJUB	MAE	PRIMA_04
5.	Aronia juice by-product (production 2019)	UNILJUB	MAE	PRIMA_05
6.	Aronia juice by-product (production 2020)	UNILJUB	MAE	PRIMA_06
7.	Cherry juice by-product (production 2020)	UNIST	MAE	PRIMA_07
8.	Rosehip extracts production by-product 2020	UNILJUB	MAE	PRIMA_08
9.	Grape pomace 2020	CUNI	MAE	PRIMA_09
10.	Olive pomace 2020	CUNI	MAE	PRIMA_10
11.	Olive leaves Lastovka FS 2020	UNIST	UAE	PRIMA_11
12.	Olive leaves Levantinka FS 2020	UNIST	UAE	PRIMA_12
13.	Olive leaves Oblica VS 2020	UNIST	UAE	PRIMA_13
14.	Olive leaves Moraiolo Toscana 2020	UNIBO	UAE	PRIMA_14

15.	Olive leaves Frantoio Toscana 2020	UNIBO	UAE	PRIMA_15
16.	Olive leaves Brisighella (Emilia Romagna) 2020	UNIBO	UAE	PRIMA_16
17.	*Juniperus oxycedrus* berries green 2020	UNIST	MAE	PRIMA_17
18.	*Juniperus oxycedrus* berries red 2020	UNIST	MAE	PRIMA_18
19.	*Juniperus oxycedrus* needles 2020	UNIST	MAE	PRIMA_19
20.	*Juniperus communis*. extract by-product 2021	UNILJUB	MAE	PRIMA_20

*MAE - microwave assisted extraction; UAE - ultrasound assisted extraction

2.1.2. The extraction procedure (UNIST)

All the dried materials were extracted in 50% EtOH using the MAE (advanced microwave extraction system ETHOS X, Milestone Srl, Sorisole, Italy, 600 W, 5 minutes) or UAE extraction in methanol (Transsonic Tp 310H, Elma Schmidbauer GmbH, Singen, Germany, triple extraction, UVZ bath, 40kHz, RT, 30 minutes) method. The choice of the method was done based on the total phenolic content of the MAE and UAE extracts of each sample established during preliminary studies. After the extraction the EtOH was evaporated and the extracts freeze dried and sent to partners for further analyses.

2.1.3. Preparation of the essential oil from the selected matrices (UNIST)

The essential oils (EOs) were prepared from samples PRIMA_02, PRIMA_19 and PRIMA_20 (Table 3.1.), by hydrodistillation of dried material (100 g) that was immersed in a flask with distilled water (1000 mL). The extraction process was performed in Clevenger apparatus during 3 h. Pentane and diethyl ether (1:1, v/v) in the inner tube of the apparatus were used for trapping of the volatile compounds carried through the system by vapour. Finally, after hydrodistillation, pentane was separated, and distillate was dried over anhydrous sodium sulfate. The samples of EOs were stored at 4°C in the dark vials until analysis.

2.1.4. HPLC identification of the compounds from extracts (UNIST)

Reagents: All used phenolic standards, reagents and solvents were appropriate analytical or HPLC grade, and were purchased from Sigma (Sigma–Aldrich GmbH, Steinheim, Germany), Merck (Darmstadt, Germany), Fluka (Buch, Switzerland) and Kemika (Zagreb, Croatia).

Instrument: HPLC-DAD Ultimate 3000, Termo Fisher Scientific, Wathman, MA, SAD
The HPLC analysis of phenolic compounds and extracts was conducted using a HPLC system equipped with a UV-Vis DAD. The separation was carried out using a Syncronis ™ C18 Columns with dimensions of 250×4.6 mm and particle size 5 µm (Termo Fisher Scientific, Wathman, MA, USA). Different gradient mobile phases were tested at different flow rates and column temperatures in order to find a suitable separation method for the standards. The gradient method that was chosen uses mixture of water containing 0,2% formic acid (A), acetonitrile (B) and methanol (C). The total runtime of the method was 80 min and the concentration gradients was varied as shown in table 3.2. The column temperature was 25 °C, the volume of the injected sample was 10 µL and flow rate 0.8 mL/min. Following the analysis of the UV-Vis spectra of the individual phenolic standards, two wavelengths 280 and 320 nm were chosen for analysis in this investigation.

Standard solutions were injected in five different concentrations (three repetitions), and each component was detected on corresponding wavelength. Table 3.3. presents summarized results including retention time of each substance, calibration curve equation, and determination coefficients. Phenolic compounds were divided in two phenolic mixtures: Mixture 1 and Mixture 2. Fig. 3.1. and 3.2. shows the chromatograms of the standard solution containing phenolic compounds obtained at 280 (Mixture 1) and 320 nm (Mixture 2).

Table 3.2. Solvent gradient flow

t, min	Gradient A, %	Gradient B, %	Gradient C, %
0	96	2	2
40	50	25	25
45	40	30	30
60	0	50	50
68	0	50	50
70	96	2	2
80	96	2	2

Table 3.3. Calibration curve data for all analyzed phenolic compounds

	Compound	**Retention time (min)**	**Calibration curve**	**Coefficient of determination (R^2)**
Mixture 1	Gallic acid	11.524	y = 2.1718x - 0.0544	0.9998
	Protocatechuic acid	16.899	y = 3.3268x - 0.1995	0.9998
	(+)-catechin	21.138	y = 7.0984x - 0.2686	0.9998
	p-hidroxybenzoic acid	22.238	y = 3.8258x - 0.4585	0.9997
	Vanilic acid	24.162	y = 3.4538x - 0.1589	0.9998
	(-)-epigallocatechin gallate	24.407	y = 3.6744x + 0.1472	0.9998
	(-)-epicatechin+Syringic acid	25.029	y = 2.0271x - 0.1374	0.9998
	Ferulic acid	31.5214	y = 1.909x - 0.0807	0.9998
	Rutin	32.438	y = 7.1137x - 0.061	0.9996
	trans-cinnamic acid	43.961	y = 0.6736x - 0.0455	0.9998
	o-cumaric acid	36.433	y = 0.9746x - 0.0136	0.9999
	Myrcetin	38.223	y = 4.0795x + 0.845	0.9995
	Quercetin	44.147	y = 4.3845x + 0.7886	0.9984
	Neringenin	46.245	y = 1.552x + 0.1398	1
	Hesperetin	47.633	y = 5.7066x + 0.0076	0.9999
Mixture 2	Chlorogenic acid	21.719	y = 1.7907x + 0.7715	0.9996
	Gentisic acid	23.287	y = 4.6269x + 0.7154	0.9996
	Caffeic acid	24.780	y = 0.9405x + 0.2204	0.9997
	Astrignin	26.101	y = 1.7311x + 1.0093	0.9996
	p-cumaric acid	30.128	y = 0.8649x + 0.1628	0.9996
	Sinapic acid + Ferulic acid	31.507	y = 0.5373x + 0.2538	0.9996
	Rutin trihydrate	32.435	y = 4.8626x + 0.4004	0.9996
	Rosmarinic acid	36.899	y = 0.7206x + 0.1231	0.9995
	Resveratrol	28.080	y = 0.6542x + 0.2192	0.9996
	Quercetin	44.231	y = 3.7747x + 0.619	0.9985
	Apigenin	48.699	y = 1.3214x + 0.2479	0.9993

Identification of individual compounds in samples was done using UV-VIS spectrum and retention time of the compound. Each compound was quantified according to the peak area measurements using calibration curves of the corresponding standards. Data are reported as means +standard deviations of two independent analyses.

