Supplementary materials

Characterization and biological activity of fiber-type *Cannabis sativa* L. aerial parts at different growth stages

Giulia Mastellone [1,†], Arianna Marengo [1,†], Barbara Sgorbini [1], Federica Scaglia [1], Francesca Capetti [1], Francesco Gai [2], Pier Giorgio Peiretti [2], Patrizia Rubiolo [1], Cecilia Cagliero [1,*]

[1] Department of Drug Science and Technology, University of Turin, I-10125, Turin, Italy; giulia.mastellone@unito.it, arianna.marengo@unito.it, barbara.sgorbini@unito.it, federica.scaglia@edu.unito.it, francesca.capetti@unito.it, patrizia.rubiolo@unito.it

[2] Institute of Sciences of Food Production, National Research Council, 10095, Grugliasco, Italy; francesco.gai@ispa.cnr.it, piergiorgio.peiretti@ispa.cnr.it

[†] Both authors contributed equally to this work

[*] Correspondence: cecilia.cagliero@unito.it; Tel.: +39 011 6707133

Table S1. Variables considered in the experimental design (2^n screening study).

Variable	Low value	High value
Sample amount (mg)	100	500
Volume of solvent (mL)	5	50
Water in the solvent (%)	0	50
Extraction time with US (min)	10	30

Table S2. Quality analytical parameters of the HPLC-UV method.

Compound	λ (nm)	Linear range (mg/L)	R^2	Calibration curve equation	Peak quantified
Luteolin-7-*O*-glucuronide	346/252	25-150	0.9989	y = 14487x - 24394	1
Apigenin-7-*O*-glucuronide	336/266	10-350	0.9986	y = 20488x + 47085	2
Apigenin	336/266	0.1-25	0.9989	y = 35166x – 2365.2	9
Diosmetin	343/252	0.1-25	0.9970	y = 37180x - 1940.9	3, 4, 10
Acacetin	267/334	1-25	0.9990	y = 32388x + 9461.5	5, 8
Coumaric acid	310	1-10	0.9950	y = 50388x + 22980	15
CBDA	220/270/310	5-50	0.9999	y = 13839x - 21.947	20, 21, 22, 23, 24, 25, 26, 27

Table S3. Classification of the hemp samples analysed in this study: acronym, growth stage, land plot and drying method.

Sample	Growth stage	Land plot[a]	Drying method
1A_OD	Mid vegetative	A	Oven-drying[b]
2A_OD	Late vegetative	A	Oven-drying
3A_OD	Shooting	A	Oven-drying
4A_OD	Early flower	A	Oven-drying
1B_OD	Mid vegetative	B	Oven-drying
2B_OD	Late vegetative	B	Oven-drying
3B_OD	Shooting	B	Oven-drying
4B_OD	Early flower	B	Oven-drying
1C_OD	Mid vegetative	C	Oven-drying
2C_OD	Late vegetative	C	Oven-drying
3C_OD	Shooting	C	Oven-drying
4C_OD	Early flower	C	Oven-drying
1A_FD	Mid vegetative	A	Freeze-drying[c]
2A_FD	Late vegetative	A	Freeze-drying
3A_FD	Shooting	A	Freeze-drying
4A_FD	Early flower	A	Freeze-drying
1B_FD	Mid vegetative	B	Freeze-drying
2B_FD	Late vegetative	B	Freeze-drying
3B_FD	Shooting	B	Freeze-drying
4B_FD	Early flower	B	Freeze-drying
1C_FD	Mid vegetative	C	Freeze-drying
2C_FD	Late vegetative	C	Freeze-drying
3C_FD	Shooting	C	Freeze-drying
4C_FD	Early flower	C	Freeze-drying

[a] 2 m^2 subplots randomly located in 2×12 m^2 plot

[b] Forced-draft oven to constant weight at 65°C

[c] Lyophilizer

Table S4. Parameters of the *in-vitro* tyrosinase inhibitory assay.

