Oleuropein derivatives from olive fruit extracts reduce α-synuclein fibrillation and oligomer toxicity

Hossein Mohammad-Beigi, Farhang Aliakbari, Cagla Sahin, Charlotte Lomax, Ahmed Tawfike, Nicholas P. Schafer, Alireza Amiri-Nowdijeh, Hoda Eskandari, Ian Max Møller, Mehdi Hosseini-Mazinani, Gunna Christiansen, Jane L. Ward, Dina Morshedi, Daniel E. Otzen

Supplementary Figures S1-14 and Tables S1-3

Supplementary Figures

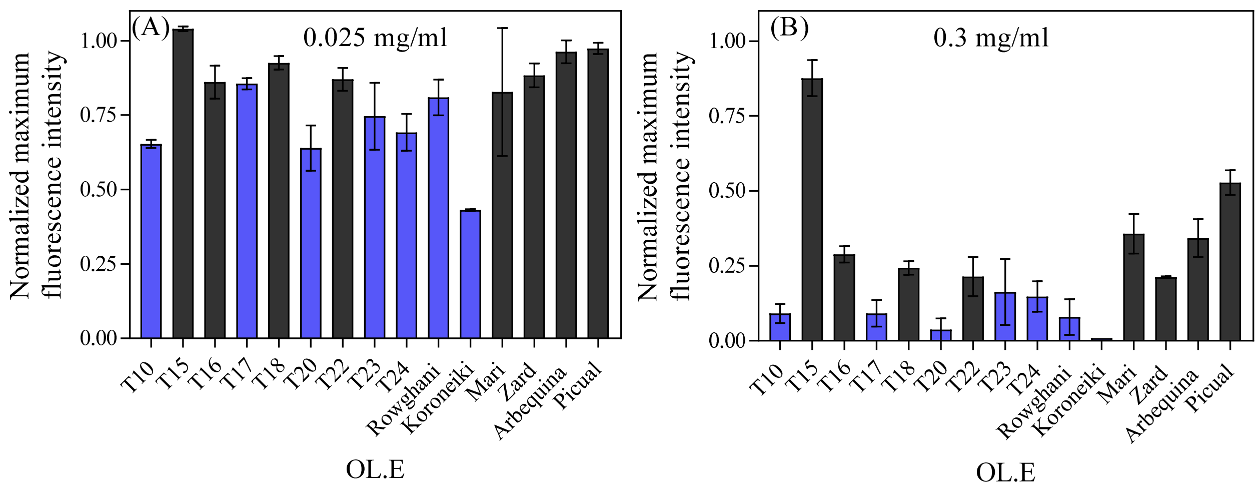


Fig. S1. The effect of different olive fruit extracts on *α*SN fibrillation. First screening: Selection of the best extracts by the effect of (A) 0.025 mg/ml extract and (B) 0.3 mg/ml extract on the end-point ThT fluorescence level at 1 mg/ml *α*SN.

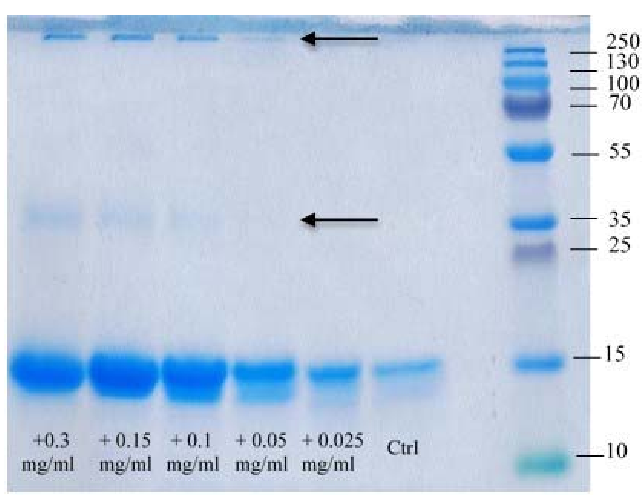
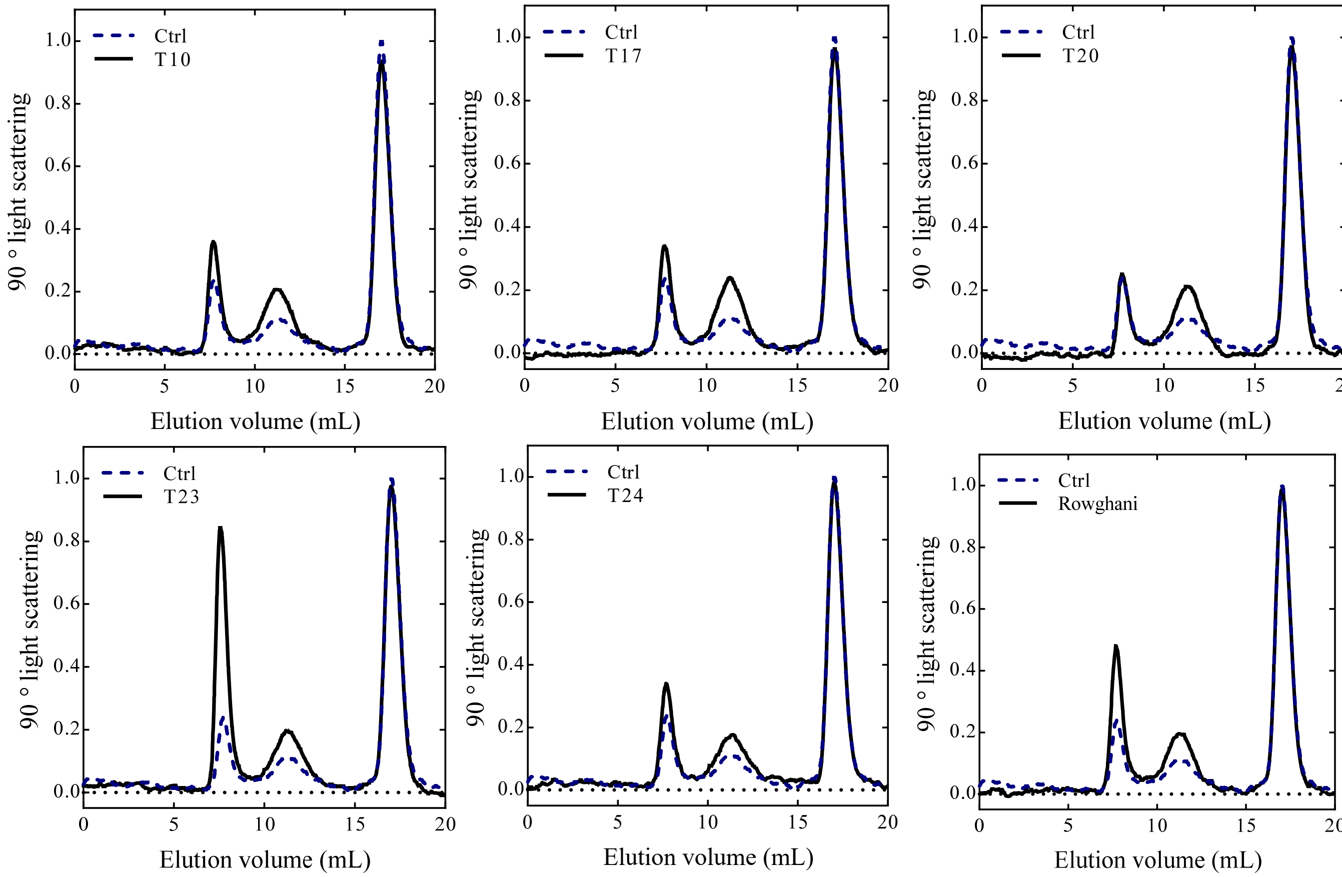


Fig. S2. SDS-PAGE analysis of the supernatants of samples of 1 mg/ml αSN incubated for 24 h in the presence of 0-0.3 mg/ml of of Koroneiki extract. Arrows highlight dimers (≈35 kDa) and oligomers (> 250 kDa).

(A)



(B)

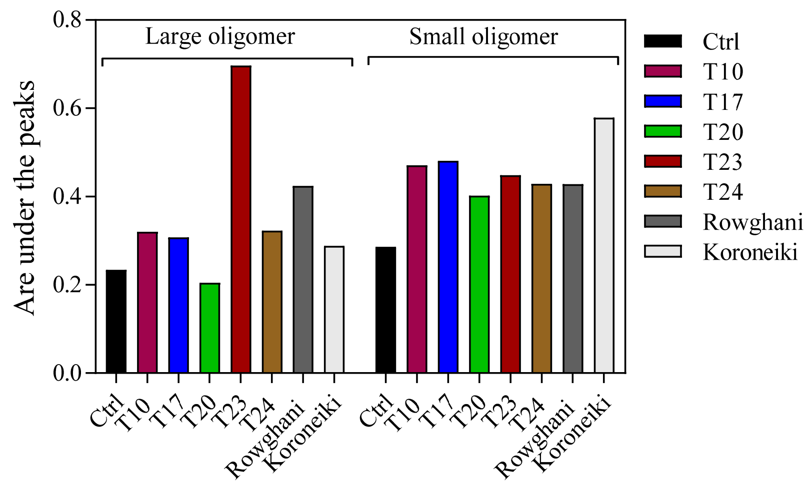


Fig. S3. Oligomerization assay. **(A)** SEC profile of the supernatants from solutions of 1 mg/ml αSN incubated for 1 h at 37ºC in the presence of different extracts. (B) Area under the peaks of small and large oligomers formed in the absence (Ctrl) and presence of 0.15 mg/ml of the best extracts.

