

**Triterpene acid and phenolics from Ancient apples of Friuli Venezia Giulia as nutraceutical ingredients:
LC-MS study and *in vitro* activities**

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Table 1. Total bioactive components and total antioxidant capacity (by phosphomolybdenum) of apple samples

	Samples	Total phenolic content (mgGAE/g)	Total flavonoid content (mgRE/g)	Phosphomolybdenum (mmolTE/g)
Pulp	A2	4.13±0.18 [*]	0.11±0.02 ^e	0.59±0.05 ^{ab}
	B1	5.60±0.21 ^c	0.15±0.02 ^{cd}	0.61±0.06 ^{ab}
	C1	5.58±0.13 ^c	0.18±0.01 ^{abc}	0.59±0.07 ^{ab}
	D2	6.38±0.06 ^b	0.12±0.02 ^{de}	0.60±0.01 ^{ab}
	E2	5.87±0.18 ^c	0.17±0.01 ^{abc}	0.48±0.03 ^{cde}
	F1	7.54±0.17 ^a	0.15±0.02 ^{cd}	0.44±0.03 ^{def}
	G1	4.12±0.07 ^e	0.15±0.01 ^{cd}	0.37±0.01 ^f
	H1	5.57±0.14 ^c	0.16±0.02 ^{bc}	0.42±0.03 ^{ef}
	I1	4.55±0.08 ^d	0.16±0.01 ^{bc}	0.53±0.08 ^{bcd}
	Golden Delicious	3.38±0.06 ^f	0.19±0.01 ^{ab}	0.63±0.07 ^a
	Red Delicious	3.99±0.09 ^e	0.11±0.02 ^e	0.43±0.01 ^{ef}
	Granny smith	4.29±0.20 ^{de}	0.20±0.01 ^a	0.49±0.03 ^{cde}
	Royal Gala	4.10±0.12 ^e	0.19±0.03 ^{ab}	0.56±0.01 ^{abc}
Peels	A2	8.77±0.08 ^h	1.97±0.02 ^h	0.50±0.04 ^f
	B1	14.13±0.21 ^b	3.86±0.01 ^d	0.61±0.01 ^{cd}
	C1	10.41±0.19 ^f	0.58±0.01 ^k	0.50±0.03 ^f
	D2	13.71±0.28 ^{bc}	2.91±0.03 ^f	0.56±0.06 ^{def}
	E2	14.29±0.22 ^b	5.31±0.03 ^b	0.54±0.01 ^{def}
	F1	15.83±0.19 ^a	4.29±0.06 ^c	0.55±0.03 ^{def}
	G1	9.75±0.34 ^g	3.45±0.02 ^e	0.50±0.02 ^f
	H1	13.30±0.39 ^{cd}	1.59±0.01 ^j	0.59±0.03 ^{de}
	I1	12.79±0.14 ^d	8.05±0.09 ^a	0.52±0.02 ^{ef}
	Golden Delicious	6.87±0.10 ⁱ	2.47±0.02 ^g	0.53±0.04 ^{ef}
	Red Delicious	11.26±0.44 ^e	1.84±0.04 ⁱ	0.72±0.02 ^{ab}
	Granny smith	9.49±0.18 ^g	3.86±0.07 ^d	0.66±0.06 ^{bc}
	Royal Gala	8.27±0.18 ^h	1.81±0.01 ⁱ	0.74±0.04 ^a

* Values expressed are means ± S.D. of three parallel measurements. GAE: Gallic acid equivalent; RE: Rutin equivalent. Different letters indicated significant differences in each group (pulp or peels) ($p < 0.05$).