Sample	Slope	R^2	Calibration curve equation
Negative control (no extract)	0.052	0.999	y = 0.052x - 0.0172
Positive control (kojic acid)	0.025	0.999	y = 0.0255x - 0.0522
MeOH hemp extract	0.035	1	y = 0.0348x + 0.0241
Acetone hemp extract	0.032	1	y = 0.032x + 0.0345

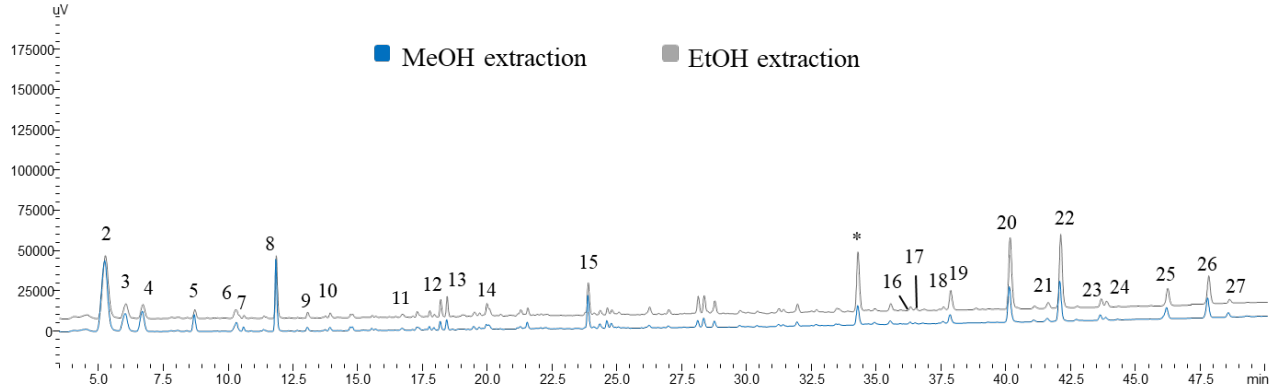
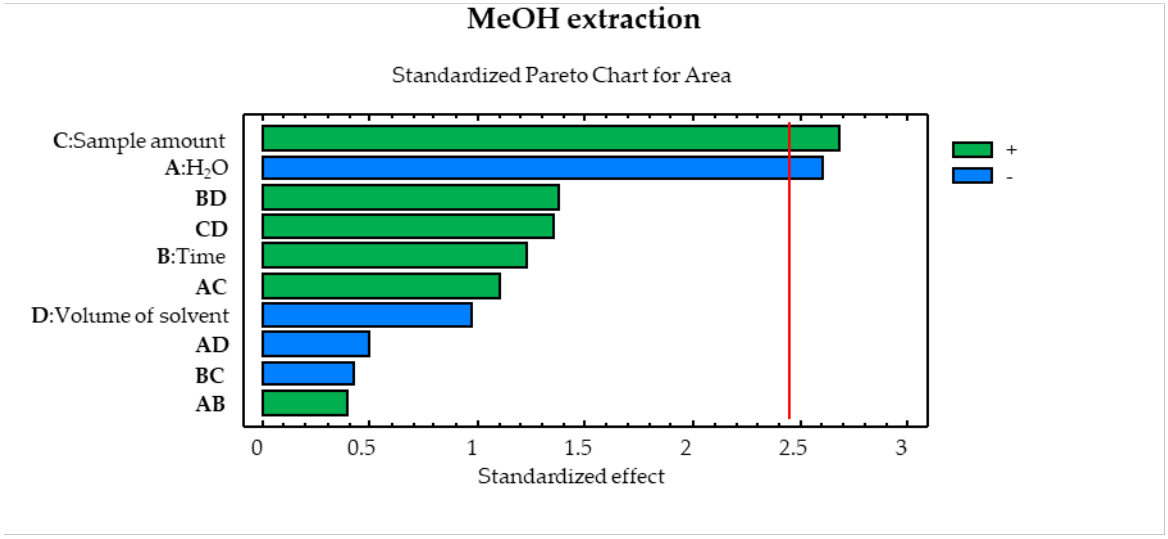
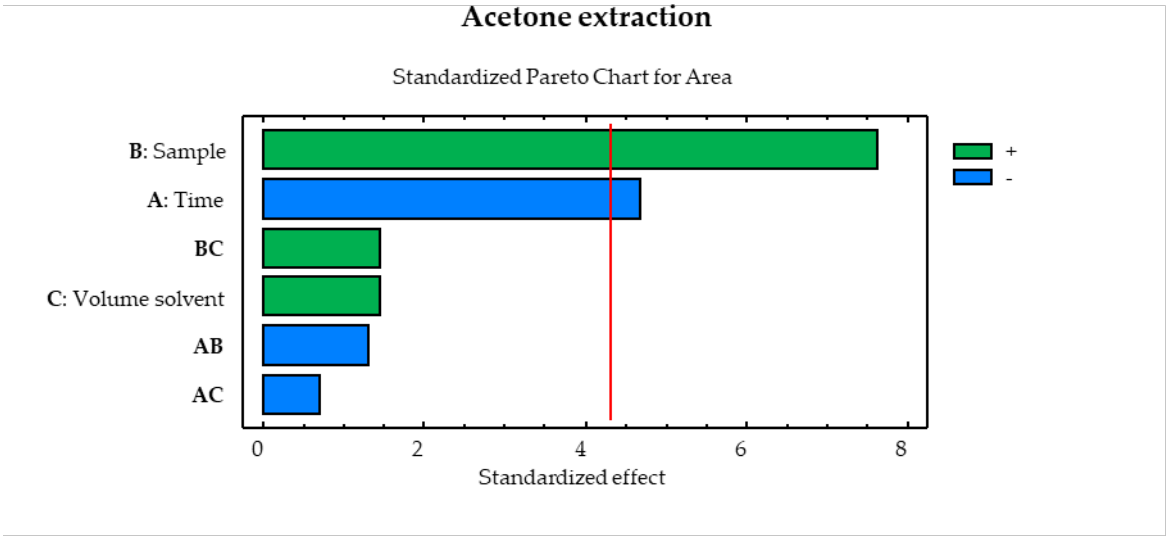