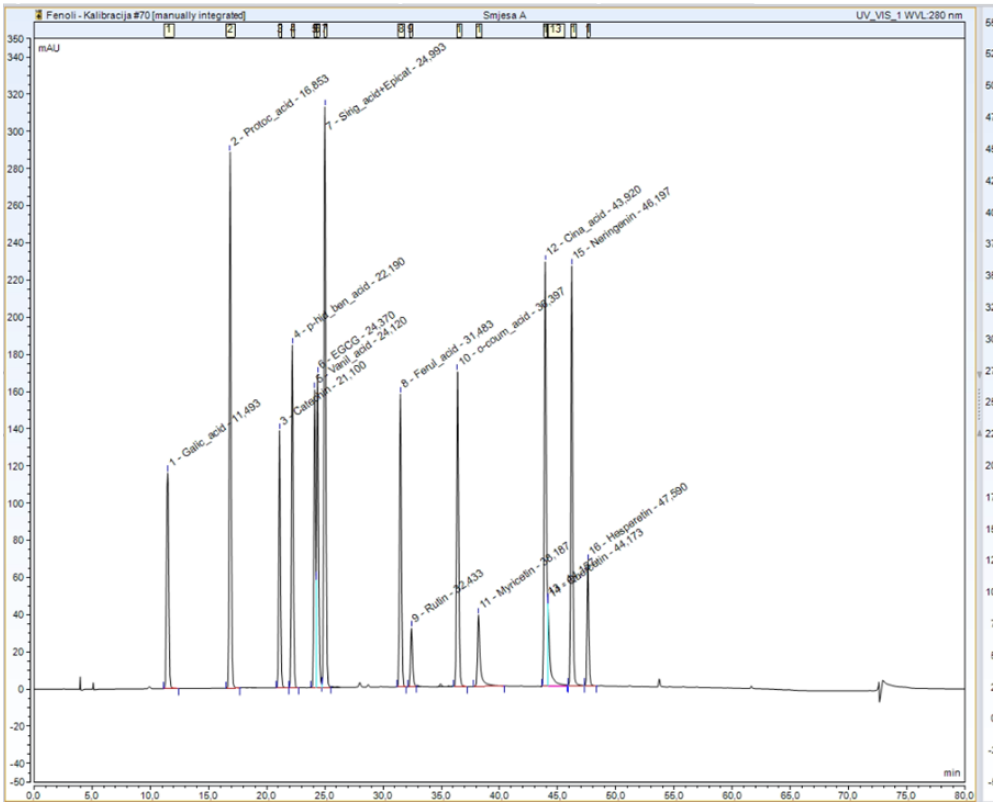

Figure 3.1. HPLC chromatogram of phenolic standards: Mixture A

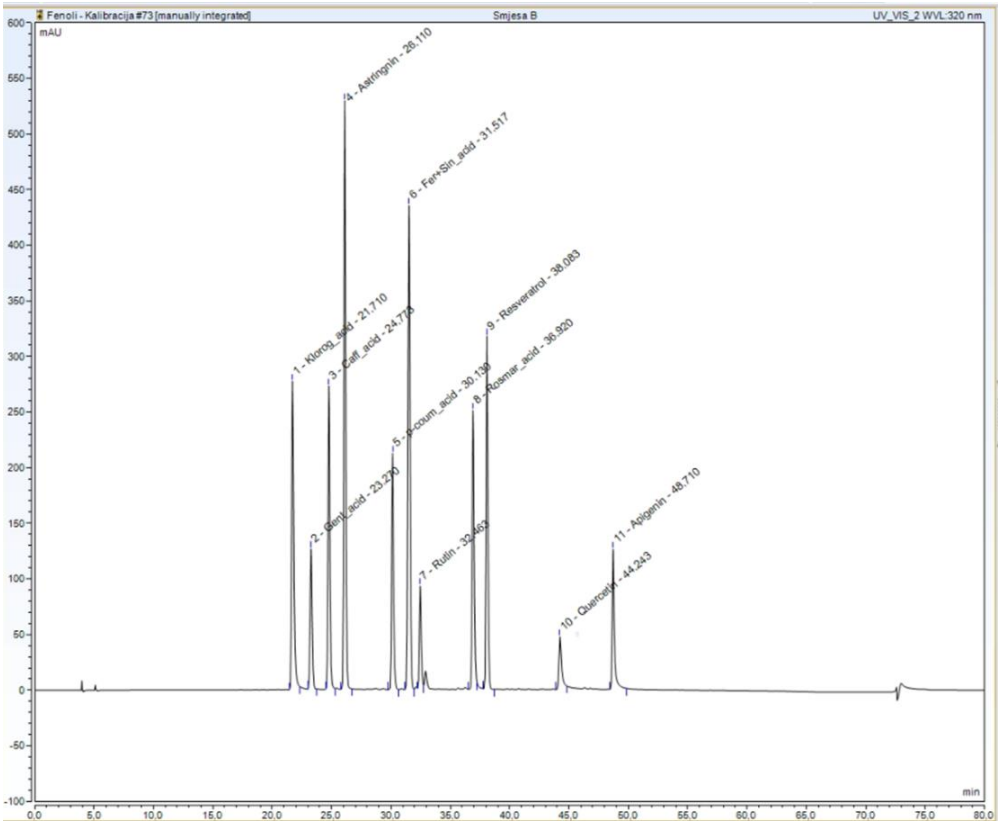

Figure 3.2. HPLC chromatogram of phenolic standards: Mixture B

2.1.5. GC/MS Characterization of food by-product extracts (but olive leaves) (UNIST)

Reagents: derivatization reagent N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA); Sigma-Adrich (St. Louis, USA)

Instrument: Shimadzu GC-MS (Kyoto, Japan); Nexis GC-203 coupled with QP2020 NX

Derivatization of polyphenols

Sample derivatization: Prepared samples were evaporated in a vacumstream of nitrogen (?), after which 50 μL of derivatizing agent (BSTFA) was added.

The GC/MS analysis of derivatized samples was carried out using Shimadzu (Kyoto, Japan) Nexis GC-2030GC coupled with Shimadzu QP2020 NX mass detector (MS), equipped with a split/splitless injection port. Analysis was preformed using Rtx-5MS (Restek) fused silica capillary column (length 30 m × inside diameter 0.25 mm i.d., film thickness 0.25 m). Ultra-pure helium was used as carrier gas with flow rate at 1 mL/min. Analysis were performed with MS full scan (35-750 m/z). The mass spectrometer was calibrated with perfluorotributylamine at an electron impact ionization energy of 70 eV. The column temperature program was: oven equilibration time 3 min; initial temperature 120 °C for 3 min, increased to 292 ◦C at a rate of 5 °C /min, then increased to 320◦ at a rate of 30 °C /min and held isothermal for 17 min. Identification of phenolic compounds in sample derivatized extracts was performed by comparing their trimethylsilyl (TMS) derivative mass spectra and GC retention times to those of the 23 derivatized standards (same standards mixtures used for HPLC), relative to series of n-hydrocarbons, as well as by the computer matching with commercial libraries (Wiley 12 and Nist 2020). GC/MS profile of tested samples was expressed as relative percentage of each single peak area with respect to the total peak area.

2.1.6. Olive leaves analysis by UPLC-PDA-ESI-QTOF (UNIBO/UNIST)

Methodology described in section 2.1.4.

2.1.7. GC/MS Characterization of selected food by-product essential oils (UNIBO)

The volatile organic compounds (VOCs) of EOs were analyzed by GC-MS (Shimadzu QP2010, Shimadzu, Kyoto, JP) equipped with an autosampler and a DB-5 60 m × 0.25 mm x 0.25 µm column (Agilent Technologies Italia Spa, Milano, Italy). The EOs were resuspended in hexane and 1 µL was injected in the following gas chromatographic conditions: injection temperature 260°C; interface temperature 280°C; ion source 220°C; carrier gas (He) flow rate 30 cm/sec; splitting ratio 1:20. The oven temperature was programmed as follows: 40°C for 4 min; from 40°C to 175°C with a 3°C/min rate of increase; from 175°C to 300°C with a 7°C/min increase, then holding for 10 min. VOCs were identified by referencing NIST 8.0 (US National Institute of Standards and Technology). For each sample, the volatile profile composition was expressed as relative percentage of each single peak area with respect to the total peak area. Data reported are the means of two repetitions.

3.2 Results

3.2.1. HPLC identification of the compounds from extracts (UNIST)

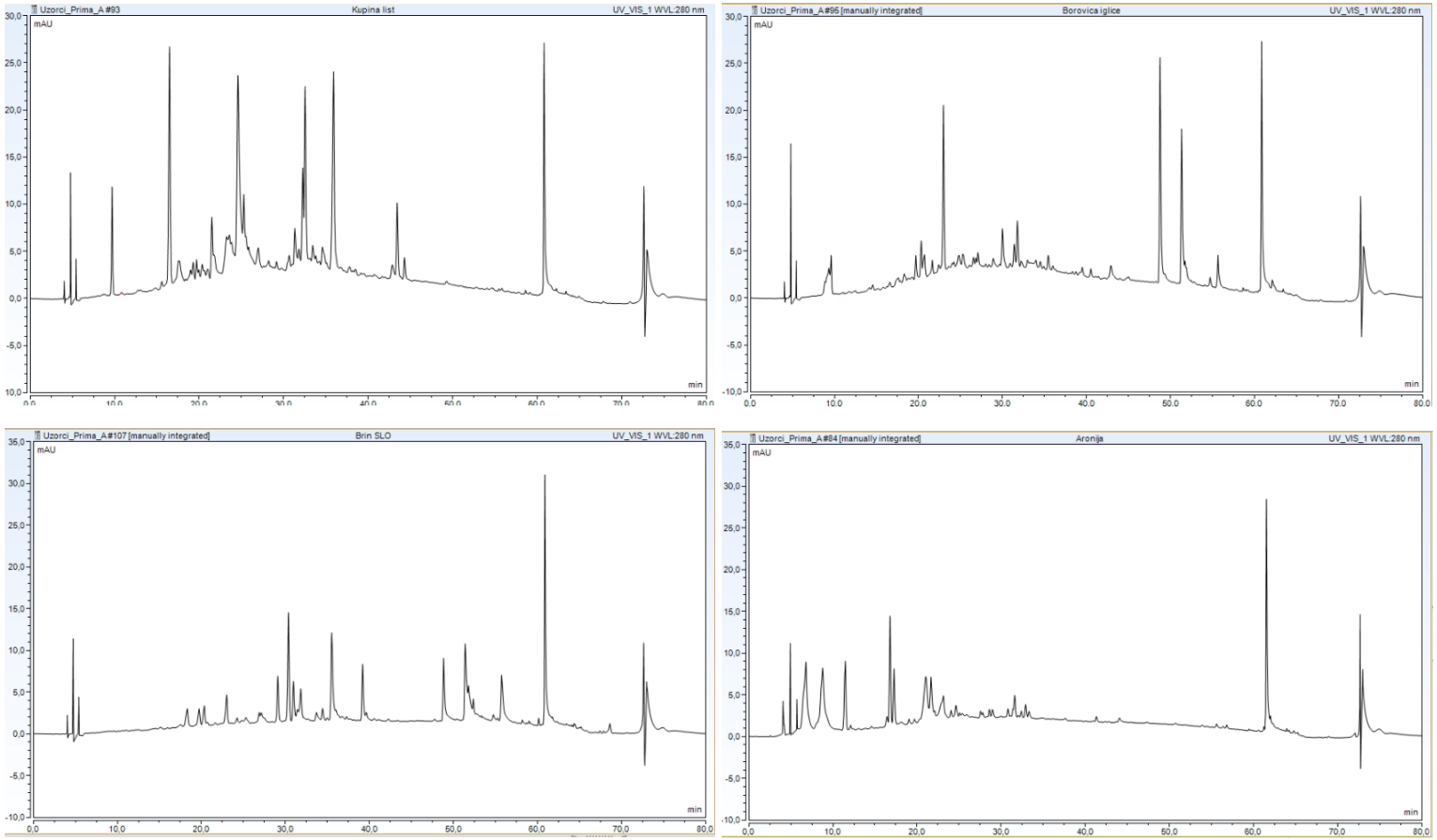

Figure 3.3. HPLC chromatogram of some extracts

Table 3.4. Results of HPLC analysis (µg/mL)

Phenolic compound	PRIMA_01	PRIMA_02	PRIMA_03	PRIMA_04	PRIMA_05	PRIMA_06	PRIMA_07	PRIMA_08	PRIMA_09	PRIMA_10	PRIMA_17	PRIMA_18	PRIMA_19	PRIMA_20
Galic acid	-	0.126±0.01	-	0.149±0.01	0.654±0.13	3.890±0.05	-	1.092±0.01	0.240±0.01	-	-	-	1.376±0.01	-
Cffeic acid	-	2.035±0.04	-	-	0.557±0.05	0.685±0.08	-	-	-	-	-	-		-
Protocatechuic acid	0.148±0.00	-	-	0.266±0.19	0.301±0.04	7.066±0.63	-	-	-	1.06±0.03	tr	0.013±0.01	0.324±0.02	-
p-hidroxybenzoic acid	-	-	-	-	-	-	-	-	-	-	-	-	0.809±0.01	-
Vanilic acid	-	-	-	-	-	-	-	-	-	-	-	-	10.508±0.16	2.589±0.02
Chlorogenic acid	-	6.223±0.07	-	-	-	4.809±0.30	-	-	-	-	-	-	-	-
p-coumaric acid	-	0.627±0.00	-	-	-	-	-	-	-	-	-	-	-	-
Syringic acid + (-)-epicatechin	-	-	-	-	0.078±0.01	-	-	-	tr	-	-	-	0.511±0.01	0.096±0.01
(+)-catechin	-	-	-	-	-	-	-	0.109±0.03	0.009±0.00	-	0.130±0.11	-	4.860±0.01	4.427±0.07
Quercetin	-	-	-	-	1.351±0.00	1.209±0.05	-	-	-	-	-	-	-	-
Rutin		29.878±0.39	0.719±0.13	1.320±0.23	1.528±0.02	1.206±0.31	0.436±0.02	-	1.264±0.11	-	0.602±0.01	-	6.952±0.01	7.158±0.112
Astringnin	-	2.410±0.02	-	-	-	-	-	-	-	-	-	-		-
Apigenin	-	-	-	-	-	-	-	-	-	0.376±0.02	1.665±0.00	0.586±0.01	7.663±0.04	3.032±0.01
(-) epigallocatechin gallate	-	-	-	-	-	-	-	0.581±0.22	-	-	-	-	0.723±0.16	0.806±0.00
sum	0.148	**41.300**	0.719	1.735	4.468	**18.864**	0.436	1.782	1.513	1.431	2.397	0.599	**33.726**	**18.108**