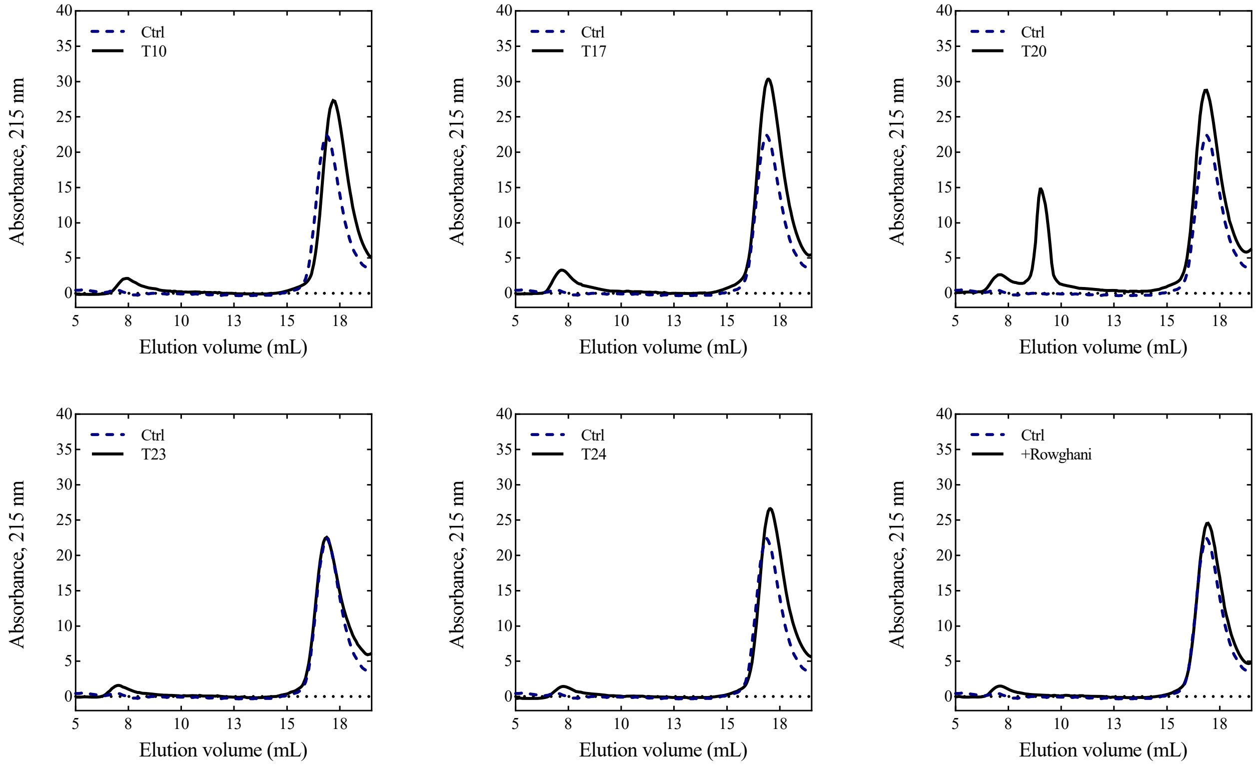


Fig. S4. Disaggregation assay. SEC profile of the supernatants of preformed αSN fibrils incubated at 0.5 mg/ml overnight at 37ºC in the absence (Ctrl) and presence of 0.15 mg/ml of the best extracts.

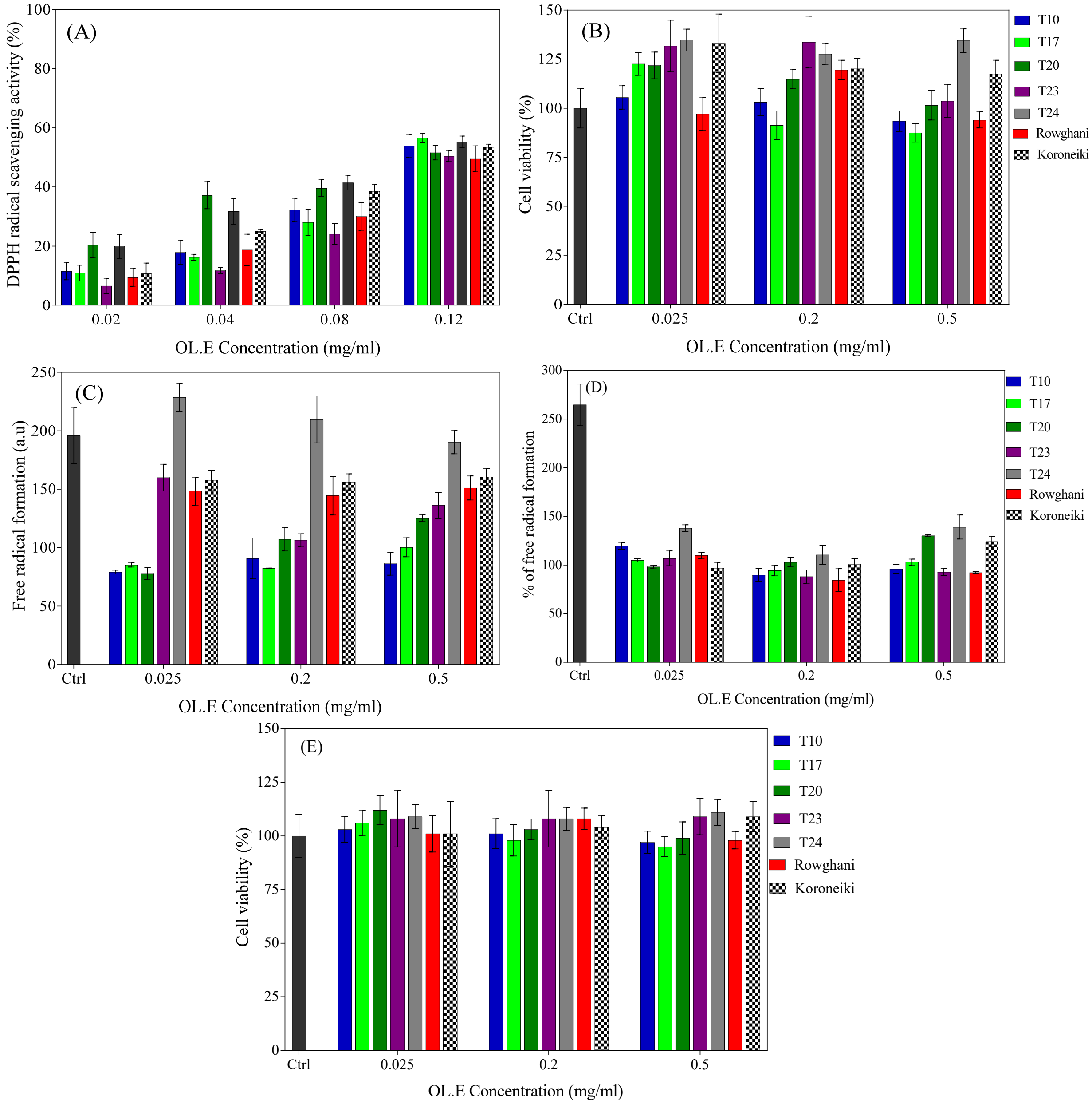


Fig. S5. Antioxidant activity and toxicity of the olive extracts. (A) Antioxidant activity of different olive fruit extracts at different concentrations (0.02, 0.04, 0.08, 0.12 mg/ml) measured by DPPH assay. (B) Viability of OLN-93 cells after 24 h incubation with the best olive extracts at different concentrations (0.025, 0.2, 0.5 mg/ml). (C) Oxidative stress in OLN-93 cells treated with olive extracts at different concentrations determined by DCFH-DA assay. (D) Free radical scavaging ability of the olive extracts measured in OLN-93 cells treated with 100 µM H_2O_2. (E) Viability of SH-SY5Y cells after 24 h incubation with 0-0.5 mg/ml of the best olive extracts.

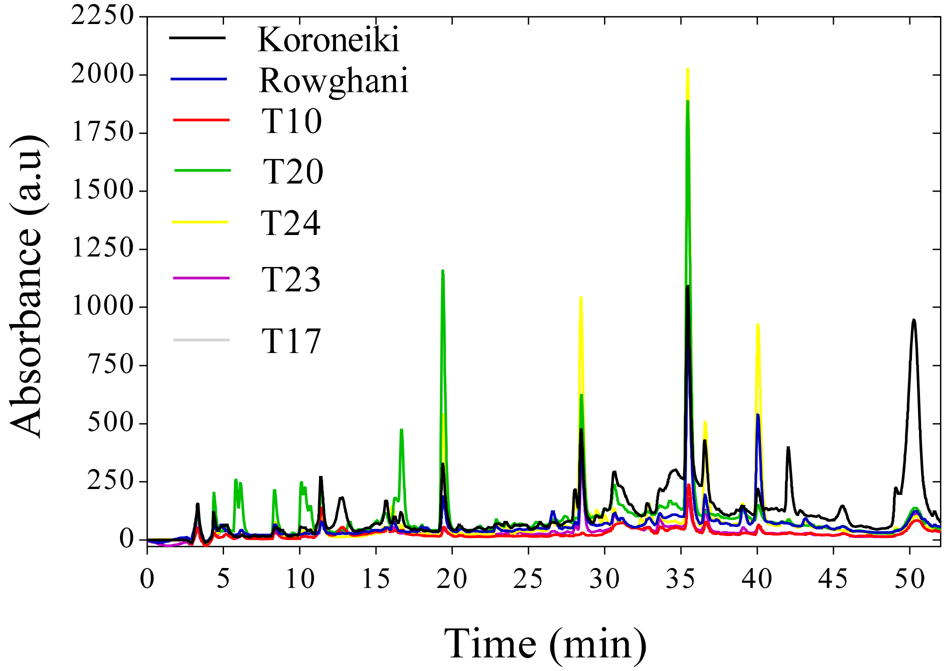


Fig. S6. HPLC chromatograms of the 7 most efficient anti-aggregative olive extracts, recorded at 230 nm.