Figure S1. Chromatographic profile at 254 nm of methanolic and ethanolic extraction of fiber-type *Cannabis sativa* L. aerial parts at 5 mg/mL. For peaks identification, see Table 1.

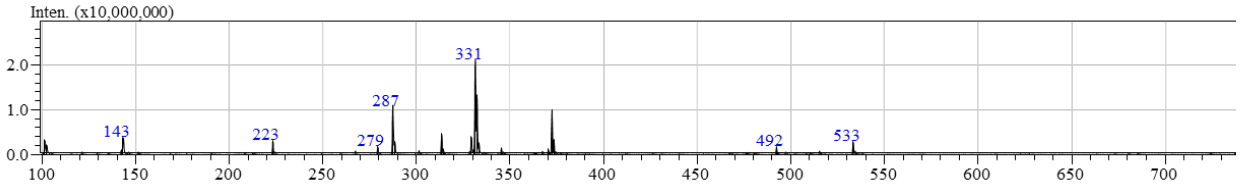


(**a**)

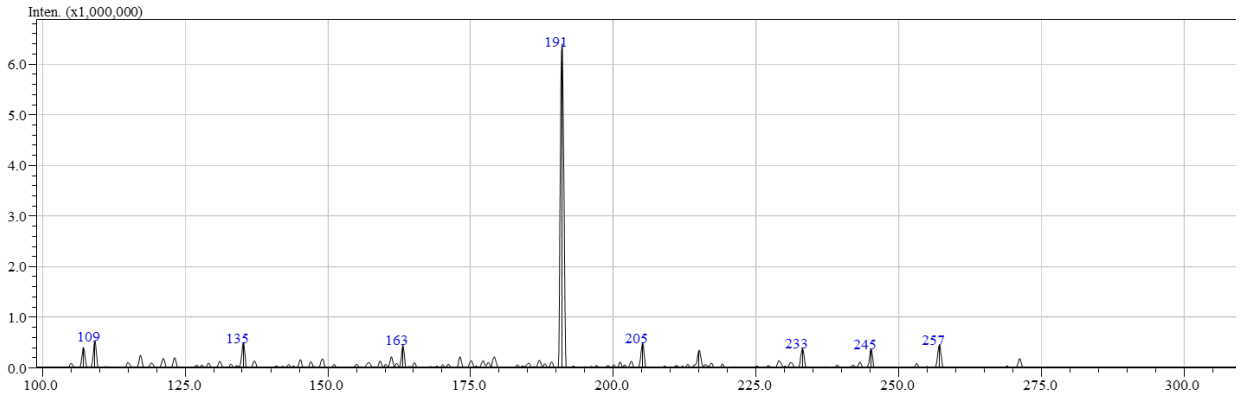


(**b**)

Figure S2. Pareto charts obtained in the screening of the main variables for the methanolic (a) and acetone extraction (b).

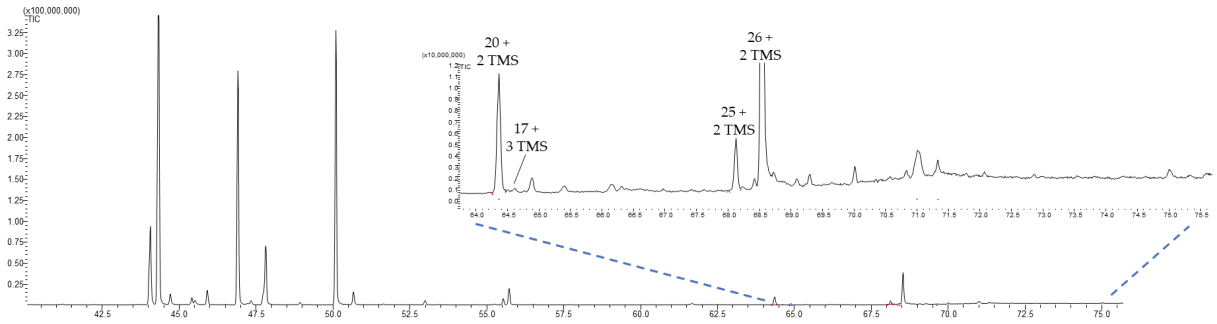


(**a**)