Table 2. Antioxidant properties of the apple samples

	Samples	DPPH (mgTE/g)	ABTS (mgTE/g)	CUPRAC (mgTE/g)	FRAP (mgTE/g)	Metal chelating (mgEDTA/g)
Pulp	A2	5.45 ±0.33 ^{e*}	7.89 ±0.81 ^d	14.15 ±0.49 ^{fg}	7.78 ±0.90 ^{ef}	4.05 ±0.26 ^{cd}
	B1	9.32 ±0.39 ^b	13.73 ±0.92 ^{bc}	20.14 ±0.62 ^{cd}	11.25 ±0.47 ^c	3.61 ±0.07 ^{cdef}
	C1	7.70 ±0.03 ^d	11.83 ±0.75 ^c	19.77 ±0.18 ^d	11.47 ±0.55 ^c	5.46 ±0.18 ^a
	D2	8.48 ±0.24 ^e	12.34 ±1.72 ^c	23.55 ±0.79 ^b	13.59 ±0.17 ^b	2.99 ±0.18 ^{efg}
	E2	7.69 ±0.04 ^d	14.48 ±0.84 ^b	21.40 ±0.41 ^c	12.47 ±0.53 ^{bc}	3.11 ±0.10 ^{efg}
	F1	11.71 ±0.11 ^a	19.42 ±0.82 ^a	28.32 ±0.92 ^a	16.44 ±0.44 ^a	5.44 ±0.90 ^a
	G1	5.38 ±0.03 ^e	8.00 ±0.43 ^d	15.04 ±0.65 ^f	8.67 ±0.31 ^{de}	2.76 ±0.36 ^{fg}
	H1	8.60 ±0.05 ^c	12.75 ±0.73 ^{bc}	21.04 ±0.53 ^{cd}	11.84 ±0.74 ^c	3.82 ±0.31 ^{cde}
	I1	5.44 ±0.16 ^e	7.27 ±0.87 ^d	16.93 ±0.50 ^e	9.20 ±0.34 ^d	2.64 ±0.39 ^g
	Golden Delicious	4.66 ±0.22 ^{fg}	6.14 ±0.30 ^d	11.50 ±0.43 ^h	6.92 ±0.74 ^f	4.46 ±0.50 ^{bc}
	Red Delicious	4.25 ±0.59 ^{gh}	6.63 ±0.34 ^d	12.96 ±0.25 ^g	7.91 ±0.28 ^{ef}	4.32 ±0.36 ^{cd}
	Granny smith	5.11 ±0.17 ^{ef}	7.63 ±0.61 ^d	13.57 ±0.79 ^g	8.74 ±0.23 ^{de}	3.50 ±0.64 ^{def}
	Royal Gala	3.77 ±0.25 ^h	6.98 ±0.59 ^d	12.77 ±0.12 ^{gh}	7.86 ±0.22 ^{ef}	5.17 ±0.37 ^{ab}
Peels	A2	14.16 ±0.13 ^{fg}	23.54 ±1.45	37.89 ±0.79 ^{gh}	21.33 ±1.74 ^d	5.05 ±0.25 ^e
	B1	20.33 ±0.05 ^a	34.26 ±0.32	53.69 ±1.63 ^e	31.59 ±0.56 ^b	5.93 ±0.30 ^{cd}
	C1	17.37 ±0.58 ^d	30.02 ±0.83	41.32 ±0.36 ^f	23.03 ±0.67 ^{cd}	6.76 ±0.24 ^b
	D2	18.11 ±0.32 ^c	33.24 ±0.33	49.53 ±1.14 ^d	30.45 ±1.73 ^b	5.01 ±0.13 ^e
	E2	19.41 ±0.16 ^b	32.31 ±0.74	56.30 ±2.03 ^b	34.74 ±0.77 ^a	4.51 ±0.26 ^{ef}
	F1	20.31 ±0.10 ^a	34.05 ±0.14	62.75 ±0.54 ^a	37.66 ±1.08 ^a	5.85 ±0.18 ^d
	G1	16.73 ±0.23 ^e	23.22 ±1.70	38.80 ±0.41 ^g	22.16 ±1.49 ^d	4.61 ±0.30 ^{ef}
	H1	20.11 ±0.04 ^a	31.77 ±1.06	54.31 ±0.44 ^{bc}	31.61 ±0.94 ^b	6.34 ±0.50 ^{bcd}
	I1	17.69 ±0.13 ^{cd}	28.26 ±1.73	49.71 ±0.49 ^d	29.27 ±1.20 ^b	4.11 ±0.39 ^f
	Golden Delicious	11.75 ±0.13 ^h	18.02 ±0.68	25.26 ±0.70 ^j	14.71 ±0.68 ^e	4.50 ±0.53 ^{ef}
	Red Delicious	19.01 ±0.10 ^b	31.67 ±1.55	45.03 ±0.43 ^e	25.37 ±1.35 ^c	6.12 ±0.30 ^{bcd}
	Granny smith	14.55 ±0.24 ^f	23.96 ±0.62	35.51 ±0.47 ^h	20.05 ±1.74 ^d	8.16 ±0.24 ^a
	Royal Gala	13.60 ±0.09 ^g	19.98 ±0.78	29.31 ±1.56 ⁱ	16.20 ±1.51 ^e	6.53 ±0.44 ^{bc}

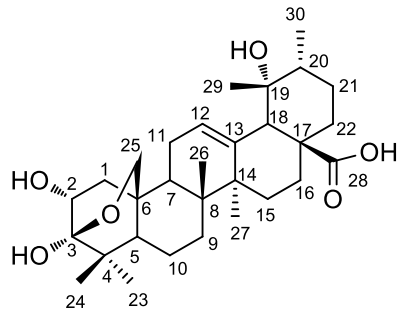
* Values expressed are means ± S.D. of three parallel measurements. TE: Trolox equivalent; EDTAE: EDTA equivalent. Different letters indicated significant differences in each group (pulp or peels) ($p < 0.05$).