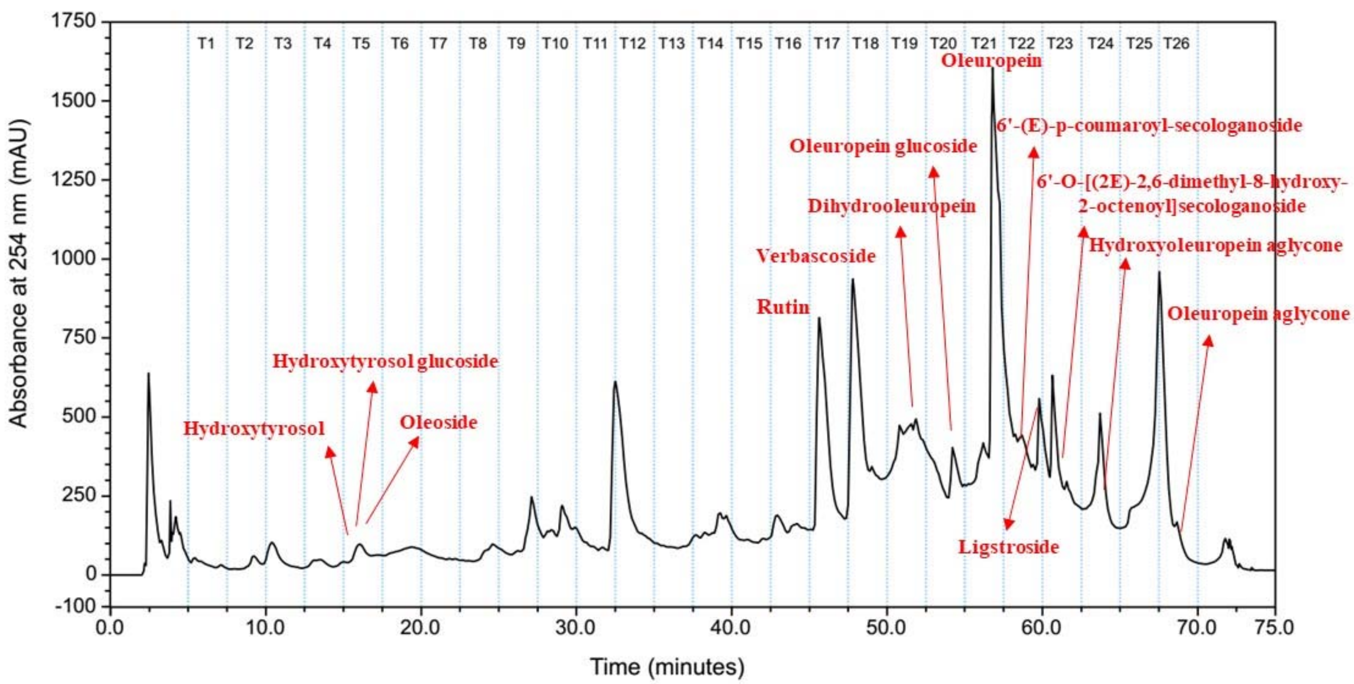
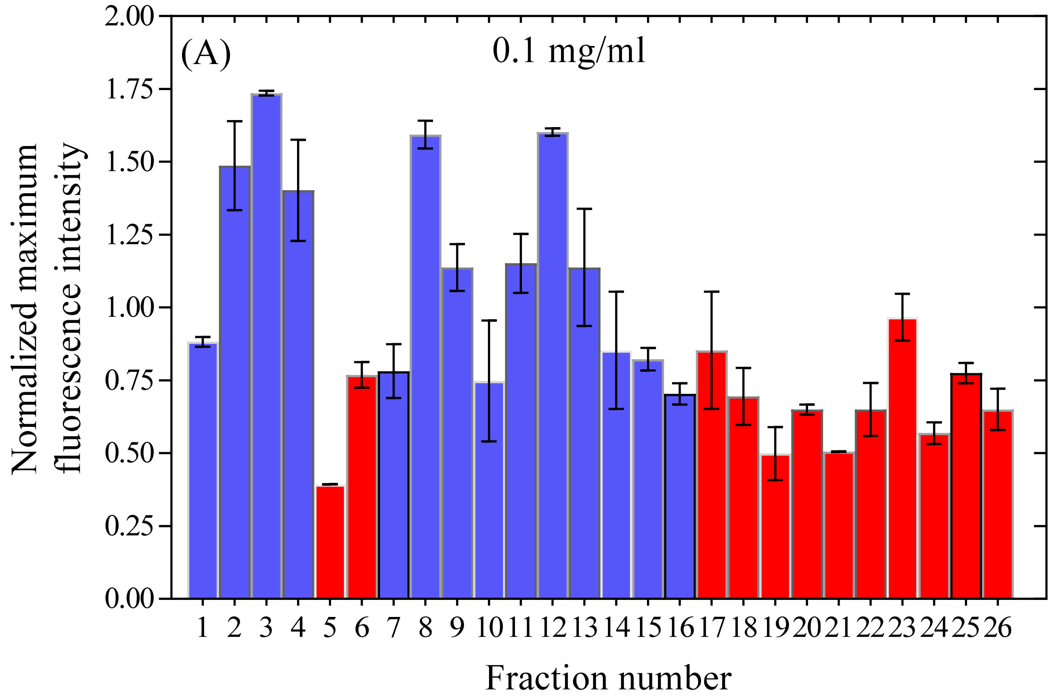


Fig. S7. Chromatogram of Koroneiki extract using HPLC. Fractions T1-26 are indicated. The different were identified by HPLC-MS.



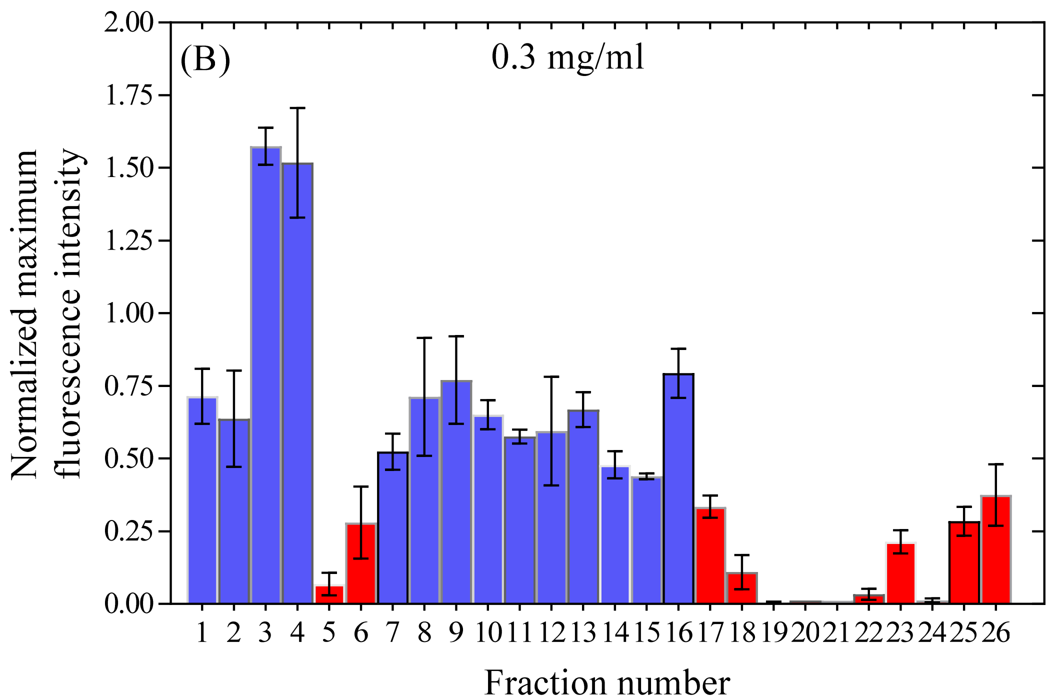


Fig. S8. The effect of (A) 1 mg/ml and (B) 3 mg/ml of the Koroneiki extract fractions on fibrillation of 1 mg/ml αSN. Maximum ThT fluorescence intensity normalized to control (absence of extract).

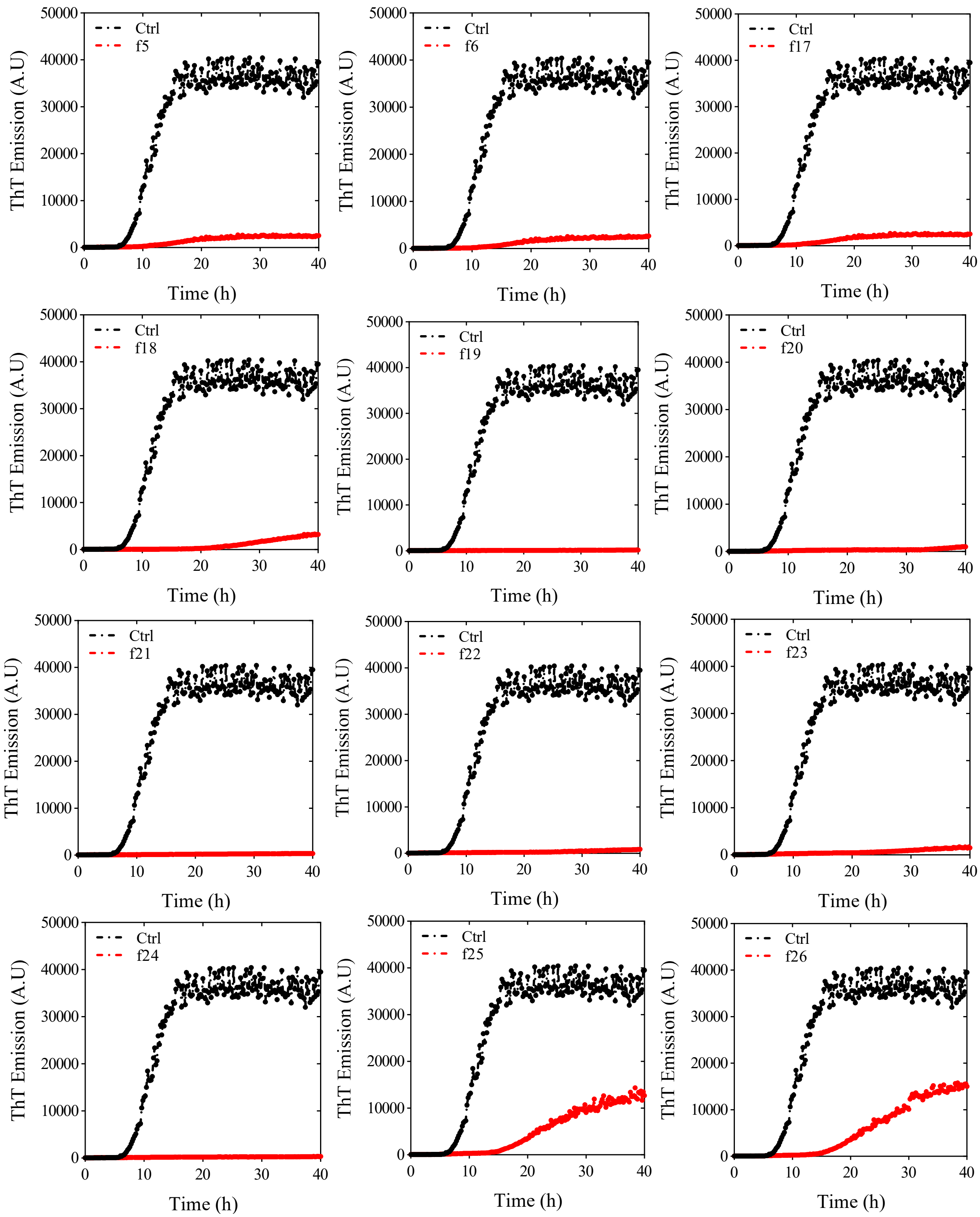


Fig. S9. The effect of Koroneiki extract fractions (3 mg/ml) on the kinetics of fibrillation of 1 mg/ml αSN monitored by ThT fluorescence.