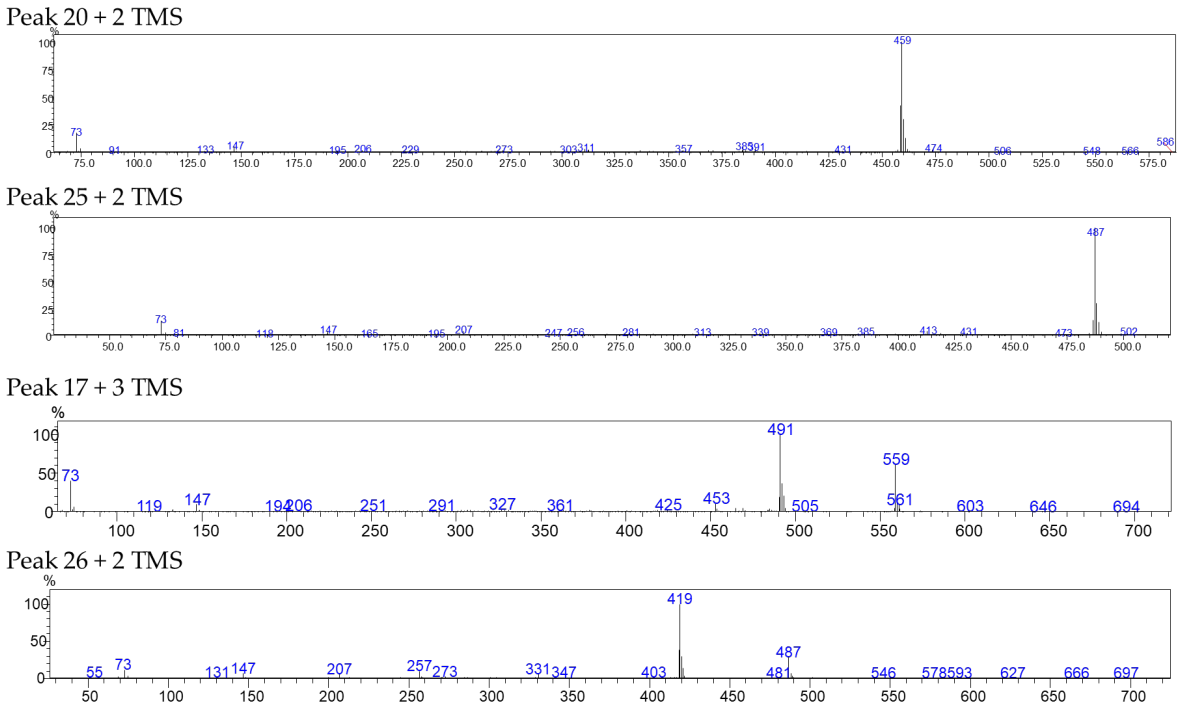


(**b**)

Figure S3. Representative MS spectrum of peak 21 in ESI+ (a) and PIS fragmentation (b) of pseudomolecular ion 331m/z (putatively identified as varinic acid derivative according to [32]).

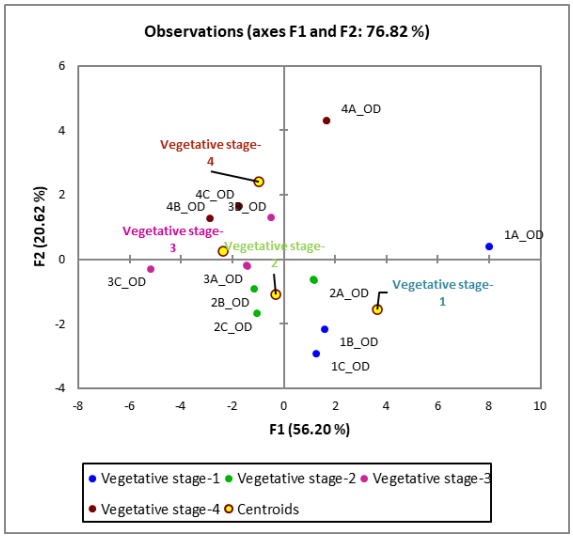


(a)

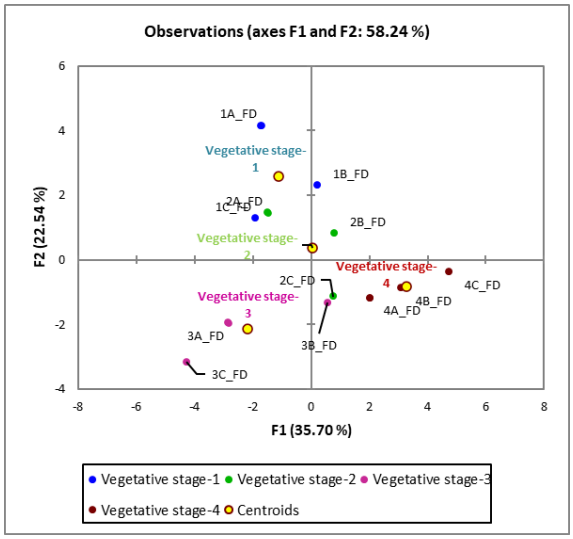


(**b**)

Figure S4. GC-MS profile of the FD acetone hemp extract, after derivatization with BSTFA (a) and MS spectra of selected peak in the hemp extract (b). TMS (trimethylsilyl). See table 1 for peak numbers.



(a)



(b)

Figure S5. PCA elaboration of oven-dried (a) and freeze-dried (b) hemp samples, according to the growth stages.