Table 3. Enzyme inhibitory effects of the apple samples

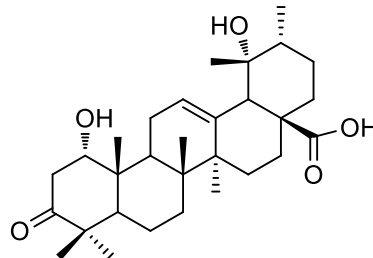
	Samples	AChE inhibition (mgGALAE/g)	BChE inhibition (mgGALAE/g)	Tyrosinase (mgKAE/g)	Amylase (mgACAE/g)	Glucosidase (mgACAE/g)
Pulp	A2	1.07 ±0.02 ^{ef*}	0.49 ±0.01 ^{cd}	25.50 ±1.36 ^g	5.74 ±0.35 ^{ab}	21.38 ±0.57 ^d
	B1	0.97 ±0.05 ^g	0.55 ±0.05 ^{bcd}	25.84 ±0.36 ^{fg}	5.02 ±0.41 ^{cde}	34.26 ±0.16 ^a
	C1	1.00 ±0.04 ^{fg}	0.46 ±0.05 ^d	26.57 ±0.83 ^{defg}	5.94 ±0.26 ^{ab}	33.03 ±0.89 ^{ab}
	D2	1.24 ±0.04 ^{ab}	0.60 ±0.09 ^{ab}	27.01 ±0.26 ^{bcd}	4.76 ±0.13 ^{def}	34.44 ±0.02 ^a
	E2	1.18 ±0.01 ^{bc}	0.68 ±0.01 ^a	26.71 ±0.06 ^{cdef}	5.71 ±0.19 ^{abc}	34.50 ±0.01 ^a
	F1	1.08 ±0.04 ^{de}	0.60 ±0.01 ^{ab}	29.25 ±0.59 ^a	6.34 ±0.18 ^a	32.81 ±0.69 ^{ab}
	G1	1.14 ±0.03 ^{cd}	0.59 ±0.03 ^{abc}	26.86 ±0.06 ^{cdef}	5.64 ±0.33 ^{abc}	31.26 ±2.12 ^b
	H1	1.11 ±0.01 ^{cde}	0.52 ±0.10 ^{bcd}	28.13 ±0.48 ^{ab}	4.61 ±0.32 ^{def}	31.50 ±1.58 ^b
	I1	1.26 ±0.01 ^a	0.59 ±0.03 ^{abc}	28.08 ±0.34 ^b	5.25 ±0.10 ^{bcd}	34.26 ±0.10 ^a
	Golden Delicious	0.98 ±0.05 ^g	0.59 ±0.01 ^{abc}	26.04 ±0.75 ^{efg}	4.35 ±0.36 ^{ef}	33.22 ±0.63 ^{ab}
	Red Delicious	1.22 ±0.05 ^{ab}	0.49 ±0.05 ^{cd}	27.73 ±0.28 ^{bcd}	4.81 ±0.11 ^{def}	25.26 ±2.87 ^c
	Granny smith	1.29 ±0.02 ^a	0.59 ±0.01 ^{abc}	27.28 ±0.35 ^{bcd}	4.28 ±0.41 ^f	32.44 ±0.77 ^{ab}
Royal Gala	1.14 ±0.04 ^{cde}	0.51 ±0.10 ^{bcd}	27.79 ±0.26 ^{bc}	4.49 ±0.26 ^{ef}	24.44 ±1.89 ^c	
Peels	A2	1.22 ±0.05 ^{ab}	0.56 ±0.01 ^{bcd}	27.60 ±0.16 ^d	6.99 ±0.26 ^a	34.11 ±0.01 ^a
	B1	1.20 ±0.04 ^{abc}	0.58 ±0.05 ^{abcd}	28.59 ±0.23 ^{bc}	6.25 ±0.21 ^{abc}	34.38 ±0.05 ^a
	C1	1.13 ±0.03 ^{de}	0.64 ±0.01 ^a	28.48 ±0.05 ^c	6.48 ±0.57 ^{ab}	34.11 ±0.04 ^a
	D2	1.23 ±0.01 ^a	0.59 ±0.02 ^{abc}	28.80 ±0.23 ^{abc}	6.68 ±0.39 ^{ab}	34.50 ±0.06 ^a
	E2	1.16 ±0.02 ^{bcd}	0.59 ±0.01 ^{abc}	29.08 ±0.41 ^{abc}	6.31 ±0.51 ^{abc}	34.45 ±0.02 ^a
	F1	1.20 ±0.02 ^{abc}	0.61 ±0.05 ^{abc}	29.27 ±0.16 ^a	6.46 ±0.23 ^{ab}	34.47 ±0.07 ^a
	G1	1.19 ±0.01 ^{abcd}	0.58 ±0.01 ^{abcd}	29.35 ±0.12 ^a	5.24 ±0.51 ^d	34.39 ±0.02 ^a
	H1	1.15 ±0.05 ^{cde}	0.64 ±0.01 ^a	29.19 ±0.30 ^{ab}	5.39 ±0.14 ^{cd}	34.30 ±0.03 ^a
	I1	1.21 ±0.01 ^{abc}	0.51 ±0.06 ^{de}	28.77 ±0.18 ^{abc}	6.19 ±0.32 ^{abc}	34.49 ±0.06 ^a
	Golden Delicious	1.19 ±0.02 ^{abcd}	0.54 ±0.06 ^{cde}	27.70 ±0.31 ^d	6.94 ±0.16 ^a	34.34 ±0.04 ^a
	Red Delicious	1.11 ±0.02 ^e	0.63 ±0.04 ^{ab}	27.20 ±0.77 ^d	6.00 ±0.18 ^{bcd}	33.07 ±0.64 ^a
	Granny smith	1.13 ±0.03 ^e	0.34 ±0.03 ^f	25.14 ±0.48 ^e	6.71 ±0.32 ^{ab}	30.72 ±1.68 ^b
Royal Gala	0.93 ±0.03 ^f	0.49 ±0.02 ^e	27.06 ±0.08 ^d	6.14 ±0.63 ^{abcd}	27.60 ±1.88 ^c	

* Values expressed are means ± S.D. of three parallel measurements. GALAE: Galatamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent. Different letters indicated significant differences in each group (pulp or peels) ($p < 0.05$).