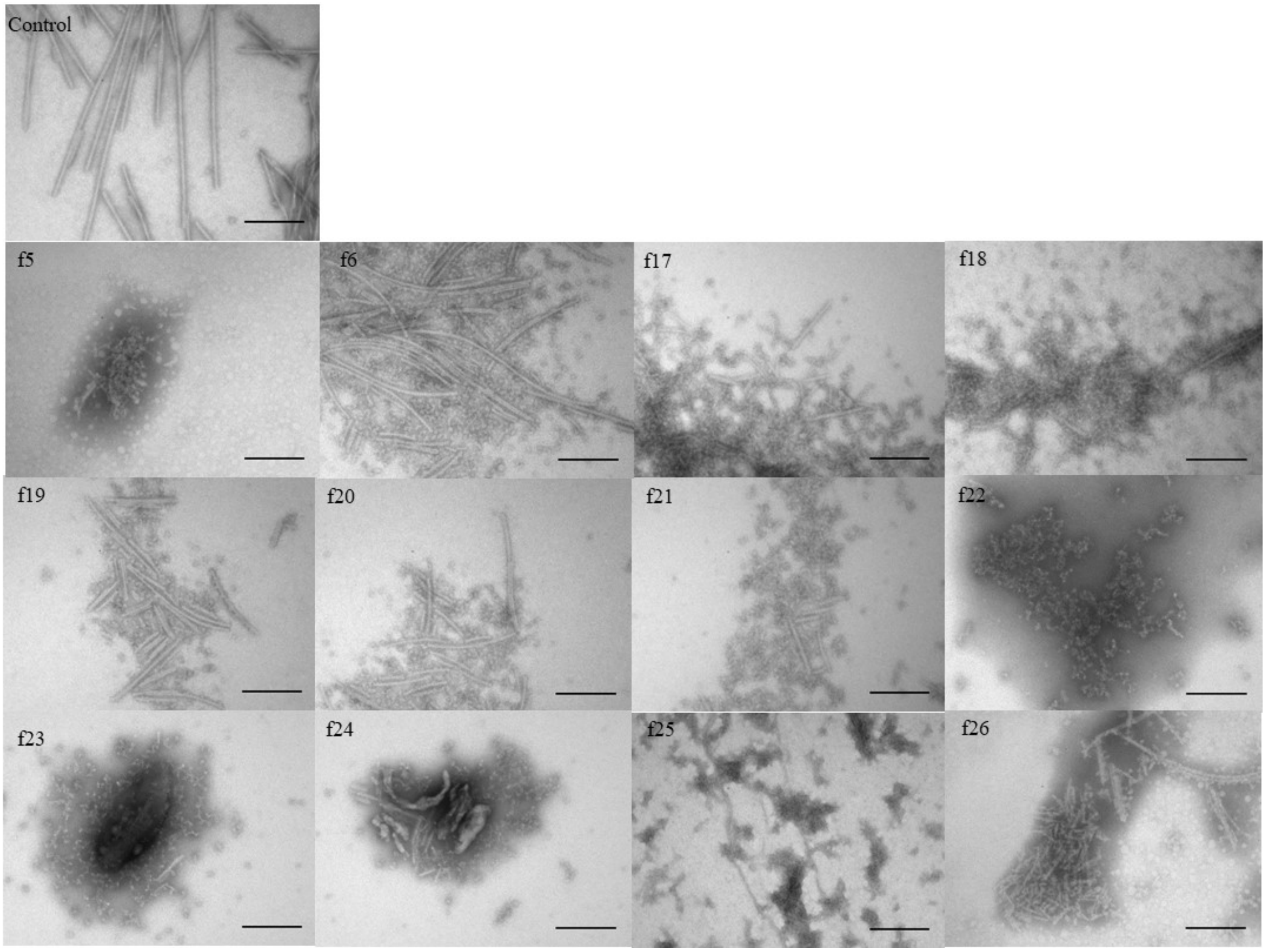
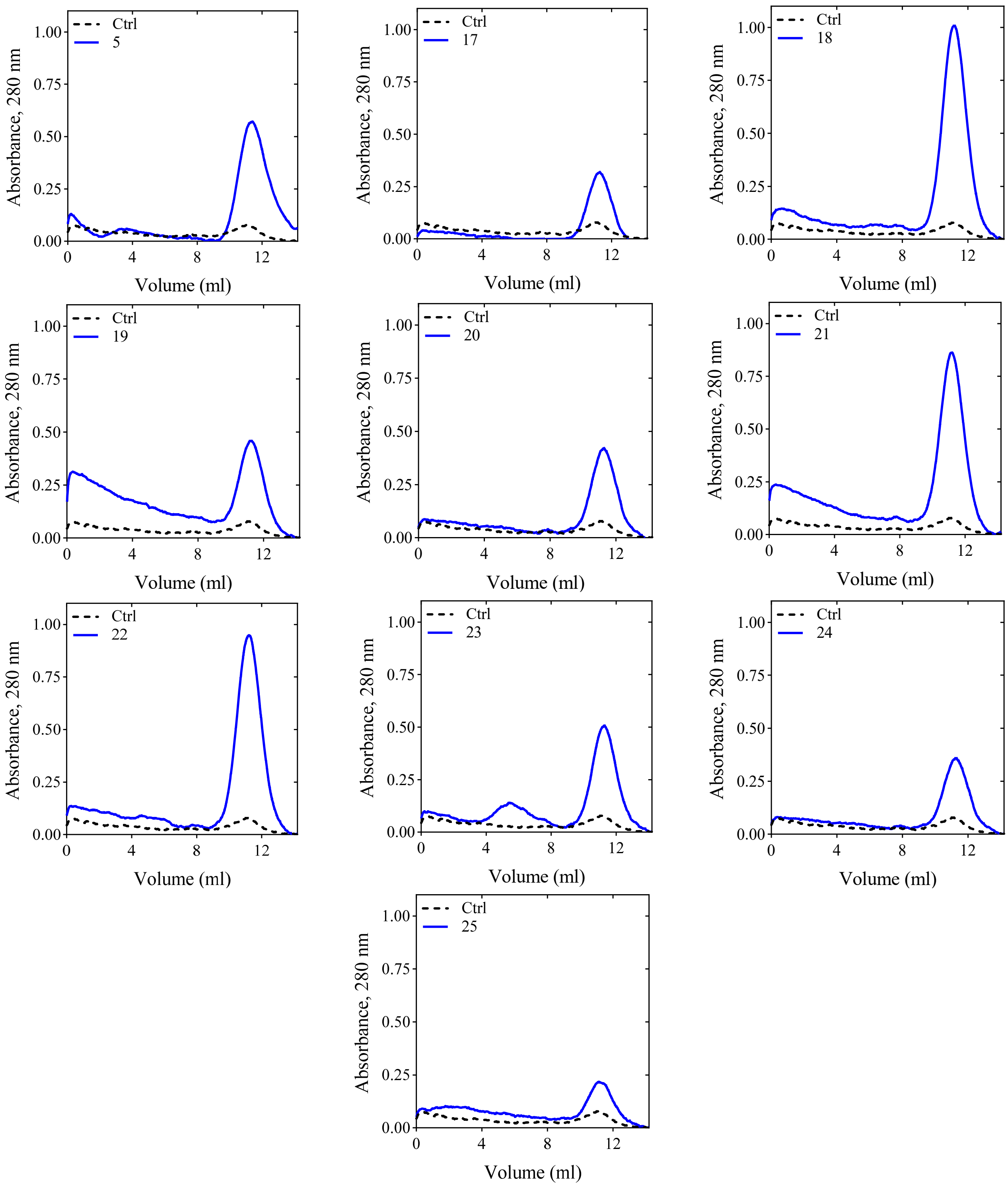


Fig. S10. TEM images of 1 mg/ml αSN incubated alone (control) and in the presence of 3 mg/ml of Koroneiki extract fractions.

(A)


Ctrl
5
Ctrl
17
Ctrl
18
Ctrl
19
Ctrl
20
Ctrl
21
Ctrl
22
Ctrl
23
Ctrl
24
Ctrl
25
Absorbance, 280 nm
Volume (ml)
0.00
0.25
0.50
0.75
1.00
0
4
8
12

(B)

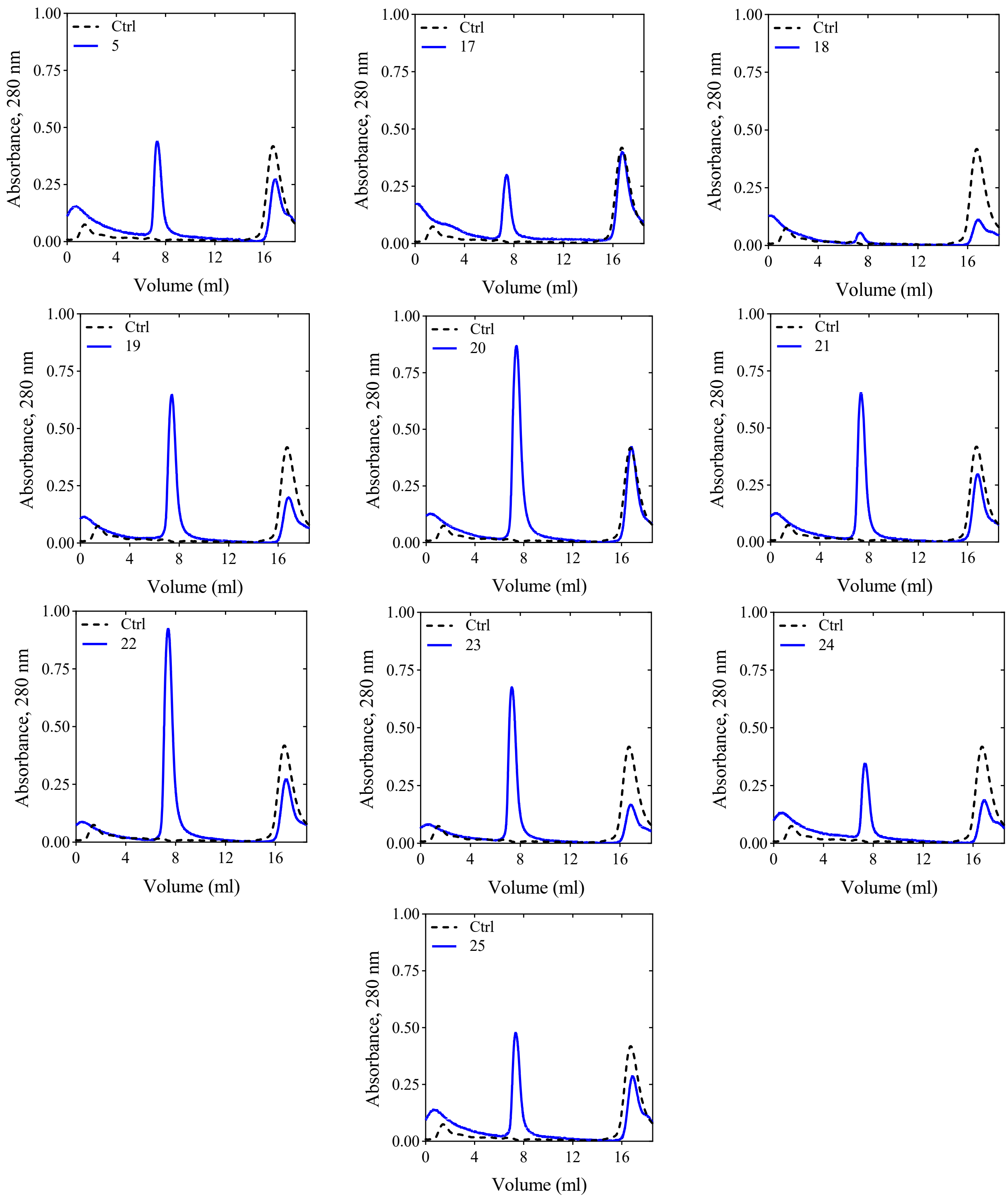


Fig. S11. SEC profiles of the supernatants of (A) samples of 1 mg/ml αSN incubated for 1 h at 37ºC in an oligomerization assay and (B) 0.5 mg/ml preformed αSN fibrils preincubated overnight at 37ºC with and without 3 mg/ml of Koroneiki extract fractions in a disaggregation assay.