tr- in traces

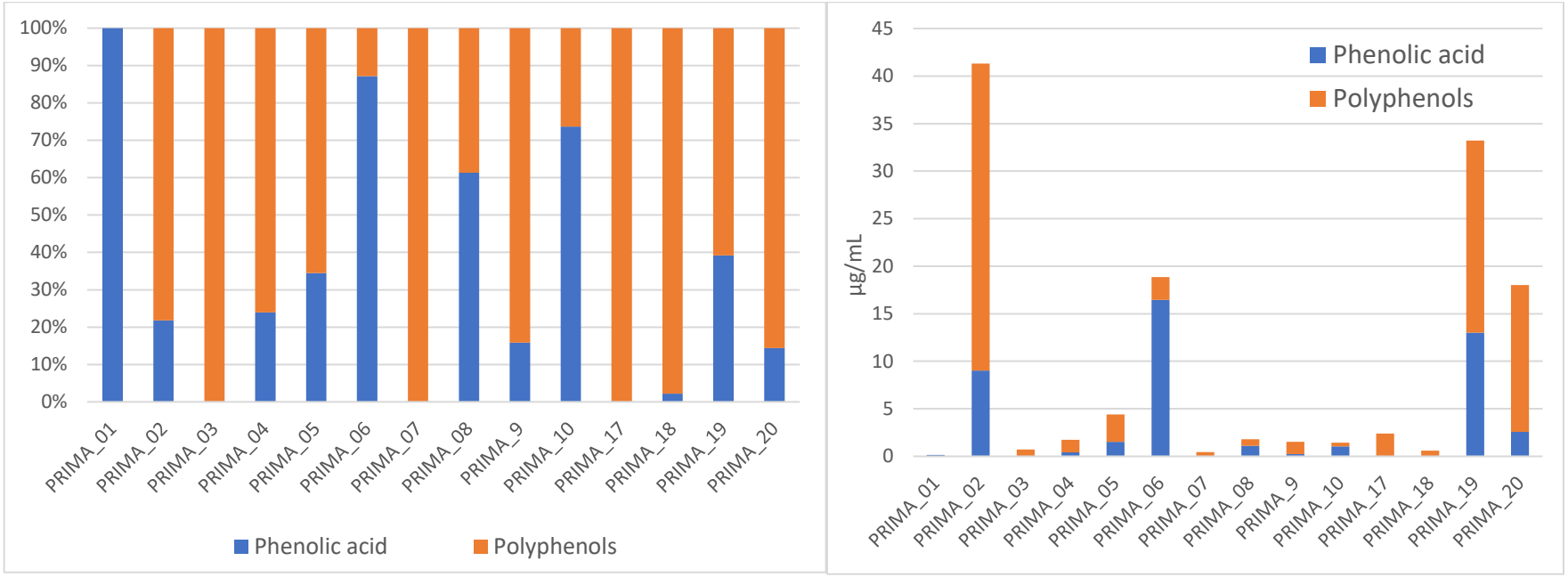

Figure 3.4. Proportion and concentration of phenolic acids and polyphenols in tested extracts.

Phenolic profile of tested by-product is shown in table 3.4. and the largest share of phenols have PRIIMA_02, PRIMA_06, PRIMA_19 and PRIMA_20 samples. As can be seen from the picture 3.4. the main group of identified phenolic compounds was polyphenols (in range to 32.3 µg/mL in PRIMA_02). From group of phenols dominant compounds were flavonol glycoside rutin (quercetin-3-rutinoside), flavon apigenin and flavan-3-ol catechin. Of the phenolic acids, the most dominant were protocatechuic and gallic acid, but not in the highest concentrations. The highest concentration of phenolic acids had PRIMA_19 and it was vanillic acid (10.51 µg/mL was acidic of a total phenols of 33.73 µg/mL). In the same sample concentration of flavonoids was 20.2 µg/mL and the main compounds were apigenin, rutin and catechin. In sample PRIMA_20 of phenolic acids only vanillin acid was identified (2.59 µg/mL), while the flavonoid content was 15.42 µg/mL and the most common compound was rutin, followed by catechin and apigenin. Compared to Prima_19 in PRIMA 17 and PRIMA_18 samples, which are berries of the same plant, no large proportion of phenolic components was identified in the extracts. From all tested agro-food by-products, the most interesting was PRIMA_02 sample, blackberry leaves. Only two polyphenols have been identified in this extract, namely rutin and astrignin, but in very high concentration (29.9 and 2.4 µg/mL, respectively). Other blackberry by-product had a very low proportion of identified phenols (0.15 to 1.74).

3.2.2. GC/MS Characterization of food by-product extracts (but olive leaves) (UNIST)

The GC/MS results of derivatized samples are reported in Table 3.5. and 3.6. The total number of identified compounds was 189 but not all compounds were identified in all samples. The smallest proportion of peak area was identified in the samples PRIMA_02, PRIMA_19 and PRIMA_20 but the sum of area of phenolics was the highest (table 3.6). Very similar results for the phenolic composition were proved by this method for sample PRIMA_19. There was a dominant amount of polyphenols (11.5%) in comparison to phenolic acids (0.5%). In relation to the HPLC method in this method the presence of more phenolic acids and some polyphenols was identified. The reason for this is most likely the method of derivatization which could have led to the separation or cracking of large polymer molecules (e.g. glycosides) and the formation of smaller and simpler compounds. Thus, for example, in this method we have not identified the presence of rutin, which was probably separated into quercetin and rutinozide by the derivatization method. In addition to phenols, GC/MS analysis showed the presence of fatty acids and esters of fatty acids. Among saturates fatty acid stand out stearic and palmitic acid in almost all samples. Of the unsaturated fatty acids, oleic, linolic and linoleic acids are present. Also, from table 3.5 it can be noticed very high amount of monopalmitin, monostearin and monostearat compounds.

Table 3.5. Results of GC/MS derivatization analysis

0	**Compound (TMS derivatives)**	**RI**	**PRIMA _01**	**PRIMA _02**	**PRIMA _03**	**PRIMA _04**	**PRIMA _05**	**PRIMA _06**	**PRIMA _07**	**PRIMA _08**	**PRIM A_9**	**PRIMA _10**	**PRIMA _19**	**PRIMA _20**
1	Octanoic acid, TMS derivative											0,08		
2	Glycerol, 3TMS derivative	1275	2.89	2.26	22	8.76	4.56	6.24	10.4		4.51	9.17	0.6	0.13
3	Benzeneacetic acid, TMS derivative	1293						0.07						
4	Butanedioic acid	1301	0.08	2.61	0.42	0.65	0.14		0.4	0.37		0.13		
5	Propylenglycol, TBS 2X	1308						0.45						
6	Glyceric acid, 3TMS derivative	1331								0.21				
7	Threonic acid,O,O,O,O-TMS	1333						0.07						
8	2-Butenedioic acid, €-, 2TMS derivative	1335										0.05		
9	Nonanoic acid, TMS derivative	1353	0.22		0.45	0.41		0.3	0.52	0.13		0.05		
10	3-Hydroxybutanal, TBDMS derivative	1361					0.1							
11	Malic acid, 2TMS derivative	1393						0.05						
12	Tartronic acid, 3TMS derivative	1394										0.02		
13	Malic acid 1-ethyl ester, 2TMS	1440								0.24				
14	Decanoic acid, TMS derivative	1454			0.07									
15	Malic acid, 4-ethyl ester, 2TMS	1460				0.75		0.08	0.35	0.23				
16	Diglycerol, 4TMS derivative	1480					0.39							
17	Malic acid, 3TMS derivative	1503	0.02	0.57	0.16	1.98		1.19	3.86	1.05	1.65	0.06	0.19	0.04
18	Erythritol, 4TMS derivative	1531								0.06		0.12		
19	Hexanedioic acid, 2TMS derivative	1515	0.02				0.66							
20	L-5-Oxoproline, 2TMS	1525										0.03		
21	Vanilin, TMS derivative	1533										0.03		
22	2-Isopropyl-3-(trimethylsilyloxy)butyric acid, trimethylsilyl ester	1539					0.07							
23	Cinnamic acid, TMS derivative	1540												0.01
24	Tyrosol, 2TMS derivative	1576						0.08						

25	Erythronic acid, tetrakis(trimethylsilyl) deriv	1585												0.02
26	3-phenyllacetic acid, 2TMS derivative	1591									0.05			
27	Tartaric acid(2R, 3R)-, 3TMS	1599									0.12			
28	Monoethyl phosphate, 2TMS derivative	1612												1.35
29	Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)-	1612								0.53				
30	p-Hydroxybenzoic acid, 2TMS derivative	1632	0.1	0.15		0.04		0.06			0.04	0.02	0.04	0.03
31	beta.-D-Tagatopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-	1635												0.04
32	tau.-Cadinol	1641												0.1
33	Arabinonic acid, 2,3,5-tris-O-(trimethylsilyl)-, .gamma.-lactone, d-	1649										0.05		
34	Dodecanoic acid, TMS derivative	1654	0.16		0.08					0.02	0.04			
35	Tridecanoic acid, TBDMS derivative	1658									0.18			
36	Phloroglucinic acid, 3TMS	1663												0.01
37	Tartaric acid, 4TMS derivative	1669					0.07			0.05	1.75	0.06		
38	Kojic acid, 2TMS derivative	1681						0.89			1.26			
39	Isocitric acid lactone, 2TMS derivative	1713	0.11		0.29	2.35				0.05				
40	Quininic acid (5TMS)	1720									0.1			
41	Pentafluoropropionic acid, pentadecyl ester	1731												0.01
42	Oplopanone	1737												0.11
43	5,6-Dimethyl-2-thiouracil, 2TMS derivative	1742					1.46	1.29						
44	D-(+)-Arabitol, 5TMS	1744										1.62		
45	Tridecanoic acid, TMS derivative	1754	0.05											
46	D-Arabino-Hexonic acid, 3-deoxy-2,5,6-tris-O-(trimethylsilyl)-, ?-lactone	1756												0.07
47	Xylitol, 5TMS	1757									0.08	0.84	0.05	
48	Adonitol, 5TMS	1760										0.1		
49	Phosphoric acid, 2-(trimethylsiloxy)-1-[(trimethylsiloxy)methyl]ethyl bis(trimethylsilyl) ester	1761												0.12

50	Vanillic Acid, 2TMS derivative	1771		0.12				0.86		0.07		0.17		
51	Mannitol TMS	1776											0.22	
52	Ethanol, (2-(3,4-dihydroxyphenyl)-, tris(trimethylsilyl)-	1779										0.16		
53	Methyl .alpha.-Arabinofuranoside, 3TMS derivative	1790					0.25	0.18						0.11
54	Phosphoric acid, bis(trimethylsilyl) 2,3-bis[(trimethylsilyl)oxy]propyl ester	1797												1.79
55	Myristic acid, TMS derivative	1816	0.09											
56	.beta.-D-Galactopyranoside, methyl 2,4-bis-O-(trimethylsilyl)-, diacetate	1822									0.16			
57	Methyl.alpha.-D-glukofuranoside, 4TMS derivative	1830									0.54			1.18
58	beta.-D-Galactofuranose, 1,2,3,5,6-pentakis-O-(trimethylsilyl)	1833												1.57
59	D-(-)-Ribofuranose, tetrakis(trimethylsilyl)ether (isomer 1)	1836									0.78			
60	Protocatechoic acid, 3TMS derivative	1836	tr.	0.03		0.14	0.34	2.78		0.18		0.36	0.1	
61	D-(+)-Talofuranose, pentakis(trimethylsilyl) ether	1837							0.57					1.93
62	L-Sorbopyranose, (1S,2R,3S)-, 5TMS	1841												0.58
63	Shikimic acid, 4TMS	1841											4.05	
64	D-Fructose, 5TMS derivative	1842		4.47							0.63			0.88
65	1,2,3,4,5-pentakis-O-(trimethylsilyl)-.beta.-D-Tagatopyranose	1851									3.22			
66	Citric acid, 3TMS	1855				0.9	3.28	0.07		3.89	0.37	0.38		
67	Galactofuranose, 2,6-di-O-methyl-1,3,5-tris-O-(trimethylsilyl)	1861												1.1
68	3-Deoxyhexitol, 5TMS derivative	1873					0.18							
69	alpha.-L-Galactofuranose, 6-deoxy-1,2,3,5-tetrakis-O-(trimethylsilyl)-	1874												0.39
70	(Z)-3-Hexenyl .beta.-glucopyranoside, 4TMS derivative	1877							1.27					
71	Methyl alpha D-glucofuranoside, 4TMS derivate	1878	0.46	2.51	3.86	2.09	1.73	0.14					1.59	0.86