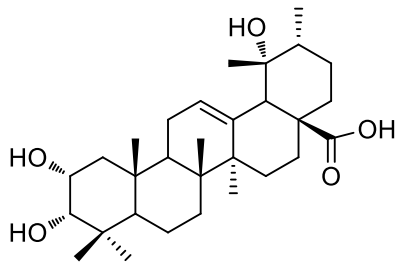
Scheme 1. Structure of main triterpene acid of apple



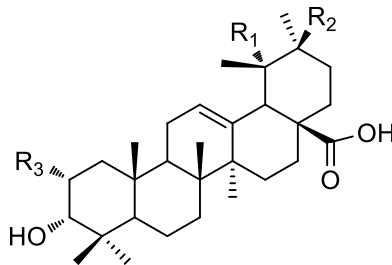
Pomaceic acid



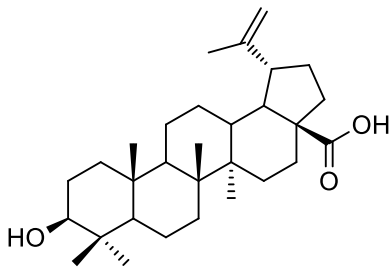
Annurcoic acid



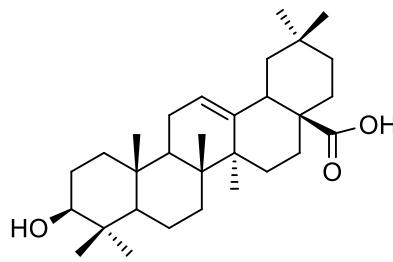
Euscaphic acid



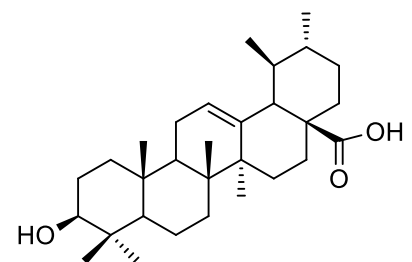
R OH, R H, R H, Pomolic acid
R₁ H, R₂ H, R₃ OH Corosolic acid



Betulinic acid



Oleanolic acid



Ursolic acid

Figure 1. MS Spectra of Pomaceic acid.