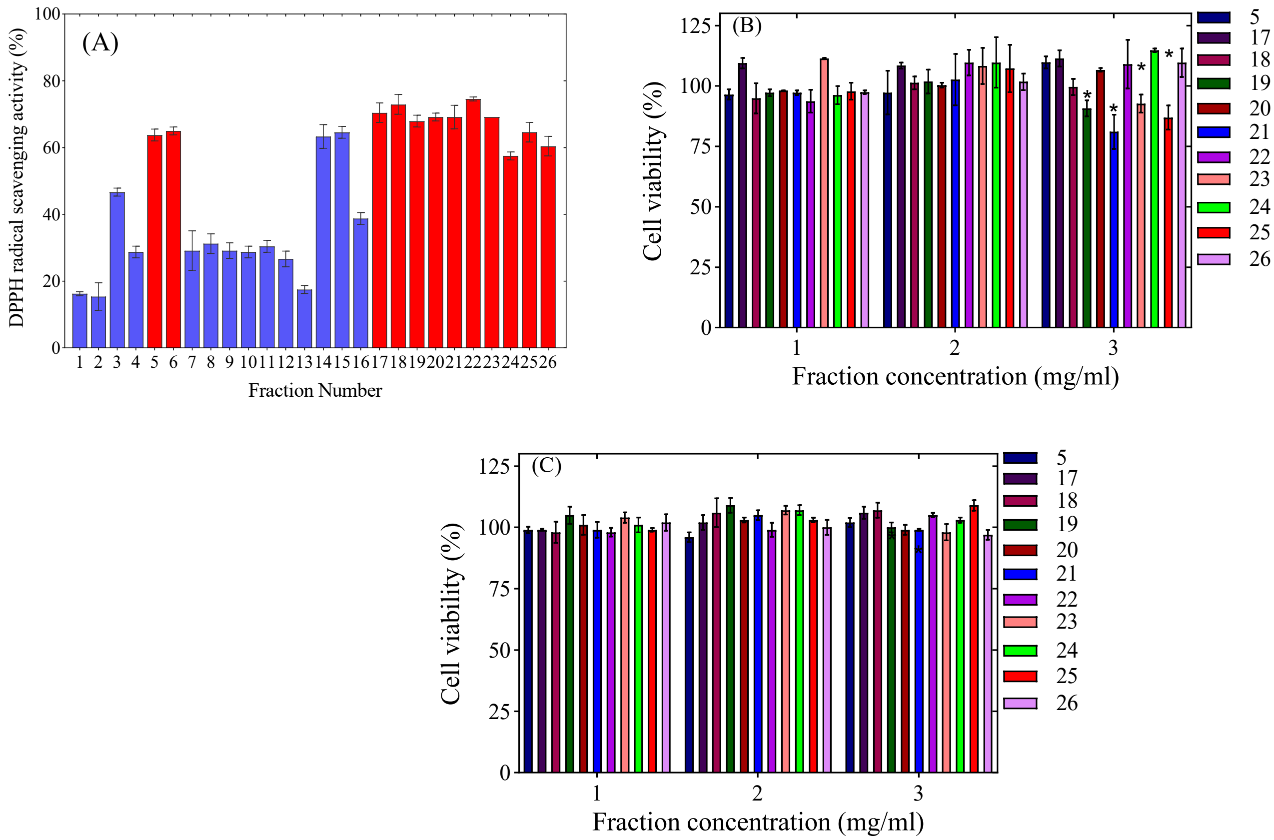


Fig. S12. (A) Antioxidant activity of Koroneiki extract fractions (3 mg/ml) measured by DPPH assay. Viability of (B) OLN-93 and (C) SH-SY5Y cells after 24 h incubation with 1-3 mg/ml Koroneiki extract fractions.