72	D-Allofuranose, pentakis(trimethylsilyl) ether	1885												0.17
73	beta.-D-Galactofuranose, 1,2,3,5,6-pentakis-O-(trimethylsilyl)-	1885		0.49										
74	Quininic acid , 5TMS	1898		0.66				0.2		0.25			3.97	
75	3,4-Dihydroxyphenylglycol, 4TMS derivative	1906										0.24		
76	Syringic acid, 2TMS	1913		0.14									0.02	
77	D-(+)-Gluconolactone, 4TMS	1917									0.18			
78	1,2,3,4,6-Pentakis-O-(trimethylsilyl)hexopyranose	1929												0.87
79	Galactonic acid, gamma.-lactone, 4TMS	1929									0.7	0.26		
80	alpha-D-Xylopyranose, 4TMS derivative	1938									0.17			
81	alpha.-D-Glactopyranose, 5TMS derivative	1939	0.2	0.75	1.43	0.74				0.43			1.91	
82	D-Galactose, 5TMS derivative	1940	0.12	0.58	1.62	0.81							0.72	
83	p-Coumaric acid, 2TMS	1945	0.01	0.08	0.01					0.05	0.02		0.02	
84	Pentadecanoic acid, TMS derivative	1950			0.14						0.13			
85	Ethyl gallate, 3TMS derivative	1961								0.84				
86	D-Glucitol, 6TMS	1979						0.12						
87	Gallic acid, 4TMS derivative	1983		0.2		0.05	0.01	0.41		0.27	0.23	0.05	0.02	
88	Palmitic acid, ethyl ester	1993												0.07
89	α-Altrofuranose, TMS	1999						0.07						
90	D-Galactonic acid, 6TMS	2003						0.05			0.39			
91	Undecanedioic acid, 2TMS derivative	2014												0.04
92	Palmitelaidic acid, TMS	2024	4.92				0.1	0.14			0.74	2.26	0.23	0.08
93	Galactopyranose	2027			0.87	0.35								
94	D-Glucose, 5TMS derivative	2028		0.77					0.19	0.55			1.77	
95	D-Gluconic acid	2044								0.17	0.15			
96	Palmitic Acid, TMS derivative	2051	32.62	1.99	2.63	1.87	2.41	1.93	1.8	2.02	2.93	8.14	0.96	0.92
97	Thunbergol	2058												0.2
98	trans-Sinapyl alcohol, 2O-TMS	2101										0.18		

99	Ferulic acid, 2TMS	2104		tr.				0.08		tr.	0.02		0.07	0.02
100	10-Heptadecanoic acid, (Z)-, TMS derivative	2126										0.33		
101	N-Acetyl-D-galactosamine, (isomer 2), 4TMS derivative	2143					0.04							
102	Heptadecanoic acid, TMS derivative	2149	1.27								0.02	0.21		0.02
103	Caffeic acid, 3 TMS derivative	2154	0.01	0.12		tr.	0.04	0.01		0.01			0.09	
104	1-Octadecanol, TMS derivative	2161									0.06			
105	Linoleic acid ethyl ester	2162												0.15
106	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	2168												0.18
107	9-Octadecadienoic acid (Z), ethyl ester	2169										0.12		
108	Phytol, TMS derivative	2183												0.25
109	9,12-Octadecadienoic acid (Z,Z), TMS derivative	2214	0.55	0.17	1.94	1.01	0.23	0.65		0.99	1.7	2.71	0.12	0.77
110	Oleic acid, trimethylsilyl ester	2219	4.87		2.14	2.28	0.38	0.61	0.89	1.19	1.96	11.62		
111	alpha Linolenic acid, TMS derivative	2221		0.95						0.67			0.54	0.68
112	Stearic acid, TMS derivate	2248	29.9	0.44	1.38	0.69	0.77	0.88	0.87	0.59	1.27	3.75	0.26	
113	Sinapinic acid, 2TMS	2258		0.03						0.01				
114	Cryptopimaric acid, TMS	2325											0.8	
115	Communic Acid, TMS derivative	2331											0.15	11.74
116	Isopimaric acid, TMS	2338											0.76	5.2
117	Ochratoxin A	2241												0.05
118	Pimaric acid TMS	2257												4.1
119	D-Erythro-Pentofuranose, 2-deoxy-1,3-bis-O-(trimethylsilyl)	2260												0.36
120	1-Naphthalenepropanol, ?-ethenyldecahydro-2-hydroxy-?,2,5,5,8a-pentamethyl-, [1R-[1?(R*),2ß,4aß,8a?]]-	2270												0.94
121	Dehydroabietinol, TMS	2316												0.76
122	Cryptopimaric acid, TMS	2328												2.7
123	Nonadecanoic acid, TMS derivative	2347	0.04											

124	D-Myo-Inositol, 1,2,4,5,6-pentakis-O-(trimethylsilyl)-, bis(trimethylsilyl) phosphate	2352												0.19
125	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl] -, methyl ester	2352									0.35			
126	9-Octadecenamide, (Z)-	2358									5.85			
127	1-Eicosanol, TMS derivative	2361												0.11
128	2-Monomyristin, 2TMS derivative	2383									0.09			
129	Dehydroabietic acid, TMS derivative	2391												0.58
130	1-Monomyristin, 2TMS derivative	2414						0.06			0.76			
131	Oleamide, TMS derivative	2415	2.03	1.29	7.02	7.88		1.22	14.46	7.46		1.01		
132	Octadecadienoic acid, [(trimethylsilyl)oxy]-, trimethylsilyl ester	2430										1.05		
133	Eicosanoic acid, TMS derivative	2445	0.87								0.08			
134	Arachidic acid, TMS derivative	2446										0.65		
135	D-Myo-Inositol, 1,2,4,5,6-pentakis-O-(trimethylsilyl)-	2469												0.52
136	3-Hydroxyferruginol, di(trimethylsilyl) ether-nema u nistu	2485												0.14
137	2-Hydroxyferruginol, di(trimethylsilyl) ether	2531												0.07
138	Retinoic acid, TMS derivative	2538												0.13
139	D-(+)-Talofuranose, pentakis(trimethylsilyl) ether (isomer 1)	2551						0.14						
140	1-Naphthalenecarboxylic acid, decahydro-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylene-, (1R,4aS,5R,8aS)-, 2TMS	2562												10.4
141	2-Palmitoylglycerol, 2TMS derivate	2576	0.06	0.51	1.21	0.16	0.38	0.58			6	0.4	0.56	0.28
142	1-Monopalmitin, 2TMS derivate	2607	0.44	20.25	14.47	7.61	11	6.79	2.33	3.71	15.86	2.58	7.36	1.67
143	Acetylimbricatolic acid, TMS- nema u nistu	2630												0.44
144	beta.-Lactose, 8TMS derivative	2638					0.05							
145	Docosanoic acid, TMS	2643	0.09									0.2		
146	Sucrose, 8 TMS derivative	2671		5.65									25.92	2.22
147	Rosiridin, 5TMS derivative	2674												0.4

148	D-Trehalose 8TMS	2708												0.3
149	cis-Resveratrol, 3TMS	2737									0.04			
150	2-Monostearin, 2TMS derivate	2767	0.78	0.62	0.62	0.69	0.73	4.41	0.86	0.4	0.88	0.29	0.16	0.05
151	1-Monolinolein, 2TMS derivative	2773												0.02
152	1-Linolenoylglycerol, 2TMS derivative	2780												0.06
153	Glycerol monostearate, 2TMS derivative	2800	4.04	12.16	19.59	32.61	34.93	24.94	40.45	33.23	11.65	3.1	5.21	1.32
154	Trehalose TMS	2813										0.06		
155	Lignoceric acid, TMS derivative	2841										0.11		0.06
156	Tetracosanoic acid, TMS derivative	2842	0.13											
157	Epicatechine, 5TMS derivative	2905		0.36						tr.	0.03		1.73	0.56
158	.delta.-Tocopherol, TMS derivative	2913	0.02			0.51								
159	Catechine, 5TMS derivative	2932		0.05		0.01				0.03	0.1		9.1	1.89
160	Epigallocatechin, 6TMS	2981											0.47	0.2
161	2,3-Dihydroxypropyl icosanoate, 2TMS derivative	2993						0.11						
162	beta- Tocopherol, TMS derivative	3005												0.1
163	gamma Tocopherol, TMS derivative	3008			0.08	0.35								
164	Hexacosanic acid, TMS derivative	3040										0.11		
165	Methyloleoside, 5TMS	3048										0.13		
166	Genistein, 2TMS derivative	3089										0.07		
167	Kaempferol, 4TMS	3112								0.11				
168	alpha Tocopherol, TMS derivative	3152				0.03								
169	Chlorogenic acid, 6TMS	3178					0.03						0.07	
170	Juniperoside III, 4TMS derivetive	3204												0.48
171	Quercetin, 5TMS	3232								0.17	0.1		0.23	0.15
172	Luteolin, 3TMS	3254										0.11		
173	.alpha.-Tocopherolhydroquinone, tris(trimethylsilyl) ether	3306												0.07
174	1,4-Dithioerythritol, 4TMS	3312												0.27

175	beta Sitosterol, TMS derivative	3321	0.03		0.15	0.63			0.32	0.43				
176	Rutin - per(trimethylsilylated) ether derivative	3331												0.38
178	Rosmarinic acid, 5O-TMS	3377											0.05	
179	Medioresinol, 2-O-TMS	3398										0.15		
180	Erythrodiol, 2O-TMS	3417										0.23		
181	Uvaol, 2O-bis-TMS	3438					0.58	0.22						
182	Betulinic acid, O,O-bis-TMS	3453										0.22		
183	Oleanolic acid 2TMS	3459					6.01	8.19		4.73	8.2	5.49		
184	Betulinic acid O,O-bis-TMS	3470					0.37			7.15				
185	Syringaresinol, 2TMS	3486										0.13		
186	Ursolic acid 2TMS	3489					4.86	10.72	1.35	7.08	1.8			
187	Oleanolic acid derivate*	3511										6.74		
188	Corosolic acid, 3TMS	3582										4.82		
189	Tormentic acid, 3TMS derivative	3597						3.05		0.8				
		sum area	87.2	60.98	82.63	76.35	76.15	80.38	80.89	80.42	77.94	71.02	70.11	65.76