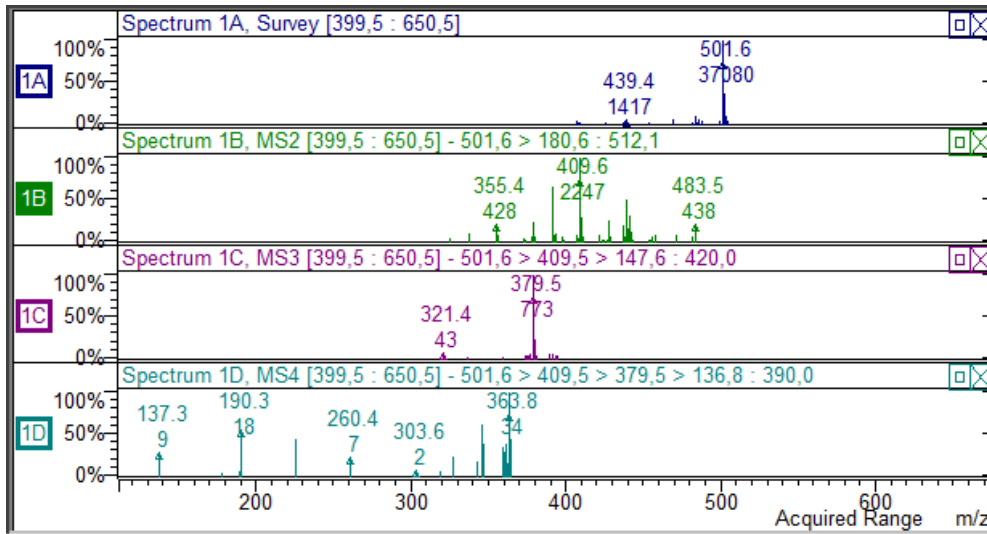


Figure 2. MS Spectra of Euscaphyc acid

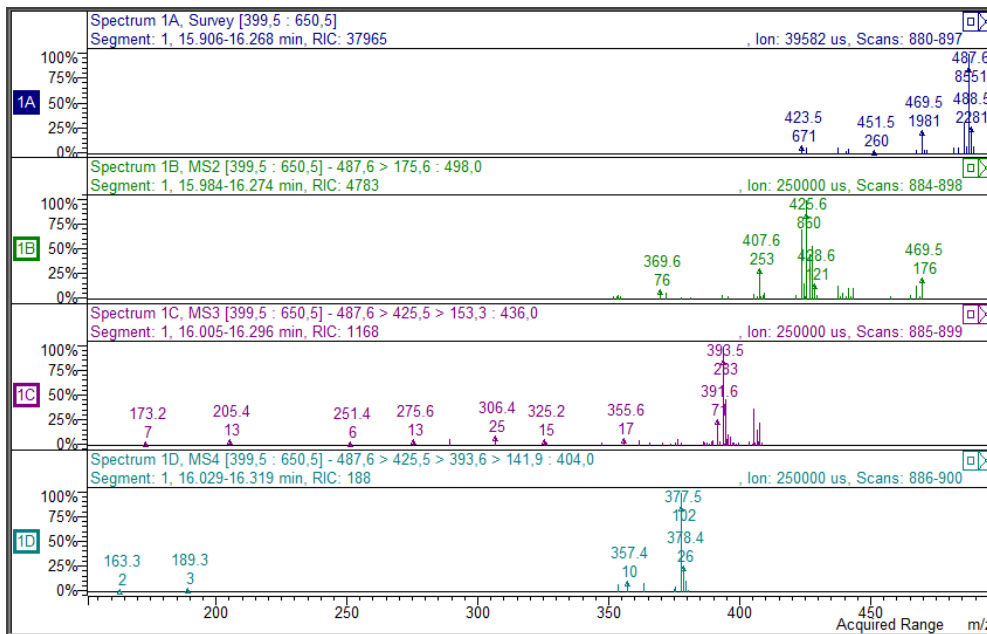


Figure 3. MS Spectra of Annurcoic acid

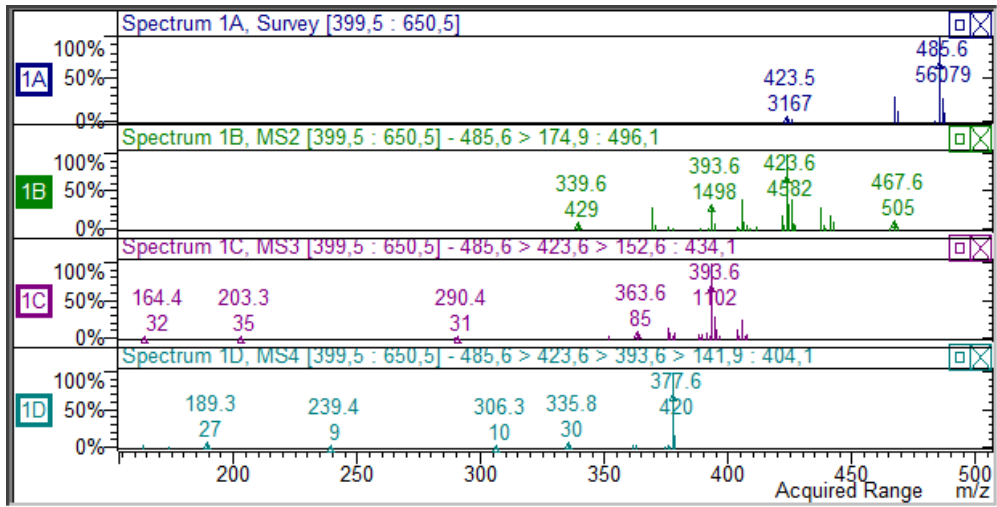


Figure 4. MS Spectra of pomolic acid.