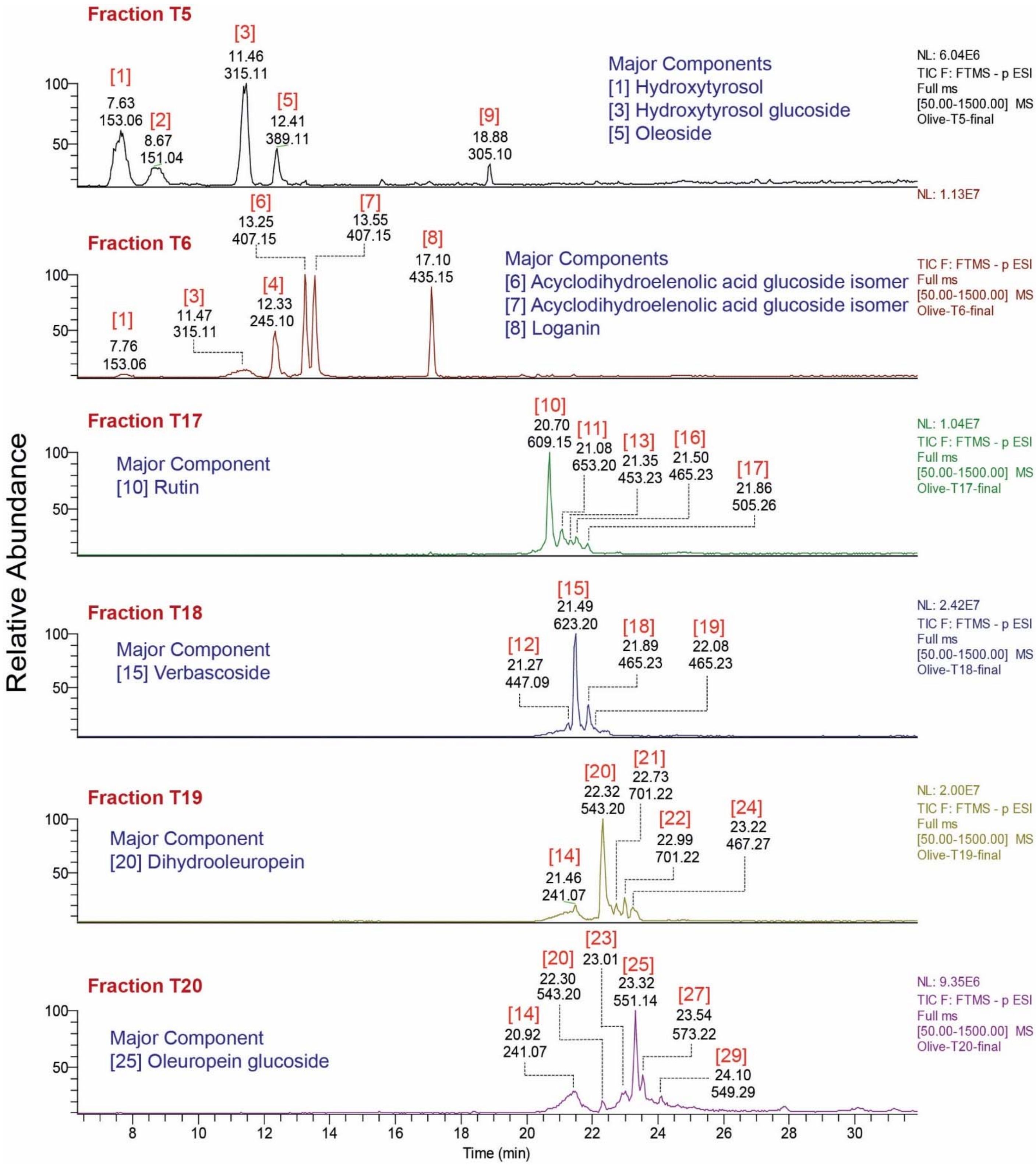


Fig. S13. Total ion chromatograms of Koroneiki fractions

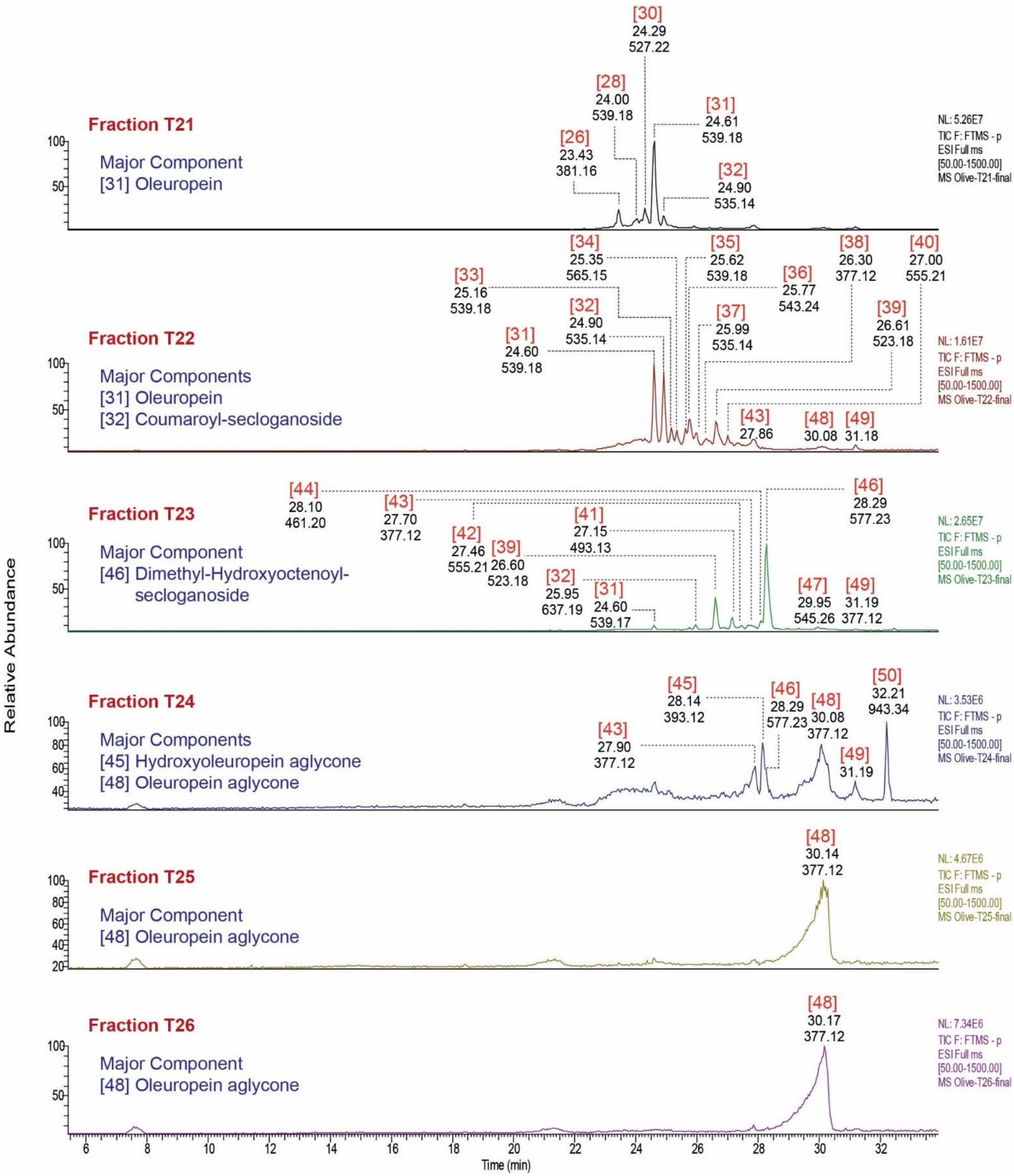


Fig. S13 (cont'd). Total ion chromatograms of Koroneiki fractions.

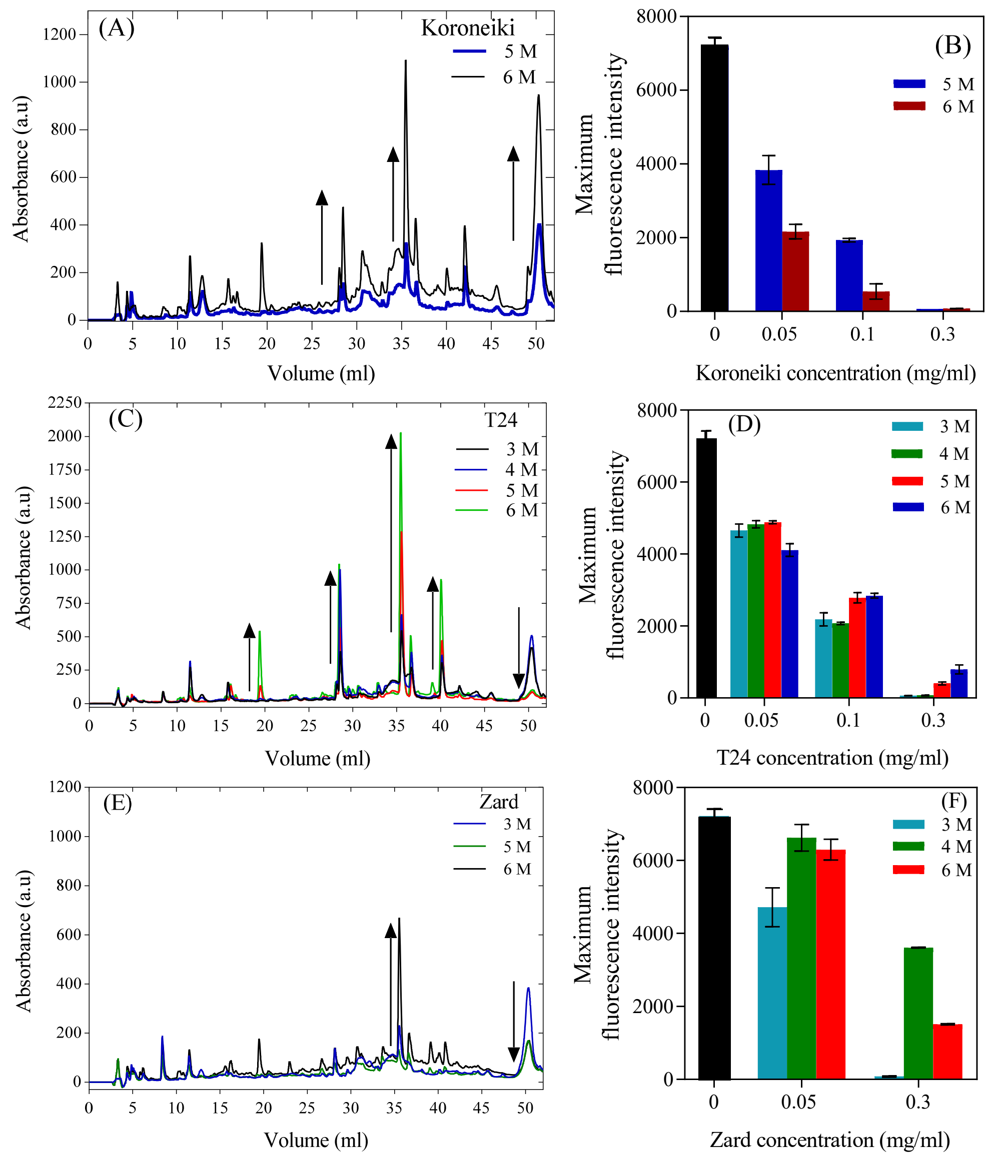


Fig. S14. Change in the level of compounds in the extracts of fruits picked at different maturation time (3, 4, 5, and 6 months after flowering) and their inhibitory effect on αSN fibrillation. HPLC chromatogram of the extracts (A, C, and E) and their effect on the maximum ThT fluorescence intensity (B, D, and F)

Table S1. Olive cultivars used in this study.

Nr	Olive Cultivar	Abbreviation	Origin	Nr	Olive Cultivar	Abbreviation	Origin
1	Koroneiki	-	Greece	9	Chenaran	T16	Iran
2	Arbequina	-	Spain	10	Khoshe	T17	Iran
3	Picual	-	Spain	11	Parseh	T18	Iran
4	Mari	-	Iran	12	Majnon	T20	Iran
5	Rowghani	-	Iran	13	Tak	T22	Iran
6	Zard	-	Iran	14	Zarin	T23	Iran
7	Yaghout	T10	Iran	15	Arghavan	T24	Iran
8	Gorgan	T15	Iran				

Table S2. LC-MS data of fractions f5, 6, and 17-26 of Koroneiki extract obtained from HPLC separation

Peak	Retention time (min)	m/z	Predicted ion formula	Delta (ppm)	MS-MS	λ_{max}	Identity or Compound class	How identified	Previously observed in *Olea* species? (reference)
1	7.63	153.0556	$[C_8 H_9 O_3]^-$	0.94	123 ($C_7H_7O_2$)	281	Hydroxytyrosol	authentic standard	Yes (1)
2	8.67	151.0401	$[C_8H_7 O_3]^-$	1.49	-	280	4-Hydroxyphenylacetate	Accurate mass.	Yes (1)
3	11.46	315.1086	$[C_{14} H_{19} O_8]^-$	1.29	153 ($C_8H_9O_3$)	278	Hydroxytyrosol glucoside	MSMS and literature data[a]	Yes (1)
4	12.33	245.1034	$[C_{11}H_{17}O_6]^-$	1.39	-	-	Unknown	-	-
5	12.41	389.1090	$[C_{16}H_{21}O_{11}]^-$	1.11	227 ($C_{10}H_{11}O_6$), 183 ($C_9H_{11}O_4$), 165 ($C_9H_9O_3$), 121 (C_8H_9O), 89 ($C_3H_5O_3$)		Oleoside	RT, MS, MSMS and literature data[b]	Yes (2)
6	13.25	407.1560	$[C_{17}H_{27}O_{11}]^-$	1.21	389 ($C_{17}H_{25}O_{10}$), 377 ($C_{16}H_{25}O_{10}$), 357 ($C_{16}H_{21}O_9$), 313 ($C_{15}H_{21}O_7$), 183 ($C_{10}H_{15}O_3$), 151 ($C_9H_{11}O_2$), 113 ($C_5H_5O_3$), 101 ($C_4H_5O_3$), 89 ($C_3H_5O_3$)		Glucosyl acyclodihydroelenolic acid isomer I	Putative from MSMS.	Yes (3)
7	13.55	407.1560	$[C_{17}H_{27}O_{11}]^-$	1.24	389 ($C_{17}H_{25}O_{10}$), 377 ($C_{16}H_{25}O_{10}$), 357 ($C_{16}H_{21}O_9$), 313 ($C_{15}H_{21}O_7$), 183 ($C_{10}H_{15}O_3$), 151 ($C_9H_{11}O_2$), 113 ($C_5H_5O_3$), 101 ($C_4H_5O_3$), 89 ($C_3H_5O_3$)		Glucosyl acyclodihydroelenolic acid.isomer II	Putative from MSMS. Identical to Peak 6	Yes (3)
8	17.10	435.1506	$[C_{18} H_{27} O_{12}]^-$	0.877	357 ($C_{16}H_{21}O_9$), 313 ($C_{15}H_{21}O_7$), 183 ($C_{10}H_{15}O_3$), 169 ($C_9H_{13}O_3$), 151 ($C_9H_{11}O_2$), 113 ($C_5H_5O_3$), 101	219	Formate adduct of Loganin($C_{17}H_{25}O_{10}$)	RT, MS, MSMS and literature data[b]	Yes (2)