Table 3.6. Phenolic content determinate GC/MS (Sum area %)

	PRIMA_01	**PRIMA_02**	**PRIMA_03**	**PRIMA_04**	**PRIMA_05**	**PRIMA_06**	**PRIMA_07**	**PRIMA_08**	**PRIMA_9**	**PRIMA_10**	**PRIMA_19**	**PRIMA_20**
Gallic acid	-	0.21	0.03	0.05	0.01	0.41	-	0.27	0.23	0.05	0.02	-
Caffeic acid	0.01	0.12	-	tr.	0.04	0.01	-	0.01	-	-	0.09	-
Protocatechuic acid	tr.	0.03	-	0.14	0.34	2.79	-	0.18	-	-	0.10	-
4-Hydroxybenzoic acid	0.10	0.25	-	0.04	tr.	0.06	-	-	0.04	0.02	0.04	0.03
Vanillic acid	-	0.12	-	-	tr.	0.86	-	0.07	-	0.17	0.04	-
Chlorogenic acid	-	-	-	-	0.03	-	-	-	-	-	0.07	-
p-coumaric acid	0.01	0.08	0.01	-	-	-	-	0.05	0.02	-	0.02	-
Rosmarinic acid	-		-	-	-	-	-	-	-	-	0.05	-
Ferulic acid	-	tr.	-	-	-	0.08	-	tr.	0.02	-	0.07	0.02
Sinapic acid	-	0.03	-	-	-	-	-	0.01	-	-	-	-
Syringic acid	-	0.07	-	-	-	-	-	-	-	-	0.02	-
Cinnamic acid	-	-	-	-	-	-	-	-	-	-	-	0.01
Epicatechin	-	0.36	-	-	-	-	-	tr.	0.03	-	1.73	0.56
Catechin	-	0.05	-	0.01	-	-	-	0.03	0.10	-	9.10	1.89
Quercetin	-	-	-	-	-	-	-	0.17	0.10	-	0.23	0.15
EGCG	-	-	-	-	-	-	-	-	-	-	0.47	0,2
Luteolin	-	-	-	-	-	-	-	-	-	0.11	-	-
Resveratrol	-	-	-	-	-	-	-	-	0,04	-	-	-
Sum	***0.12***	***1.32***	***0.04***	***0.24***	***0.42***	***4.21***	***0.000***	***0.79***	***0.54***	***0.24***	***12.05***	***2.86***

tr- in traces

3.2.3. Olive leaves analysis by UPLC-PDA-ESI-QTOF (UNIBO/UNIST)

In olive leave samples (PRIMA 10-16) the total phenols ranged from 4 to 22 mg/g leaves, with Croatian Oblica leaves (PRIMA13) having the highest flavonoids and secoiridoids content.

Table 3.7. Compounds Analysis of olive leaves by UPLC-PDA-ESI-QTOF.

		Quantification (mg analyte/g leaves)											
		Moraiolo Toscana		*Frantoio Toscana*		*Oblica Vida*		*Lastovka Strikic*		*Levatinka Strikic*		*Jar. Brisighella*	
	Compounds	**x**	**SD**	**x**	**SD**	**x**	**SD**	**x**	**SD**	**x**	**SD**	**x**	**SD**
1	Hydroxytyrosol-hexose	0.079	0.008	0.102	0.006	0.617	0.020	0.070	0.001	0.152	0.009	0.197	0.008
2	Oleoside	0.104	0.000	0.113	0.002	0.387	0.012	0.098	0.000	**<LOQ**		0.119	0.006
3	Hydroxytyrosol	0.123	0.000	0.117	0.006	0.090	0.004	0.009	0.002	0.021	0.005	0.155	0.004
4	Oleoside/secologanoside	0.266	0.037	0.154	0.011	0.466	0.006	0.061	0.003	**<LOQ**		0.184	0.014
5	Gallocatechin	0.107	0.021	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**	
6	Elenolic acid glucoside isomer a	0.003	0.004	**<LOQ**		0.067	0.004	**<LOQ**		**<LOQ**		0.013	0.001
7	Elenolic acid glucoside isomer b	0.100	0.007	0.082	0.001	0.097	0.001	0.085	0.000	0.024	0.006	0.086	0.000
8	Elenolic acid glucoside isomer c	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**	
9	Oleuropein aglycon	0.010	0.014	0.004	0.006	0.093	0.004	0.093	0.002	**<LOQ**		0.085	0.006
10	Luteolin rutinoside isomer a	0.046	0.003	0.067	0.001	0.090	0.000	0.061	0.000	0.096	0.003	0.061	0.003
11	Luteolin-diglucoside isomer a	0.119	0.000	0.106	0.001	0.205	0.002	0.157	0.003	0.179	0.004	0.173	0.012
12	Elenolic acid glucoside isomer d	0.049	0.000	**<LOQ**		0.081	0.003	**<LOQ**		**<LOQ**		**<LOQ**	
13	Luteolin-diglucoside isomer b	0.020	0.001	0.019	0.000	0.082	0.000	0.036	0.000	0.059	0.001	0.084	0.005
14	Demethyloleuropein	0.081	0.052	0.284	0.024	0.037	0.005	**<LOQ**		**<LOD**		0.046	0.007
15	Rutin	0.266	0.015	0.227	0.010	0.399	0.024	0.146	0.018	0.179	0.006	0.271	0.000
16	Hydroxyoleuropein isomer a	0.313	0.011	0.171	0.003	0.037	0.001	0.208	0.002	0.089	0.016	0.250	0.003
17	Hydroxyoleuropein isomer b	0.333	0.010	0.178	0.004	0.033	0.002	0.203	0.007	0.088	0.013	0.248	0.002

18	Hydroxyoleuropein isomer c	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**	
19	Luteolin rutinoside isomer b	0.073	0.000	0.035	0.000	0.079	0.001	0.083	0.001	0.097	0.001	0.086	0.005
20	Luteolin glucoside isomer a	0.459	0.015	0.478	0.008	0.557	0.003	0.424	0.002	0.473	0.016	0.464	0.016
21	Luteolin rutinoside isomer c	0.176	0.008	0.078	0.001	0.099	0.005	0.115	0.003	0.163	0.006	0.089	0.004
22	Hydroxyoleuropein isomer d	0.004	0.001	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		0.015	0.005
23	Verbascoside isomer a	0.562	0.018	0.595	0.053	0.860	0.047	**<LOQ**		**<LOQ**		0.970	0.022
24	Hydroxyoleuropein isomer e	0.010	0.004	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		0.004	0.005
25	Hydroxyoleuropein isomer f	**<LOQ**		0.025	0.003	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**	
26	Luteolin glucoside isomer b	0.170	0.010	0.099	0.004	0.191	0.012	0.051	0.001	0.063	0.004	0.063	0.002
27	Oleuropein glucoside isomer a	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**	
28	Apigenin rutinoside isomer a	0.096	0.002	0.113	0.001	0.120	0.000	0.167	0.000	0.195	0.001	0.127	0.006
29	Luteolin rutinoside isomer d	0.053	0.002	0.001	0.000	0.077	0.001	0.022	0.001	0.030	0.003	0.091	0.004
30	Luteolin glucoside isomer c	0.478	0.016	0.530	0.001	0.507	0.006	0.409	0.001	0.459	0.013	0.461	0.001
31	Verbascoside isomer b	**<LOD**		0.004	0.001	0.224	0.024	**<LOD**		**<LOD**		0.008	0.002
32	Apigenin glucoside	0.140	0.008	0.222	0.000	0.257	0.003	0.219	0.007	0.323	0.015	0.183	0.005
33	Oleuropein glucoside isomer b	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**	
34	Oleuropein glucoside isomer c	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOD**		**<LOQ**	
35	Comselogoside	0.004	0.005	**<LOQ**		0.039	0.004	**<LOQ**		0.024	0.006	0.013	0.003
36	Verbascoside isomer c	0.087	0.016	0.120	0.010	0.251	0.017	**<LOD**		**<LOD**		0.297	0.041
37	Apigenin rutinoside isomer b	0.047	0.005	0.039	0.001	0.014	0.001	0.040	0.000	0.054	0.001	0.012	0.001
38	Oleuropein glucoside isomer d	0.023	0.003	0.017	0.001	0.125	0.007	**<LOQ**		**<LOQ**		0.117	0.011
39	Oleuropein glucoside isomer e	0.024	0.002	0.008	0.001	0.125	0.002	0.018	0.001	0.022	0.002	0.056	0.007
40	Chrysoeriol-7-Oglucoside	0.189	0.008	0.329	0.007	0.342	0.002	0.117	0.005	0.244	0.019	0.238	0.008
41	Luteolin glucoside isomer d	0.121	0.008	0.152	0.002	0.375	0.003	0.137	0.001	0.240	0.011	0.280	0.015
42	Oleuropein glucoside isomer f	0.227	0.002	0.086	0.001	0.279	0.001	0.075	0.003	0.059	0.008	0.241	0.020
43	Oleuropein isomer a	**<LOQ**		**<LOQ**		0.131	0.002	**<LOQ**		**<LOQ**		**<LOQ**	
44	Hydro-oleuropein	**<LOQ**		**<LOQ**		0.097	0.002	**<LOQ**		**<LOD**		**<LOQ**	
45	Oleuropein isomer b	0.022	0.003	**<LOQ**		0.171	0.003	**<LOQ**		**<LOQ**		**<LOQ**	
46	2''-Methoxyoleuropein isomer a	0.174	0.019	0.113	0.002	**<LOQ**		0.042	0.001	0.018	0.009	0.007	0.002