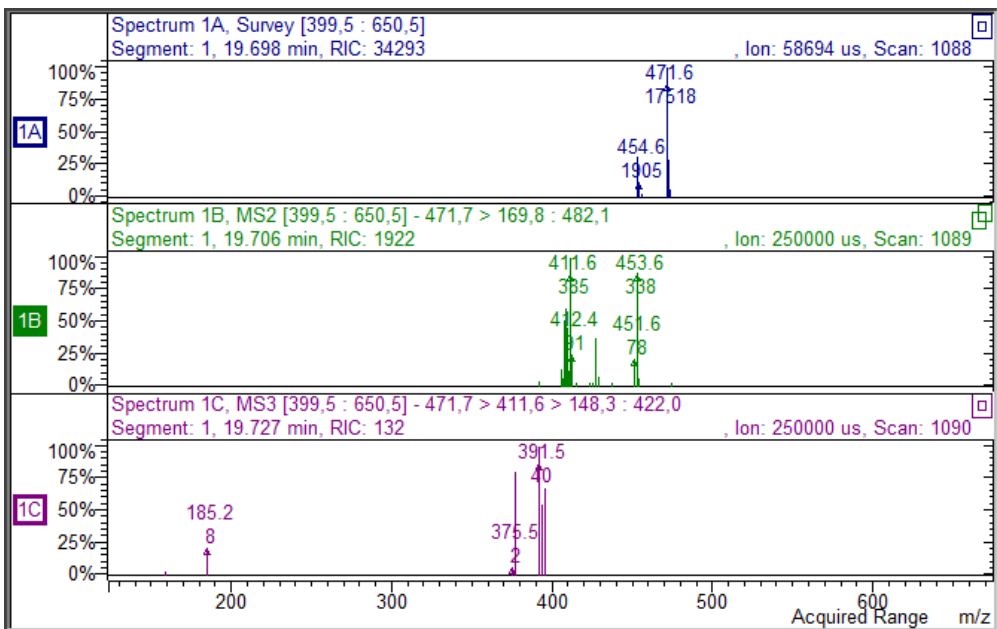


Figure 5. MS Spectra of maslinic acid

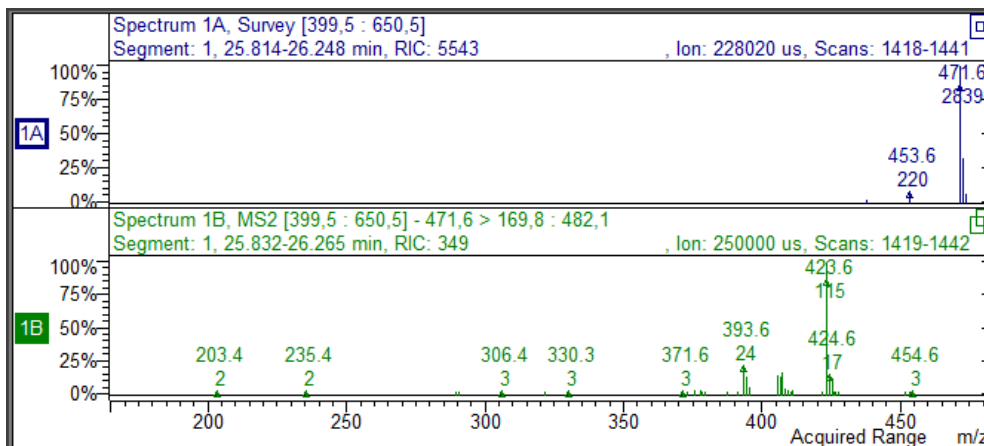


Figure 6. MS Spectra of corosolic acid

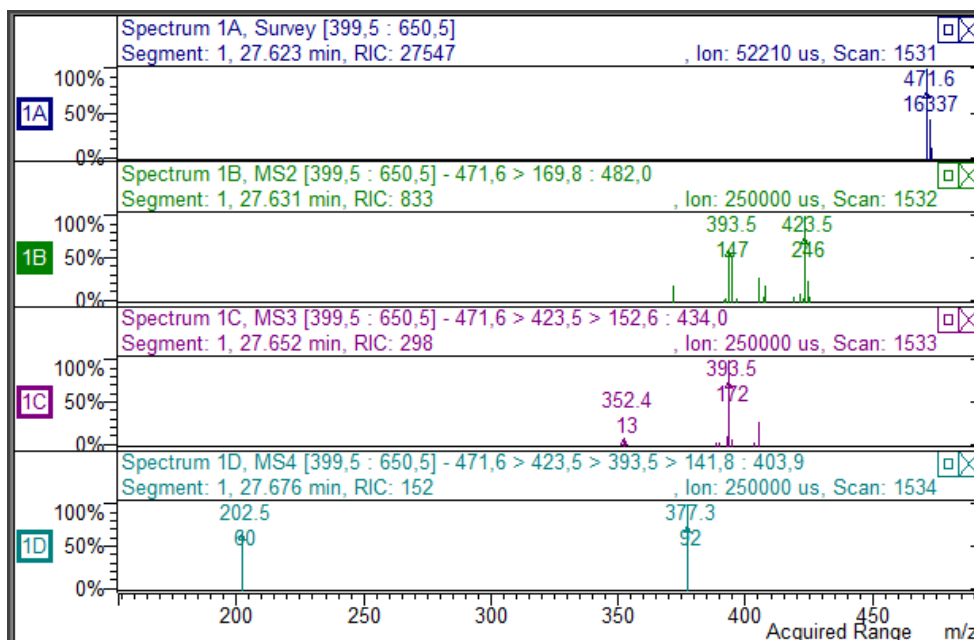


Figure 7. MS Spectra of betulinic acid

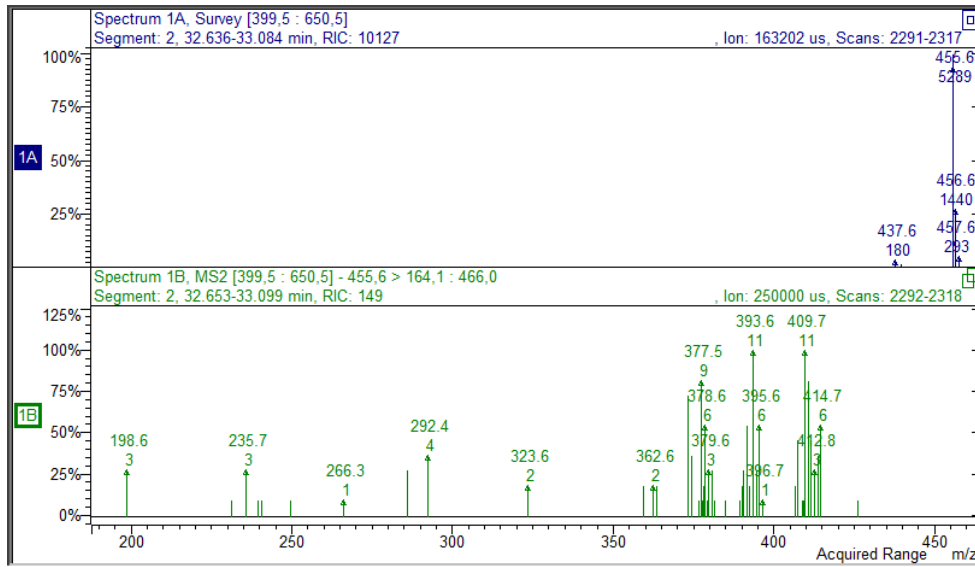