					($C_4H_5O_3$), 89 ($C_3H_5O_3$)				
9	18.88	305.1038	$[C_{16}H_{17}O_6]^-$	1.82	153 ($C_8H_9O_3$), 151 ($C_8H_7O_3$), 123 ($C_7H_7O_2$)	217	Conjugate of hydroxytyrosol and hydroxyphenyl acetate (ie 4-(hydroxyphenyl)ethyl 2-(4-hydroxyphenyl)acetate) OR conjugate of Vanillin and hydroxy tyrosol	Putative based on MS/MSMS fragmentation	-
10	20.70	609.1514	$[C_{27}H_{29}O_{16}]^-$	1.17	300 ($C_{15}H_8O_7$)	253, 354	Rutin	Authentic standard	Yes (2)
11	21.08	653.2030	$[C_{30}H_{37}O_{16}]^-$	1.30	621 ($C_{29}H_{33}O_{15}$), 459 ($C_{20}H_{27}O_{12}$), 179 ($C_9H_7O_4$), 161 ($C_9H_5O_3$), 151 ($C_8H_7O_3$)		Methoxyverbascoside	RT, MS, MSMS and literature data[b]	Yes (2)
12	21.27	447.0934	$[C_{21}H_{19}O_{11}]^-$	1.18	285 ($C_{15}H_9O_6$)	346	Luteolin-7-glucoside	Authentic standard	Yes (2)
13	21.35	453.2337	$[C_{20}H_{37}O_{11}]^-$	0.63	321 ($C_{15}H_{29}O_7$), 233 ($C_9H_{13}O_7$), 191 ($C_7H_{11}O_6$), 161 ($C_6H_9O_5$), 113 ($C_5H_5O_3$), 101 ($C_4H_5O_3$)	218	Unknown	-	-
14	21.46	241.0719	$[C_{11}H_{13}O_6]^-$	0.169	139 ($C_6H_3O_4$)	219.,328	Unknown	-	
15	21.49	623.2022	$[C_{22}H_{39}O_{20}]^-$	-0.71	461 ($C_{16}H_{29}O_{15}$), 179 ($C_9H_7O_4$), 161 ($C_9H_5O_3$)	330	Verbascoside	RT, MS, MSMS and literature data[a]	Yes (1)
16	21.50	505.2626	$[C_{24}H_{41}O_{11}]^-$		251 ($C_9H_{15}O_8$), 191 ($C_7H_{11}O_6$), 149 ($C_5H_9O_5$), 131 ($C_5H_7O_4$), 101 ($C_4H_5O_3$), 89 ($C_3H_5O_3$)		Unknown diglycoside	-	-
17	21.86	505.2625	$[C_{24}H_{41}O_{11}]^-$		373 ($C_{19}H_{33}O_7$), 233 ($C_9H_{13}O_7$), 161 ($C_6H_9O_5$), 89 ($C_3H_5O_3$)		Unknown diglycoside	-	-
18	21.89	465.2323	n.d.		333 ($C_{16}H_{29}O_7$), 233 ($C_9H_{13}O_7$), 161 ($C_6H_9O_5$), 113 ($C_5H_5O_3$), 101	218	Unknown		-

					($C_4H_5O_3$), 89 ($C_3H_5O_3$)				
19	22.08	465.2332	n.d.		333 ($C_{16}H_{29}O_7$), 233 ($C_9H_{13}O_7$), 161 ($C_6H_9O_5$), 113 ($C_5H_5O_3$), 101 ($C_4H_5O_3$), 89 ($C_3H_5O_3$)	218	Unknown, isomer of 18	-	-
20	22.32	543.2047	$[C_{25}H_{35}O_{13}]^-$	-2.55	377 ($C_{16}H_{25}O_{10}$), 357 ($C_{16}H_{21}O_9$), 313 ($C_{15}H_{21}O_7$), 197 ($C_{10}H_{13}O_4$), 101 ($C_4H_5O_3$)		Dihydrooleuropein	RT, MS, MSMS. And ref[d]	Yes (4)
		623.2041	$[C_{22}H_{39}O_{20}]^-$	1.21	461 ($C_{16}H_{29}O_{15}$), 179 ($C_9H_7O_4$), 161 ($C_9H_5O_3$)		Verbascoside isomer		Yes (2)
21	22.73	701.2235	$[C_{31}H_{41}O_{18}]^-$	-5.92	377 ($C_{19}H_{21}O_8$), 307 ($C_{15}H_{15}O_7$), 275 ($C_{15}H_{15}O_5$), 221 ($C_8H_{13}O_7$), 179 ($C_6H_{11}O_6$), 149 ($C_8H_5O_3$), 101 ($C_4H_5O_3$)	219	Oleuropein glycoside isomer	RT, MS, MSMS. And ref [b]	Yes (2)
22	22.99	701.2213	$[C_{31}H_{41}O_{18}]^-$	-7.44	377 ($C_{19}H_{21}O_8$), 307 ($C_{15}H_{15}O_7$), 275 ($C_{15}H_{15}O_5$), 221 ($C_8H_{13}O_7$), 179 ($C_6H_{11}O_6$), 149 ($C_8H_5O_3$), 101 ($C_4H_5O_3$)	219	Oleuropein glycoside isomer	RT, MS, MSMS. And ref [b]	Yes (2)
23	23.01	447.0924	$[C_{21}H_{19}O_{11}]^-$	-0.91	285 ($C_{15}H_9O_6$)	328	Luteolin glycoside isomer	By MSMS	Yes (2)
24	23.22	467.2479	$[C_{21}H_{39}O_{11}]^-$	-0.81	335 ($C_{16}H_{31}O_7$), 233 ($C_9H_{13}O_7$), 161 ($C_6H_9O_5$), 101 ($C_4H_5O_3$), 89 ($C_3H_5O_3$)	219	Terpene diglycoside (Putative)	-	-
25	23.32	551.1416	$[C_{21}H_{27}O_{14}]^-$	1.94	161 ($C_9H_5O_3$)	219	cinnamoyl hydroxyloganin (PUTATIVE)	Putative;	
		701.2344	$[C_{21}H_{39}O_{11}]^-$	5.70	539, 371 ($C_{16}H_{19}O_{10}$), 307 ($C_{15}H_{15}O_7$), 275 ($C_{15}H_{15}O_5$), 223	220, 328	Oleuropein-glucoside or Aleuricine A/B		

					($C_{11}H_{11}O_5$), 179 ($C_6H_{11}O_6$), 149 ($C_8H_5O_3$)				
26	23.43	381.1555	$[C_{19}H_{25}O_8]^-$	1.11	231 ($C_{10}H_{15}O_6$), 201 ($C_9H_{13}O_5$), 183 ($C_9H_{11}O_4$), 151 ($C_9H_{11}O_{2,}$), 139 ($C_8H_{11}O_2$)		HT-ACDE. (Hydroxytyrosylacyldihydro -elenolate)	RT, MS, MSMS.	Yes (5)
27	23.54	573.2135	n.d.		345 ($C_{15}H_{21}O_9$), 225 ($C_{12}H_{17}O_4$), 209 ($C_{10}H_9O_5$), 183 ($C_9H_{11}O_4$), 165 ($C_9H_9O_3$), 141 ($C_7H_9O_3$), 121 (C_8H_9O)	219	Unknown	-	-
28	24.00	539.1771	$[C_{25}H_{31}O_{13}]^-$	1.21	403 ($C_{13}H_{23}O_{14}$), 223 ($C_{11}H_{11}O_5$), 179 ($C_6H_{11}O_6$), 119 ($C_4H_7O_4$), 113 ($C_5H_5O_3$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)	346	Oleuroside isomer	MS, MSMS	-
29	24.10	549.2870	n.d	2.32	417 ($C_{21}H_{37}O_8$), 233($C_9H_{13}O_7$), 161 ($C_6H_9O_5$).	219	Unknown		
		377.1241	$[C_{19}H_{21}O_8]^-$	0.96	307 ($C_{15}H_{15}O_7$), 275 ($C_{14}H_{11}O_6$), 149 ($C_8H_5O_3$), 139 ($C_7H_7O_3$), 127 ($C_6H_7O_3$), 111 ($C_5H_3O_3$), 111 ($C_5H_3O_3$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)		Oleuropein aglycone isomer	RT, MS, MSMS. And ref [b]	Yes (2)
30	24.29	569.1924	$[C_{26}H_{33}O_{14}]^-$	-4.11	537 ($C_{25}H_{29}O_{13}$) 403 ($C_{17}H_{23}O_{11}$), 371 ($C_{16}H_{19}O_{10}$), 305 ($C_{15}H_{13}O_7$), 223 ($C_{11}H_{11}O_5$), 151 ($C_8H_7O_3$)		Methoxyoleuropein	RT, MS, MSMS. And ref [b]	Yes (2)
		527.2092	$[C_{25}H_{35}O_{12}]^-$	4.72	377 ($C_{16}H_{25}O_{10}$), 313 ($C_{15}H_{21}O_7$), 101($C_4H_5O_3$)		Coumaroyl bearing derivative	MSMS	

		377.1241			307 ($C_{15}H_{15}O_7$), 275 ($C_{14}H_{11}O_6$), 197 ($C_{10}H_{13}O_4$), 165 ($C_9H_9O_3$), 149 ($C_8H_5O_3$), 139 ($C_7H_7O_3$), 121 (C_8H_9O), 111 ($C_5H_3O_3$), 111 ($C_5H_3O_3$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)		Oleuropein aglycone isomer	RT, MS, MSMS. And ref [b]	Yes (2)
31	24.61	539.1745	$[C_{25}H_{31}O_{13}]^-$	-1.44	403 ($C_{13}H_{23}O_{14}$), 371 ($C_{16}H_{19}O_{10}$), 307 ($C_8H_{19}O_{12}$), 275 ($C_{15}H_{15}O_5$), 223 ($C_{11}H_{11}O_5$), 179 ($C_6H_{11}O_6$), 149 ($C_8H_5O_3$) 119 ($C_4H_7O_4$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)	222, 282	Oleuropein	RT, MS, MSMS. And ref [b]	Yes (2)
32	24.90	535.1430	$[C_{25}H_{27}O_{13}]^-$	-1.61	389 ($C_{16}H_{21}O_{11}$), 345 ($C_{15}H_{21}O_9$), 307 ($C_{15}H_{15}O_7$), 265 ($C_{13}H_{13}O_6$), 235 ($C_{12}H_{11}O_5$), 205 ($C_{11}H_9O_4$), 163 ($C_9H_7O_3$) 145 ($C_9H_5O_2$), 121 (C_8H_9O)	219, 312	6'-(E)-p-coumaroyl-secologanoside	MS, MSMS	Yes (4)
33	25.16	539.1730	$[C_{25}H_{31}O_{13}]^-$	1.91	403 ($C_{13}H_{23}O_{14}$), 327 ($C_{18}H_{15}O_6$), 307 ($C_{15}H_{15}O_7$), 275 ($C_{15}H_{15}O_5$), 223 ($C_{11}H_{11}O_5$), 197 ($C_{10}H_{13}O_4$), 165 ($C_9H_0O_3$), 149 ($C_8H_5O_3$) 139 ($C_7H_7O_3$), 119 ($C_4H_7O_4$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)	219	Oleuropein Isomer	RT, MS, MSMS. And ref [b]	Yes (2)
34	25.35	565.1511	$[C_{26}H_{29}O_{14}]^-$ [	-4.04	345 ($C_{15}H_{21}O_9$), 295 ($C_{14}H_{15}O_7$), 235	220, 327	Unknown	-	-