47	2"-Methoxyoleuropein isomer b	0.187	0.020	0.122	0.002	0.017	0.000	0.040	0.000	0.019	0.008	0.020	0.002
48	Oleuropein glucoside isomer g	0.086	0.007	0.063	0.002	0.136	0.004	**<LOQ**		**<LOQ**		0.131	0.011
49	Oleuropein isomer c	3.162	0.082	2.928	0.017	10.217	0.148	0.414	0.037	0.325	0.055	4.146	0.106
50	Oleuropein isomer d	0.008	0.002	**<LOQ**		0.023	0.002	**<LOQ**		**<LOQ**		**<LOQ**	
51	Oleuropein isomer e	0.141	0.009	0.164	0.003	0.675	0.006	**<LOQ**		**<LOQ**		0.281	0.012
52	Luteolin	0.132	0.038	0.080	0.013	**<LOQ**		0.149	0.003	0.308	0.014	0.118	0.001
53	Oleuropein isomer f	0.460	0.023	0.546	0.007	2.456	0.042	0.037	0.008	0.021	0.012	0.798	0.060
54	Lucidumoside C isomer a	0.257	0.019	0.248	0.001	0.139	0.005	0.136	0.006	0.051	0.013	0.063	0.009
55	Lucidumoside C isomer b	0.266	0.015	0.241	0.008	0.127	0.000	0.139	0.009	0.046	0.012	0.069	0.008
56	Ligstroside	0.156	0.010	0.152	0.007	0.639	0.012	0.047	0.008	0.019	0.007	0.283	0.005
57	Hydroxyoleuropein isomer g	0.047	0.001	**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**		**<LOQ**	
58	Lucidumoside C isomer c	0.027	0.002	0.041	0.002	0.010	0.001	0.001	0.002	**<LOQ**		**<LOQ**	
59	Oleuroside methyl ether	**<LOQ**		**<LOQ**		0.013	0.001	**<LOQ**		**<LOQ**		**<LOQ**	
60	Resinoside isomer a	0.012	0.000	**<LOQ**		0.004	0.000	0.054	0.003	0.005	0.000	0.010	0.001
61	Oleuropein isomer g	0.093	0.000	0.015	0.000	**<LOQ**		0.002	0.001	**<LOQ**		**<LOQ**	
62	Oleuropein isomer h	0.096	0.001	0.020	0.001	**<LOQ**		0.008	0.002	**<LOQ**		**<LOQ**	
63	Oleuropein isomer i	0.072	0.005	0.008	0.001	**<LOQ**		0.009	0.003	**<LOQ**		**<LOQ**	
64	Oleuropein isomer j	0.065	0.003	0.005	0.001	**<LOQ**		0.007	0.000	**<LOQ**		**<LOQ**	
65	Resinoside isomer b	0.012	0.002	0.050	0.002	0.085	0.001	0.082	0.007	0.041	0.000	0.030	0.001
66	Resinoside isomer c	0.014	0.001	0.011	0.000	0.030	0.000	0.038	0.002	0.027	0.000	0.019	0.001
	Simple Phenols	0.202	0.008	0.219	0.000	0.707	0.024	0.079	0.000	0.173	0.014	0.352	0.011
	Flavonoids	2.730	0.064	2.635	0.003	3.514	0.036	2.508	0.047	3.234	0.066	2.861	0.081
	Secoiridoids	6.720	0.258	5.705	0.023	16.472	0.242	1.638	0.079	0.779	0.148	7.177	0.271
	Elenolic acid derivatives	0.152	0.011	0.082	0.001	0.245	0.006	0.085	0.000	0.024	0.006	0.099	0.001
	Other phenolic compounds	0.648	0.034	0.720	0.061	1.334	0.088	0.000	0.000	0.000	0.000	1.274	0.065
	Total phenols	**10.443**	**0.162**	**9.361**	**0.082**	**22.273**	**0.395**	**4.310**	**0.127**	**4.209**	**0.233**	**11.764**	**0.428**

3.2.4. GCMS identification of the essential oils (UNIBO)

The results regarding the volatile organic compounds (VOCs) of blackberry leaves EO are reported in Table 3.5. An example of the chromatogram obtained is shown in Figure 3.5.

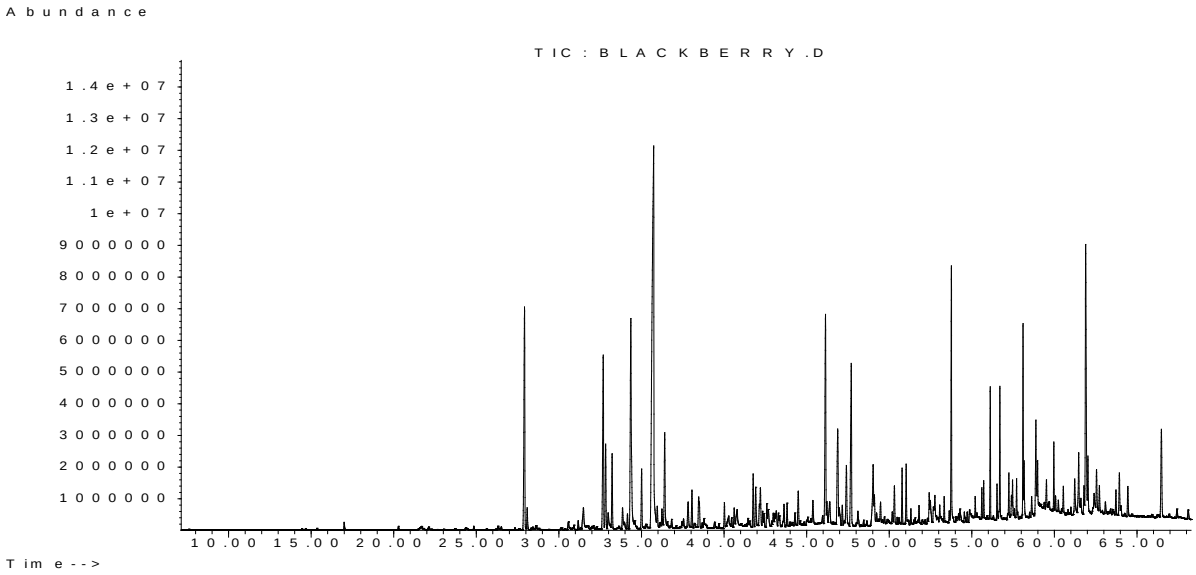

Figure 3.5. GC-MS chromatogram of blackberry leaves EO.

The analytical protocol allowed to discriminate 119 different compounds, accounting for about 93% of the total peaks identified. The VOCs belonged to different chemical classes such as alcohols, aldehydes, terpenes and terpenoids, esters, acid, hydrocarbons.

The main constituents were: geraniol (13.04%), phytol (4.65%), beta-cytronellol (4.40%), linalool (3.94%), β-Ionone (3.51%), hexadecanal (3.12%), dodecanoic acid (2.97%) alpha-terpineol (2.91%), citral (2.29%). Interestingly, some of these compounds are known to exert antimicrobial activity against foodborne microorganisms. For example, the antimicrobial activity of geraniol, the most abundant compound, has been recently reviewed and reported effect on both Gram positive and Gram negative bacteria and fungi (Pereira de Lira et al., 2020). Also phytol and linalool, accounting for about 4.5 and 4 % of the total peaks area, respectively, showed antibacterial activity against *Pseudomonas aeruginosa* by inducing oxidative cell death (Lee et al., 2016; Liu et al., 2020), while citral has both antifungal and antibacterial effect, widely reported in literature (Tabanelli et al., 2014; Chueca et al., 2016; OuYang et al., 2018). It is noteworthy that some of these compounds, although present in low amounts, can act in an additive or even synergistic way, increasing the total antimicrobial effect.

Table 3.8. VOCs composition of Blackberry leaves EO (sample PRIMA_02). The data are expressed as relative percentage of each single peak area with respect to the total peak area. Results are the mean of two replicates and standard deviations are reported in brackets.

No.	RT (min)	Compounds	Blackberry
1	17.00	2-heptanol	0.12 (± 0.01)
2	20.29	Benzaldehyde	0.06 (± 0.01)
3	22.11	2-methyl-6-hepten-1-ol	0.07 (± 0.01)
4	23.70	(S)-3-Ethyl-4-methylpentanol	0.05 (± 0.02)
5	24.36	Benzyl alcohol	0.07 (± 0.01)
6	24.85	Benzeneacetaldehyde	0.06 (±0.01)
7	26.31	1-octanol	0.10 (± 0.01)
8	26.52	cis-linaloloxide	0.09 (±0.01)
9	**27.92**	**Linalool**	**3.94** (± 0.33)
10	28.08	Nonanal	0.30 (± 0.02)
11	28.60	Phenylethyl alcohol	0.10 (±0.01)
12	30.58	2,6-nonadienal	0.16 (±0.01)
13	30.91	2-nonenal	0.07 (±0.01)
14	31.17	3-ethyl-benzaldehyde	0.14 (±0.01)
15	31.49	1-nonanol	0.57 (± 0.03)
16	31.84	Octanoic acid	0.55 (± 0.32)
17	32.01	4-carvomenthenol	0.05 (± 0.03)
18	32.09	3-methyl,bicyclo[4.1.0]heptane	0.08 (± 0.04)
19	32.30	1-(4-methylphenyl)-ethanone	0.08 (±0.01)
20	**32.69**	**alpha-terpineol**	**2.91** (± 0.29)
21	**32.83**	**2-hydroxy-benzoic acid, methyl ester**	**1.19** (± 0.02)
22	32.98	Myrtenol	0.21 (± 0.05)
23	33.22	Decanal	0.99 (± 0.12)
24	33.86	2,3-Dihydrobenzofuran	0.36 (±0.01)
25	**34.36**	**beta-cytronellol**	**4.40** (± 0.15)
26	**35.01**	**citral**	**2.29** (± 0.22)
27	**35.70**	**Geraniol**	**13.04** (±1.09)
28	35.96	2-decenal	0.19 (± 0.9)
29	36.24	gamma-ionone	0.24 (± 0.01)
30	36.58	Citronellyl formate	0.07 (± 0.03)
31	36.83	5-methyl-3-(1-methylethenyl),trans-cyclohexene	0.10 (±0.01)
32	37.46	2,4-decadienal	0.11 (±0.01)
33	37.82	Geranyl formate	0.48 (± 0.12)
34	38.05	Undecanal	0.55 (± 0.09)
35	38.46	2-methoxy-4-vinylphenol	0.40 (± 0.03)
36	38.50	2,4-decadienal	0.32 (±0.08)
37	38.68	4-(1-methylpropyl)-phenol	0.07 (±0.01)
38	39.69	3-none-2-one	0.08 (±0.01)
39	40.02	2,3-diethyl-cyclohexane-1,3-diene	0.37 (± 0.06)

40	40.29	Geranic acid	0.34 (± 0.04)
41	40.46	1,2-Dihydro-1,1,6-trimethyl-naphthalene	0.36 (± 0.22)
42	40.62	2-undecenal	0.34 (± 0.04)
43	40.75	*n*-decanoic acid	0.99 (± 0.5)
44	41.45	Neryl acetate	0.17 (± 0.01)
45	41.76	(E)-beta-damascenone	0.78 (± 0.13)
46	42.20	Tetradecane	0.56 (± 0.13)
47	42.34	Jasmone	0.17 (± 0.15)
48	42.42	5,6-diethyl-cyclohexa-1,3-diene	0.21 (±0.02)
49	42.59	Dodecanal	0.34 (± 0.04)
50	43.29	2-ethyl-1,3-dimethyl-benzene	0.22 (± 0.02)
51	43.58	Caryophyllene	0.08 (±0.01)
52	43.63	alpha-ionone	0.32 (± 0.07)
53	43.82	meta-cymene	0.35 (± 0.05)
54	44.49	Geranylacetone	0.60 (± 0.11)
55	44.85	Undecane	0.09 (±0.01)
56	45.00	2-ethenyl-1,3,3-trimethyl-cyclohexene	0.08 (±0.01)
57	45.38	1,4-Diisopropylbenzene	0.46 (±0.01)
58	45.96	Dehydro-beta-ionone	0.16 (±0.01)
59	**46.14**	**β-Ionone**	**3.51** (± 0.55)
60	46.23	1-Adamantyl methyl ketone	0.35 (± 0.02)
61	46.40	Pentadecane	0.44 (± 0.2)
62	**46.88**	**alpha-farnesene**	**1.92** (± 0.24)
63	46.98	Tridecanal	0.26 (± 0.13)
64	47.16	Butylated hydroxytoluene	0.24 (± 0.03)
65	47.41	3-Amino-2-cyclohexen-1-one	0.91 (± 0.04)
66	**47.70**	**Olivetol**	**2.88** (± 0.24)
67	48.11	Dihydroactinidolide	0.26 (± 0.07)
68	48.46	2-cyclohexenyl cyclohexanone	0.08 (± 0.01)
69	**49.03**	**Dodecanoic acid**	**2.97** (± 1.66)
70	49.10	Nerolidol	0.98 (± 0.59)
71	49.47	3-hexen-1-ol benzoate	0.35 (± 0.04)
72	49.71	Benzoic acid, hexyl ester	0.14 (±0.01)
73	50.32	Hexadecane	0.60 (± 0.09)
74	50.59	Ledol	0.11 (±0.01)
75	51.03	Linalyl acetate	0.73 (± 0.11)
76	51.57	Benzophenone	0.10 (±0.01)
77	51.80	gamma-eudesmol	0.26 (± 0.02)
78	52.32	Hexamethyl benzene	0.10 (±0.01)
79	52.42	β-Eudesmol	0.39 (± 0.02)
80	52.49	alpha-eudesmol	0.29 (± 0.07)
81	53.33	Heptadecane	0.30 (± 0.03)
82	**53.76**	**Hexadecanal**	**3.12** (± 0.4)
83	54.20	9-octadecyne	0.08 (± 0.02)
84	54.29	3-tetradecene	0.18 (±0.01)