Figure 8. MS Spectra of oleanolic acid

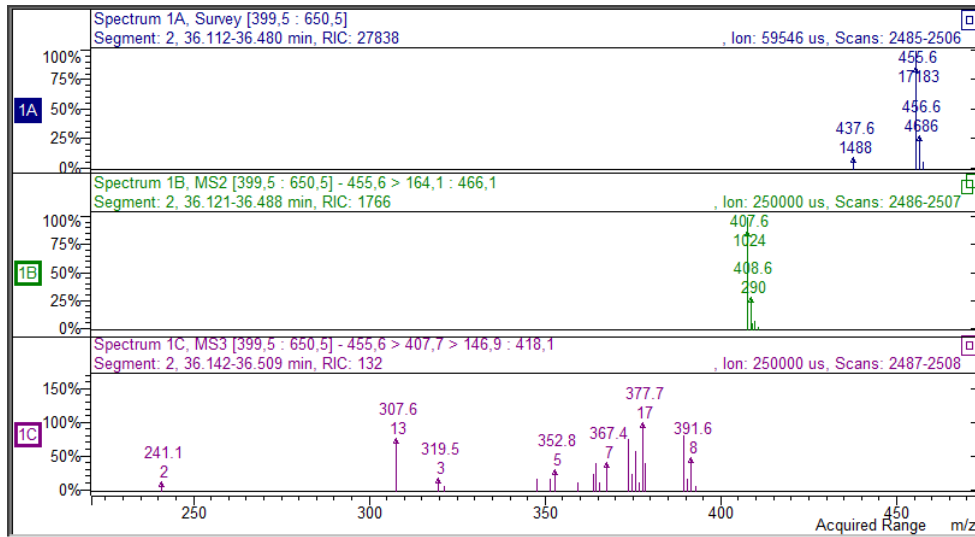


Figure 9. MS Spectra of ursolic acid

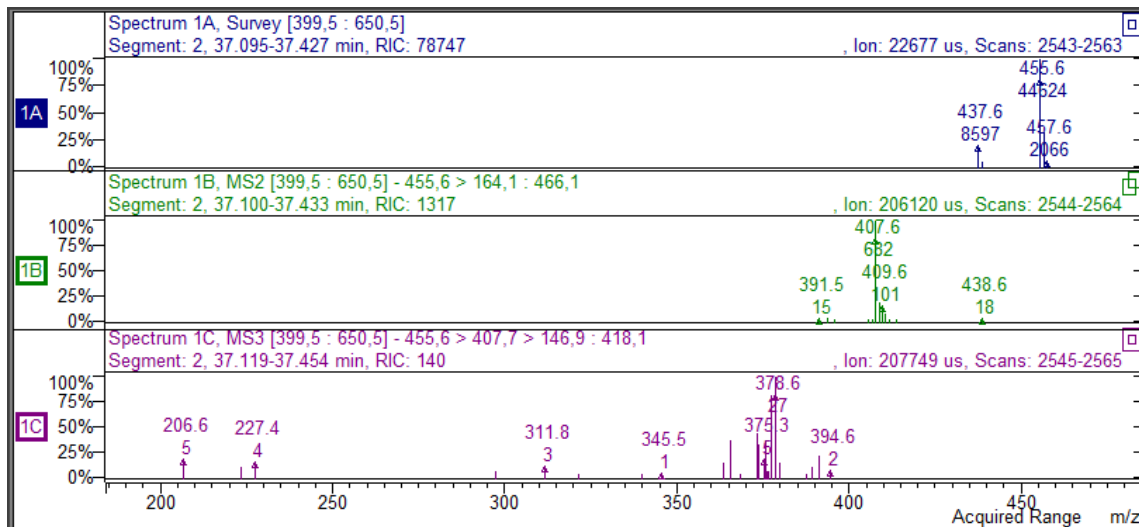
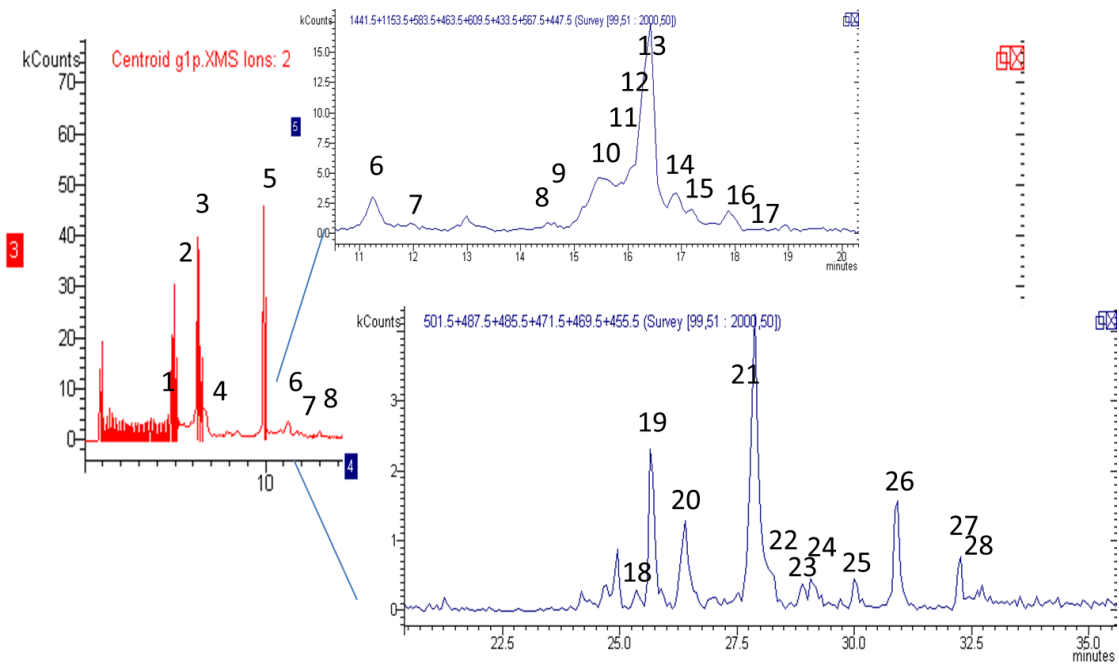
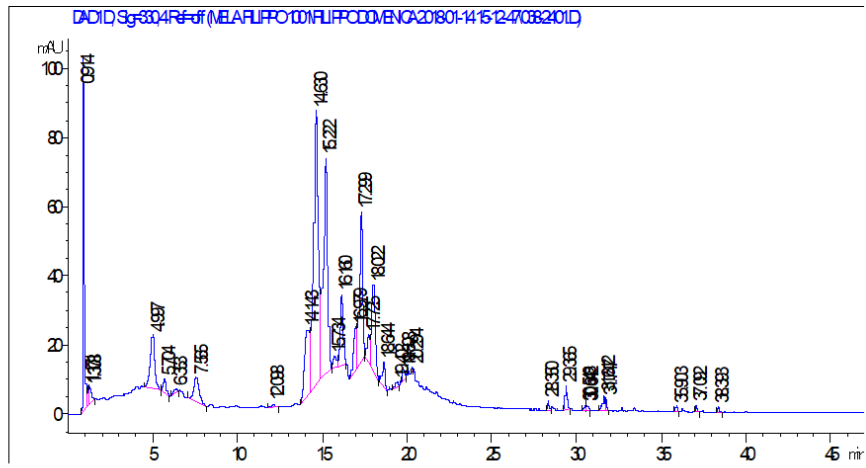