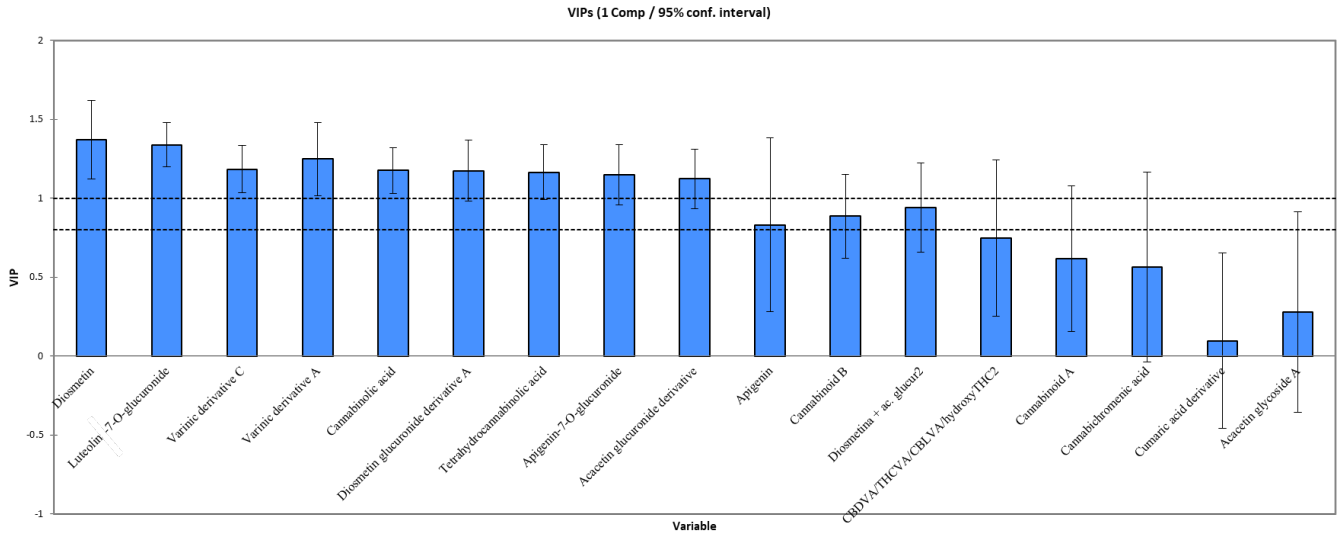
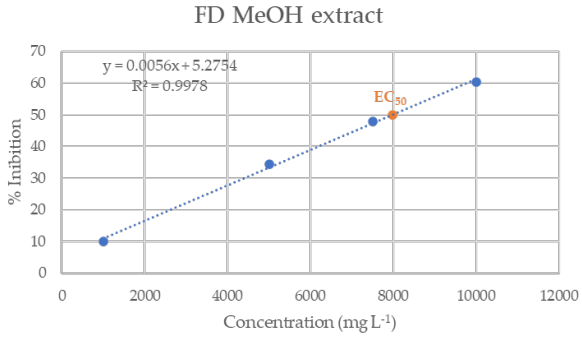
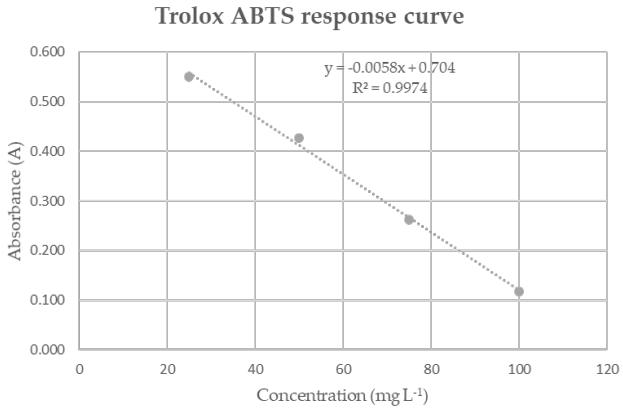


Figure S6. Variables importance in the projection (VIP) for the discrimination of oven-dried and freeze-dried samples.