85	54.60	Aromadendrene, dehydro	0.05 (± 0.05)
86	54.73	Geranyl Propionate	0.20 (± 0.02)
87	54.86	Tetradecanoic acid	0.77 (± 0.45)
88	55.21	Benzyl benzoate	0.33 (±0.01)
89	55.31	Cyclotetradecane	0.14 v 0.08)
90	55.60	alpha-cedrene	0.35 (± 0.02)
91	55.72	Octadecane	0.43 (± 0.06)
92	**56.12**	**Tetradecanal**	**2.08** (± 0.25)
93	56.53	Tridecanedial	0.37 (± 0.06)
94	**56.70**	**Hexahydrofarnesyl acetone**	**1.27** (± 0.19)
95	57.24	Phtalic acid, isobutyl octyl ester	0.47 (± 0.03)
96	57.40	Cyclohexadecane	0.33 (± 0.02)
97	57.47	4-benzyloxybenzoic acid	0.43 (±0.01)
98	57.71	Eicosane	0.76 (± 0.14)
99	57.79	2-heptadecanone	0.08 (±0.01)
100	**58.11**	**16-octadecenal**	**1.78** (±0.28)
101	58.17	Farnesyl acetone	0.62 (± 0.05)
102	58.63	Isophytol	0.36 (± 0.09)
103	**58.88**	***n*-hexadecanoic acid**	**1.11** (± 1.00)
104	**58.99**	**Dibutyl phthalate**	**4.61** (± 4.62)
105	59.97	Hexadecanal	0.69 (± 0.13)
106	60.24	2-cis,6-trans-Farnesol	0.18 (±0.01)
107	60.54	13-epimanoyl oxide	0.29 (± 0.07)
108	61.23	5-octadecene	0.54 (± 0.03)
109	61.47	Heneicosane	0.85 (± 0.25)
110	61.61	Methyl linolenate	0.18 (± 0.09)
111	**61.90**	**Phytol**	**4.65** (± 0.79)
112	62.03	Hexadecanal	0.77 (± 0.14)
113	**62.49**	**Oleic acid**	**1.93** (± 1.38)
114	63.09	Ethyl linolenate	0.21 (±0.01)
115	63.53	1-nonadecene	0.09 (± 0.01)
116	**63.73**	**Eicosane**	**2.02** (± 0.39)
117	64.45	Octadecanal	0.49 (± 0.03)
118	67.42	Tetradecanal	0.17 (± 0.01)
119	68.10	Neryl acetate	0.18 (± 0.01)
		Total identified compounds	**92.96**

The chemical composition of volatile organic compounds (VOCs) of Juniperus needles EOs is reported in Table 3.6. An example of the chromatogram obtained is shown in Figure 3.6.

The analytical protocol allowed to discriminate 114 different compounds, accounting for about 90% of the total peaks identified. The VOCs belonged to different chemical classes: such as terpenes and terpenoids, alcohols, aldehydes, esters, acid, hydrocarbons.

The main constituents were: limonene (13.59%), alpha-pinene (10.79%), manoyl oxide (8.41%); 3-carene (4.12%), alpha-curcumene (3.50%), bicyclosesquiphellandrene (3.24%), androst-5-en-4-one (2.59%), alpha-cedrene (1.94%),

Limonene, the most abundant compound in this EO, is one of the most common terpenes found in various plants (black pepper, lemon and orange, etc.) and has a broad-spectrum bactericidal activity. Indeed., it can effectively inhibit the growth of pathogen bacteria such as *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* (Han et al., 2019: Kim et al., 2013). Also α-pinene, whose presence accounts for about 10% of total peak areas, showed a good antimicrobial activity against both Gram-positive and Gram-negative bacteria, especially against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* (Ghavam et al., 2020).

As stated above for blackberry EO, also in this case the presence of a wide array of terpenes and terpenoids, can improve the effect of Juniperus EO. Indeed, these compounds, although present at low extent when considered individually, represent the most abundant chemical class of this EO. Because of their ability to work in additive or synergistic way, even small amounts of specific molecules can therefore increase the total antimicrobial effect.

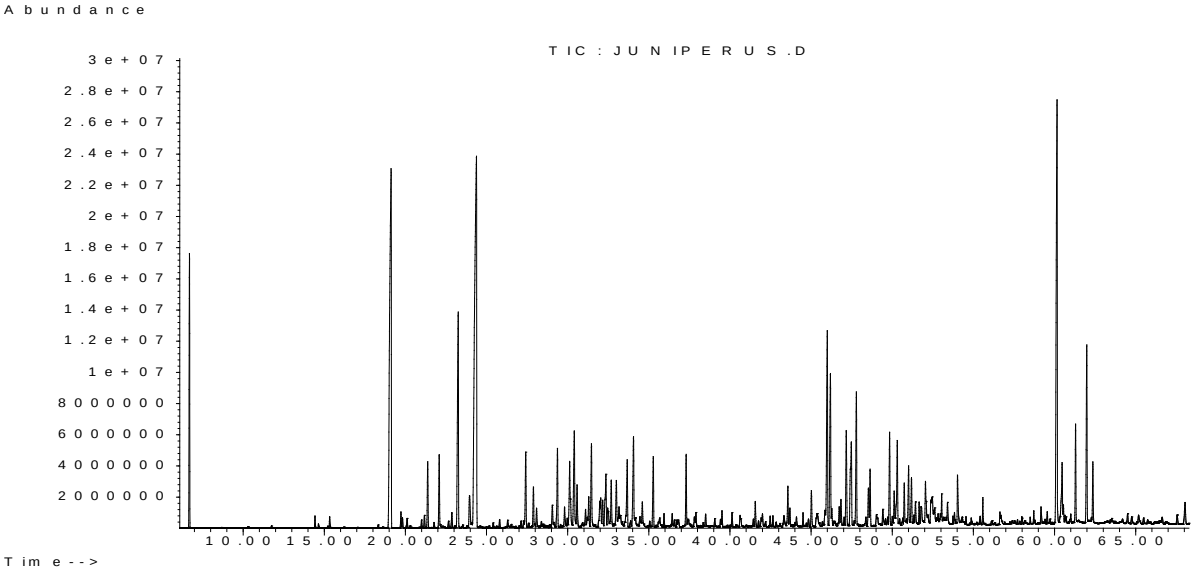

Figure 3.6. GC-MS chromatogram of Juniperus needles EO.

Table 3.9. VOCs composition of *Juniperus oxycedrus* needles by products EO (sample PRIMA_19). The data are expressed as relative percentage of each single peak area with respect to the total peak area. Results are the mean of two replicates and standard deviations are reported in brackets.

No.	RT (min)	Compounds	Juniperus
1	14.43	2-hexenal	0.14 (±0.01)
2	14.64	3-hexen-1-ol	0.07 (±0.01)
3	15.33	1-hexanol	0.13 (±0.01)
4	**18.33**	**3-carene**	**4.12** (±0.10)
5	18.60	alpha-phellandrene	0.23 (±0.01)
6	**19.12**	**alpha-pinene**	**10.79** (±0.21)
7	19.73	alpha-fenchene	0.19 (±0.01)
8	19.82	Camphene	0.31 (±0.01)
9	20.11	2,4-thujadiene	0.10 (± 0.03)
10	21.00	3,7,7-trimethyl-1,3,5-Cycloheptatriene	0.10 (±0.01)
11	21.17	beta-phellandrene	0.16 (±0.01)
12	21.38	beta-pinene	0.88 (± 0.02)
13	21.77	6-methyl-5-hepten-2-one	0.06 (±0.01)
14	22.08	beta-myrcene	0.94 (± 0.04)
15	22.66	2-carene	0.08 (±0.01)
16	23.95	1-methyl-2-(1-methylethyl)-benzene	0.61 (± 0.03)
17	**24.38**	**Limonene**	**13.59** (±0.13)
18	25.41	3,3,5-trimethyl-1,5-heptadiene	0.06 (±0.01)
19	25.80	p-mentha-1,4-diene	0.10 (±0.01)
20	26.30	1-octanol	0.09 (±0.01)
21	27.13	Citral	0.10 (±0.01)
22	**27.41**	**p-Menth-4(8)-ene**	**1.14** (±0.01)
23	27.89	Linalool	0.56 (±0.01)
24	28.08	Nonanal	0.25 (±0.01)
25	28.38	trans-p-mentha-2,8-dien-1-ol	0.32 (±0.02)
26	**29.37**	**Campholenic Aldehyde**	**1.18** (± 0.05)
27	29.81	cis-p-mentha-2,8-dien-1-ol	0.26 (± 0.02)
28	29.98	Limonene oxide	0.09 (±0.01)
29	**30.12**	**trans-pinocarveol**	**1.00** (±0.01)
30	30.18	S-cis-verbenol	0.26 (± 0.02)
31	**30.42**	**Verbenol**	**1.68** (± 0.06)
32	30.58	1,3-Cycloheptadiene	0.55 (±0.01)
33	31.10	1-Methyl-1,4-cyclohexadiene	0.20 (±0.01)
34	31.21	Isopinocamphone	0.12 (±0.01)
35	31.30	Pinocarvone	0.37 (± 0.05)
36	**31.47**	**1,3,5-heptatriene**	**1.29** (± 0.03)
37	31.98	2-Isopropenyl-5-methylhex-4-enal	0.31 (± 0.07)
38	32.03	4-terpinenol	0.33 (± 0.06)