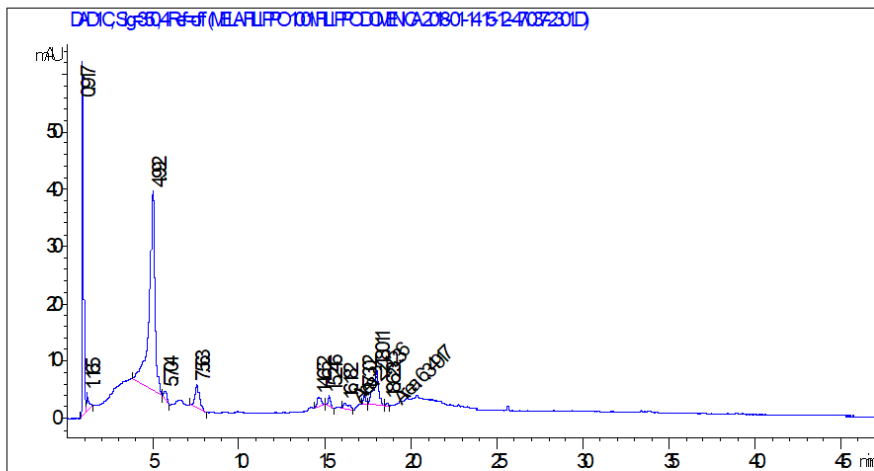


Figure 10. Representative chromatogram of apple peels; the principal classes of phytoconstituents are highlighted; numbers of the identified metabolites are those reported in Table 1.





Peels sample E1 della Rosa



Pulp sample E1 della Rosa