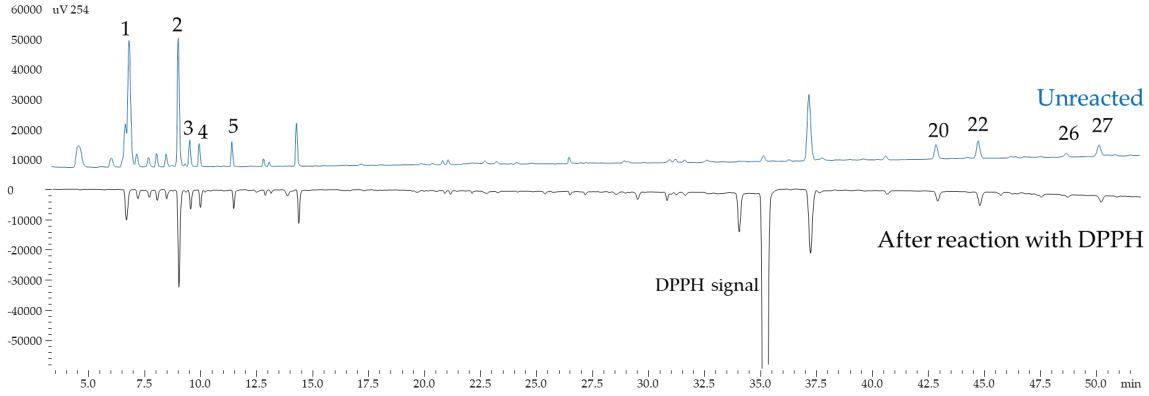


(a)

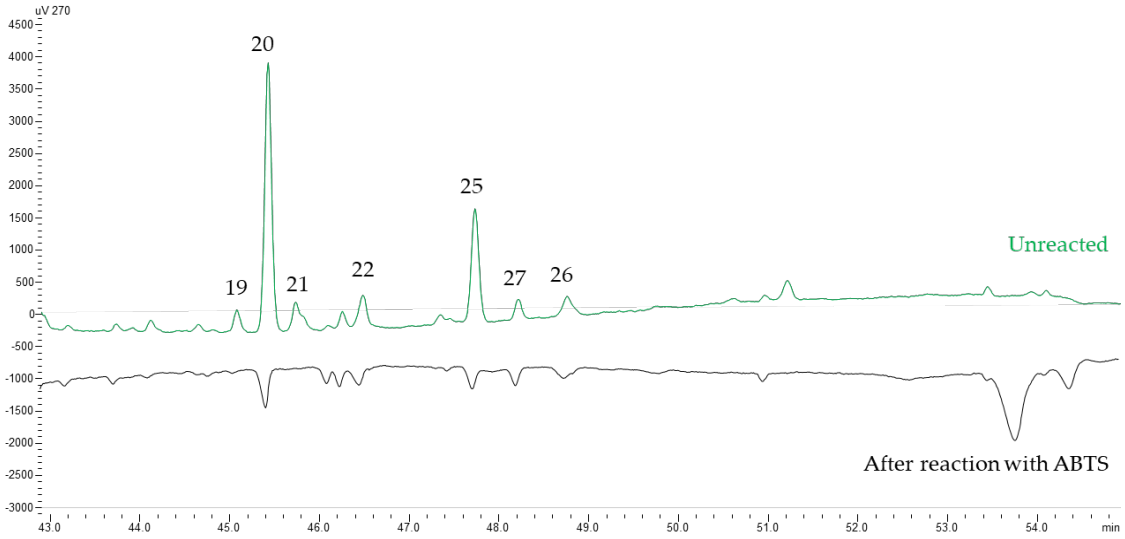


(**b**)

Figure S7. Representative response curve of FD MeOH extract (a) and Trolox (b), used to measure the scavenging effect on DPPH• and $ABTS^{+\bullet}$ radicals, respectively.



(a)



(b)

Figure S8. Representative chromatogram of FD MeOH hemp profile before and after the reaction with DPPH$^{\bullet}$ radicals on C18 column (a) and FD acetone hemp profile before and after the reaction with $ABTS^{+\bullet}$ on RP-Amide column (b).