39	**32.15**	**α,α,4-trimethyl-benzenemethanol**	**1.06** (± 0.05)
40	32.31	1-(4-methylphenyl)-ethanone	0.32 (± 0.02)
41	32.47	4-(1-methylethyl)2-Cyclohexen-1-one	0.35 (± 0.03)
42	32.69	p-menth-1-en-8-ol	0.65 (±0.01)
43	33.00	alpha-thujenal	0.73 (± 0.73)
44	**33.25**	**Bornyl acetate**	**1.21** (±0.01)
45	**33.67**	**(-)-verbenone**	**1.11** (± 0.07)
46	**34.06**	**cis-carveol**	**1.68** (± 0.03)
47	34.45	Iso-carveol	0.13 (±0.01)
48	34.60	trans-carveol	0.35 (±0.01)
49	35.06	2-methyl-3-phenyl-propanal	0.06 (±0.01)
50	**35.27**	**(+)-Carvone**	**1.03** (±0.01)
51	35.59	Geraniol	0.06 (±0.01)
52	35.93	2-decenal	0.17 (±0.01)
53	36.44	Cinnamaldehyde	0.18 (±0.01)
54	36.58	(S)-isopiperitenone	0.10 (±0.01)
55	36.73	(-)-Perillaldehyde	0.09 (±0.01)
56	36.81	Phellandral	0.15 (±0.01)
57	37.40	4-Isopropylbenzyl alcohol	0.08 (±0.01)
58	37.81	2-ethenyl-1,3,3-trimethyl-cyclohexene	0.16 (±0.01)
59	37.90	trans-pinocavyl acetate	0.20 (±0.01)
60	38.50	2,4-decadienal	0.17 (±0.01)
61	39.06	Myrtenyl acetate	0.11 (±0.01)
62	39.51	cis-Carveyl acetate	0.23 (±0.01)
63	40.13	α-Terpinene	0.21(±0.01)
64	40.62	2-hexenal	0.16 (± 0.03)
65	40.67	trans-Carveyl acetate	0.09 (±0.01)
66	41.44	Geraniol acetate	0.11 (± 0.02)
67	41.56	Copaene	0.38 (± 0.02)
68	42.00	alphabourbonene	0.20 (± 0.03)
69	42.19	(-)-beta-elemene	0.07 (±0.01)
70	42.48	gamma-4-dimethyl-benzenebutanal	0.14 (±0.01)
71	42.65	alpha-zingiberene	0.14 (± 0.02)
72	**42.84**	**alpha cedrene**	**1.94** (± 0.06)
73	43.33	Cedrene	0.22 (±0.01)
74	43.57	Caryophillene	0.60 (±0.01)
75	43.69	beta-cedrene	0.29 (±0.01)
76	**44.01**	**Bicyclosesquiphellandrene**	**3.24** (± 0.04)
77	44.10	Thujopsene	0.15 (±0.01)
78	44.50	Geranylacetone	0.19 (±0.01)
79	45.02	Alpha caryophillene	0.53 (±0.01)
80	45.33	Isobornylacetate	0.06 (± 0.08)
81	45.36	Cyclododecane	0.30 (±0.01)
82	45.86	Ylangene	0.27 (±0.01)
83	**46.00**	**alpha-curcumene**	**3.50** (± 0.02)

84	46.84	alpha-muurolene	0.40 (± 0.03)
85	47.04	beta-bisabolene	0.09 (±0.01)
86	**47.48**	**alpha-amorphene**	**2.03** (± 0.08)
87	**47.79**	**delta-cadinene**	**2.04** (± 0.03)
88	48.37	cis-alpha-bisabolene	0.09 (± 0.02)
89	48.53	alpha-copaene-11-ol	0.55 (± 0.01)
90	**48.63**	**alpha-calacorene**	**1.04** (± 0.07)
91	50.12	Dodecanoic acid ethyl ester	0.46 (± 0.06)
92	**50.31**	**Caryophillene oxide**	**1.38** (±0.01)
93	51.00	Cedrol	0.84 (± 0.05)
94	52.08	alpha-himachalene	0.17 (± 0.02)
95	52.46	beta-himachalene	0.35 (± 0.04)
96	52.62	Cyclododecene	0.16 (± 0.02)
97	52.97	Isoaromadendrene epoxide	0.12 (±0.01)
98	53.04	Cadalene	0.32 (±0.01)
99	53.74	Hexadecanal	0.10 (±0.01)
100	54.02	Farnesol	0.75 (±0.01)
101	54.54	(E,E)-farnesal	0.10 (±0.01)
102	55.42	Cedryl acetate	0.15 (±0.01)
103	55.58	Tetradecanoic acid, ethyl ester	0.32 (± 0.02)
104	56.64	trans,trans-Farnesyl acetate	0.11 (±0.01)
105	57.99	Sclareol oxide	0.06 (±0.01)
106	59.55	Octadecane	0.15 (±0.01)
107	**60.15**	**Manoyl oxide**	**8.41** (± 0.07)
108	60.55	13-epimanoyl oxide	0.17 (± 0.04)
109	60.72	Eicosane	0.17 (± 0.08)
110	**61.30**	**ar-abietatriene**	**1.19** (±0.01)
111	**61.98**	**Androst-5-en-4-one**	**2.59** (± 0.05)
112	64.76	Estrone	0.17 (±0.01)
113	66.63	Dehydroabietal	0.12 (± 0.05)
114	67.56	9,10 dehydro-cycloisolongifolene	0.17 (± 0.02)
		Total identified compounds	**90.70**

The third EO that was characterized derived from *Juniperus communis* extract by-product (sample PRIMA_20) and its composition in volatile organic compounds (VOCs) is reported in Table 3.10.

The analytical protocol allowed to discriminate 70 different compounds, accounting for about 96% of the total peaks identified. The VOC composition was mostly represented by terpenes and terpenoids.

The main constituents were alpha-phellandrene (31.65%), trans-2,7-dimethyl,3-Octen-5-yne (15.63 %), limonene (6.00 %), beta-myrcene (5.78 %), terpinolene (4.45 %), 4-Terpineol (4.39 %), gamma-terpinene (3.81), (+)-4-Carene (3.62%) and germacrene (3.43 %).

Table 3.10. VOCs composition of *Juniperus sp.* by products EO (sample PRIMA_20). The data are expressed as relative percentage of each single peak area with respect to the total peak area. Results are the mean of two replicates and standard deviations are reported in brackets.

No.	RT (min)	Compounds	Juniperus (PRIMA_20)
1	17.83	alpha-pinene	0.07 (±0.01)
2	**18.12**	**alpha-phellandrene**	**31.65 (±2.13)**
3	**18.55**	**trans-2,7-dimethyl, 3-Octen-5-yne**	**15.63 (±0.93)**
4	19.30	Camphene	0.22 (±0.05)
5	**20.94**	**beta-pinene**	**1.63 (±0.07)**
6	**21.62**	**beta-myrcene**	**5.78 (±0.26)**
7	**22.17**	**(+)-4-Carene**	**3.62 (±1.96)**
8	22.69	delta-3-Carene	0.32 (±0.03)
9	**23.43**	**meta-cymene**	**1.36 (±0.05)**
10	**23.72**	**Limonene**	**6.00 (±0.32)**
11	24.68	Ocimene	0.10 (±0.02)
12	**25.32**	**gamma-terpinene**	**3.81 (±0.19)**
13	25.75	cis-beta-terpineol	0.77 (±0.06)
14	**26.92**	**Terpinolene**	**4.45 (±1.72)**
15	27.68	Isoamyl valerianate	0.07 (±0.03)
16	28.37	Thujone	0.14 (±0.08)
17	29.86	Camphor	0.05 (±0.03)
18	30.30	Isomenthone	0.04 (±0.02)
19	**31.55**	**4-Terpineol**	**4.39 (±0.17)**
20	31.81	p-Cymen-8-ol	0.03 (±0.01)
21	32.15	alpha-terpineol	0.18 (±0.05)
22	32.42	trans-p-Menth-1-en-3-ol	0.06 (±0.01)
23	32.46	(-)-Myrtenol	0.03 (±0.01)
24	32.99	trans-Piperitol	0.08 (±0.01)
25	33.65	alpha-fenchyl acetate	0.02 (±0.01)
26	33.84	beta-Citronellol	0.10 (±0.01)
27	34.23	Thymyl methyl ether	0.02 (±0.01)
28	34.30	Verbenyl acetate	0.04 (±0.01)
29	34.75	(-)-Carvone	0.20 (±0.08)
30	34.88	Isopentyl hexanoate	0.02 (±0.01)
31	35.19	alpha-cyclogeraniol acetate	0.05 (±0.01)
32	35.27	Piperitone	0.02 (±0.01)

33	35.40	(S)-(-)-Citronellic acid, methyl ester	0.03 (±0.01)
34	36.81	Bornyl acetate	0.66 (±0.09)
35	37.01	Myrtenyl acetate	0.15 (±0.03)
36	37.35	Carvacrol	0.05 (±0.02)
37	37.42	Terpinene 4-acetate	0.22 (±0.04)
38	38.97	alpha-Terpineol acetate	0.94 (±0.08)
39	39.84	alpha-Cubebene	0.09 (±0.01)
40	41.11	Copaene	0.08 (±0.03)
41	41.32	(-)-cis-Myrtanyl acetate	0.05 (±0.02)
42	41.75	beta-Elemene	0.71 (±0.09)
43	**43.11**	**Caryophyllene**	**1.18 (±0.13)**
44	43.53	gamma-Elemene	0.13 (±0.02)
45	43.64	Thujopsene	0.53 (±0.04)
46	44.29	beta-Farnesene	0.04 (±0.02)
47	44.57	alpha-Caryophyllene	0.93 (±0.10)
48	44.93	Bicyclosesquiphellandrene	0.36 (±0.06)
49	45.43	gamma-Cadinene	0.65 (±0.27)
50	45.51	alpha-Curcumene	0.15 (±0.05)
51	**45.72**	**Germacrene**	**3.43 (±1.61)**
52	45.96	beta-Eudesmene	0.08 (±0.01)
53	46.38	alpha-Amorphene	0.66 (±0.08)
54	46.73	Cedr-8-ene	0.27 (±0.01)
55	47.07	Beta-ionone epoxide	0.07 (±0.02)
56	**47.33**	**delta-Cadinene**	**1.37 (±0.10)**
57	47.95	alpha-Muurolene	0.11 (±0.01)
58	48.35	alpha-elemol	0.06 (±0.01)
59	48.70	trans-Nerolidol	0.10 (±0.01)
60	49.62	Spathulenol	0.16 (±0.08)
61	49.89	Caryophyllene oxide	0.28 (±0.08)
62	50.60	Cedrol	0.03 (±0.01)
63	50.72	trans-beta-Ionone	0.07 (±0.01)
64	50.91	Cubenol	0.03 (±0.01)
65	51.33	Cedrene	0.06 (±0.01)
66	51.73	alpha-Cadinol	0.96 (±0.09)
67	51.84	delta-Cadinol	0.08 (±0.03)
68	53.26	Carotol	0.05 (±0.01)
69	53.72	trans-Farnesol	0.06 (±0.01)
70	61.14	Cembrene	0.11 (±0.06)
		Total identified compounds	**95.96 (**±0.06)

4. CONCLUSIONS

The HPLC-qTOF-MS analyses of od brown algae showed a large number of compounds found in algae samples, but no targeted phenolic compounds. Although the TPC analyses reviled some phenolics, they could not be characterized by the HPLC-qTOF-MS method dur to the complexity of the phenolics in algae. The agro-food by-product matrices PRIMA_02, PRIMA_19 and PRIMA 20 seems to be promising low-cost sources of phenolics. They contain a very high concentrations of polyphenols, especially flavonoids (catehin, quercetin, rutine) that are known as good antioxidants and antimicrobial agents.

Moreover, the analysis of the essential oils obtained from these matrices showed the presence of molecules endowed with recognized antimicrobial activity, especially in blackberry leave (PRIMA_02) essential oils. These compounds can act synergistically in these oils, enhancing the bioactivity. For these reasons this essential oils and extract have been proposed to be used in Task 3.4 (alone or in combination with bioprotective culture supernatants) to better highlight their mode of action and antimicrobial activity.