					($C_{12}H_{11}O_5$), 193 ($C_{10}H_9O_4$), 175 ($C_{10}H_7O_3$), 161 ($C_9H_5O_3$)				
35	25.62	539.1793	$[C_{25}H_{31}O_{13}]^-$	3.35	403 ($C_{13}H_{23}O_{14}$), 371 ($C_{16}H_{19}O_{10}$), 327 ($C_{18}H_{15}O_6$), 307 ($C_{15}H_{15}O_7$), 275 ($C_{15}H_{15}O_5$), 223 ($C_{11}H_{11}O_5$), 197 ($C_{10}H_{13}O_4$), 165 ($C_9H_0O_3$), 149 ($C_8H_5O_3$) 139 ($C_7H_7O_3$), 119 ($C_4H_7O_4$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)		Oleuroside Isomer	MS,MSMS	
36	25.77	543.2459	$[C_{26}H_{39}O_{12}]^-$	2.31	375 ($C_{16}H_{23}O_{10}$), 357 ($C_{16}H_{21}O_9$), 227 ($C_{12}H_{19}O_4$), 213 ($C_{10}H_{13}O_5$), 199 ($C_{11}H_{19}O_3$), 185 ($C_{10}H_{17}O_3$), 169 ($C_9H_{13}O_3$), 151 ($C_9H_{11}O_2$) 125 ($C_7H_9O_2$), 113 ($C_5H_5O_3$)	220	Dihydro oleuropein	RT, MS, MS-MS	Yes (2)
37	25.99	535.1479	$[C_{25}H_{27}O_{13}]^-$	3.33	389 ($C_{16}H_{21}O_{11}$), 345 ($C_{15}H_{21}O_9$), 307 ($C_{15}H_{15}O_7$), 265 ($C_{13}H_{13}O_6$), 235 ($C_{12}H_{11}O_5$), 205 ($C_{11}H_9O_4$), 163 ($C_9H_7O_3$) 145 ($C_9H_5O_2$), 121 (C_8H_9O)	220, 300	Coumaroyl-secologanoside isomer	MS. MS-MS	
38	26.30	377.1243	$[C_{19}H_{21}O_8]^-$	0.29	307 ($C_{15}H_{15}O_7$), 275 ($C_{14}H_{11}O_6$), 171 ($C_7H_7O_5$), 149 ($C_8H_5O_3$), 139 ($C_7H_7O_3$), 127 ($C_6H_7O_3$), 113 ($C_5H_5O_3$) 111	220, 286	Isomer of oleuropein aglycone	MSMS	

					($C_5H_3O_3$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)				
39	26.60	523.1780	$[C_{25}H_{31}O_{12}]^-$	3.12	361 ($C_{19}H_{21}O_7$), 291 ($C_{15}H_{15}O_6$), 259 ($C_{15}H_{15}O_4$), 101 ($C_4H_5O_3$)	220, 276	Ligstroside (or isomer)	MSMS	Yes (6)
40	27.00	555.2100	$[C_{26}H_{35}O_{13}]^-$	2.74	511 ($C_{25}H_{35}O_{11}$) 345 ($C_{15}H_{21}O_9$), 327 ($C_{15}H_{19}O_8$), 225 ($C_{12}H_{17}O_4$), 197 ($C_{11}H_{17}O_3$), 183 ($C_{10}H_{15}O_3$), 165 ($C_9H_9O_3$), 155 ($C_5H_5O_3$), 139 ($C_6H_3O_4$), 121 (C_8H_9O)	220	Hydroxyoleuroside	RT, MS, MSMS and ref[b]	Yes (2)
41	27.15	493.1330	$[C_{23}H_{25}O_{12}]^-$	-1.04	327 ($C_{15}H_{19}O_8$), 209 ($C_{10}H_9O_5$), 183 ($C_9H_{11}O_4$), 165 ($C_9H_9O_3$), 135 ($C_8H_7O_2$), 121 ($C_7H_5O_2$)	220	Unknown		
42	27.46	555.2040	$[C_{26}H_{35}O_{13}]^-$	-3.20	345 ($C_{15}H_{21}O_9$), 327 ($C_{15}H_{19}O_8$), 225 ($C_{12}H_{17}O_4$), 197 ($C_{11}H_{17}O_3$), 183 ($C_{10}H_{15}O_3$), 165 ($C_9H_9O_3$), 155 ($C_5H_5O_3$), 139 ($C_6H_3O_4$), 121 (C_8H_9O)	220	Hydroxyoleuroside isomer	MS, MSMS	
43	27.70	377.1243	$[C_{19}H_{21}O_8]^-$	0.21	307 ($C_{15}H_{15}O_7$), 275 ($C_{14}H_{11}O_6$), 191 ($C_{10}H_7O_4$), 171 ($C_7H_7O_5$), 149 ($C_8H_5O_3$), 139 ($C_7H_7O_3$), 127 ($C_6H_7O_3$), 111 ($C_5H_3O_3$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)	220	Isomer of oleuropein aglycone	RT, MS, MSMS and ref[b]	Yes (2)
44	28.10	461.2013	$[C_{21}H_{33}O_{11}]^-$	-0.43	167 ($C_{10}H_{15}O_2$)		Unknown	-	-

45	28.14	393.1191	$[C_{19}H_{21}O_9]^-$	1.05	317 ($C_{17}H_{17}O_6$), 181 ($C_9H_9O_4$), 137 ($C_8H_9O_2$)	220	Hydroxyoleuropein aglycone	Putative from MSMS data	-
46	28.29	557.2192	$[C_{26}H_{37}O_{13}]^-$	-3.68	345 ($C_{15}H_{21}O_9$), 227 ($C_{12}H_{19}O_4$), 199 ($C_{11}H_{19}O_3$), 185 ($C_{10}H_{17}O_3$), 165 ($C_9H_9O_3$), 139 ($C_6H_3O_4$), 121 (C_8H_9O)	220,	6'-O-[(2E)-2,6-dimethyl-8-hydroxy-2-octenoyl]secologanoside	MSMS data	Yes (4)
47	29.95	545.2563	n.d.	-	-	-	Unknown	-	-
48	30.08	377.1242	$[C_{19}H_{21}O_8]^-$	1.10	307 ($C_{15}H_{15}O_7$), 275 ($C_{14}H_{11}O_6$), 191 ($C_{10}H_7O_4$), 171 ($C_7H_7O_5$), 149 ($C_8H_5O_3$), 139 ($C_7H_7O_3$), 127 ($C_6H_7O_3$), 111 ($C_5H_3O_3$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)		Oleuropein aglycone	RT, MS, MSMS and ref[b]	Yes (2)
49	31.19	377.1243	$[C_{19}H_{21}O_8]^-$	0.24	307 ($C_{15}H_{15}O_7$), 275 ($C_{14}H_{11}O_6$), 191 ($C_{10}H_7O_4$), 171 ($C_7H_7O_5$), 149 ($C_8H_5O_3$), 139 ($C_7H_7O_3$), 127 ($C_6H_7O_3$), 111 ($C_5H_3O_3$), 101 ($C_4H_5O_3$), 95 (C_6H_7O)		Isomer of oleuropein aglycone	MSMS data	
50	32.21	943.3442	$[C_{43}H_{59}O_{23}]^-$	0.05	513 ($C_{35}H_{29}O_4$), 345 ($C_{15}H_{21}O_9$), 227 ($C_{12}H_{19}O_4$), 209 ($C_{10}H_9O_5$), 185 ($C_{10}H_{17}O_3$), 165 ($C_9H_9O_3$), 149 ($C_8H_5O_3$), 139 ($C_6H_3O_4$), 121 (C_8H_9O), 101 ($C_4H_5O_3$)	222	Unknown	-	-

Table. S3. Major compounds identified in fractions from the Koroneiki extract

Nr	Fraction	Major compounds
1	5	Hydroxytyrosol, Hydroxytyrosol glucoside, and Oleoside
2	6	Acyclodihydroelenolic acid glucoside isomer, Loganin
3	17	Rutin
4	18	Verbascoside
5	19	Dihydro oleuropein
6	20	Oleuropein glucoside
7	21	Oleuropein
8	22	Oleuropein and Coumaroyl-secloganoside
9	23	Dimethyl- Hydroxyoctenoyl-secologanoside
10	24	Hydroxyoleuropein aglycone, Oleuropein aglycone
11	25	Oleuropein aglycone
12	26	Oleuropein aglycone

References

1. Maalej, A., Bouallagui, Z., Hadrich, F., Isoda, H., and Sayadi, S. (2017) Assessment of Olea europaea L. fruit extracts: Phytochemical characterization and anticancer pathway investigation. *Biomedicine & Pharmacotherapy* **90**, 179–186

2. Michel, T., Khlif, I., Kanakis, P., Termentzi, A., Allouche, N., Halabalaki, M., and Skaltsounis, A.-L. (2015) UHPLC-DAD-FLD and UHPLC-HRMS/MS based metabolic profiling and characterization of different Olea europaea organs of Koroneiki and Chetoui varieties. *Phytochemistry Letters* **11**, 424–439

3. Rubio-Senent, F., Martos, S., Lama-Muñoz, A., Fernández-Bolaños, J. G., Rodriguez-Gutiérrez, G., and Fernández-Bolaños, J. (2015) Isolation and identification of minor secoiridoids and phenolic components from thermally treated olive oil by-products. *Food chemistry* **187**, 166–173

4. Obied, H. K., Prenzler, P. D., Ryan, D., Servili, M., Taticchi, A., Esposto, S., and Robards, K. (2008) Biosynthesis and biotransformations of phenol-conjugated oleosidic secoiridoids from *Olea europaea* L. *Natural product reports* **25**, 1167–1179

5. Obied, H. K., Karuso, P., Prenzler, P. D., and Robards, K. (2007) Novel secoiridoids with antioxidant activity from Australian olive mill waste. *Journal of agricultural and food chemistry* **55**, 2848–2853

6. Gariboldi, P., Jommi, G., and Verotta, L. (1986) Secoiridoids from Olea europaea. *Phytochemistry* **25**, 865–869