Supplementary Information

Multistep Approach Points to Compounds Responsible for the Biological Activity and Safety of Hydrolates from Nine *Lamiaceae* Medicinal Plants on Human Skin Fibroblasts

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Table S1: Percentage of volatile organic compounds in hydrolates

Hydrolate	% of VOC content*
(1) MP - *M. piperita*	0.04 ± 0.01
(2) RO - *R. officinalis*	0.05 ± 0.02
(3) LO - *L. officinalis*	0.05 ± 0.02
(4) TV - *T. vulgaris*	0.11 ± 0.01
(5) SS - *S. sclarea*	0.04± 0.01
(6) SM - *S. montana* ssp. *variegata*	0.10 ± 0.02
(7) LI - *L. intermedia*	0.07 ± 0.02
(8) OV - *O. vulgare*	0.09 ± 0.03
(9) MO - *M. officinalis*	0.06 ± 0.01

*Values represent average value of triplicates ± SD.

Table S2. Correlation matrix for antioxidative properties by DPPH and ABTS+ test and total phenolic content of nine hydrolates

Correlation Pearson r	DPPH	ABTS+	TPC	Correlation P values	DPPH	ABTS+	TPC
DPPH		0.927	0.960	DPPH	0.0003	0.0003	0.0004
ABTS+	0.927		0.952	ABTS+			0.0007
TPC	0.960	0.952		TPC	0.00004	0.0001	

Legend: r – Pearson correlation coefficient; ABTS+ - antioxidative test; DPPH - 2,2-diphenyl-1-picrylhydrazyl antioxidative test; TPC – total phenolic content determination.

Table S3. Protein and peptide concentration determination via micro and macro method of bicinchoninic acid (BCA) assay

Method	Standard curve with bovine serum albumin as standard (mg/mL)	Sample/Hydrolate	SpeedVac concentrated hydrolates samples (Fig. 1) (mg/mL)	TCA/acetone precipitated samples (Fig. S2) (mg/mL)
	Blank (zero)	MP - *M. piperita*	as blank	as blank
micro	0.0025	RO - *R. officinalis*	as blank	as blank
	0.005	LO - *L. officinalis*	as blank	as blank
	0.01	TV - *T. vulgaris*	as blank	as blank
	0.02	SS - *S. sclarea*	as blank	as blank
macro	0.04	SM - *S. montana* ssp	as blank	as blank
	0.08	LI - *L. intermedia*	as blank	as blank
	1.20	OV - *O. vulgare*	as blank	as blank
	1.60	MO - *M. officinalis*	as blank	as blank
		Ambrosia extract 2x diluted	N/A	1.25

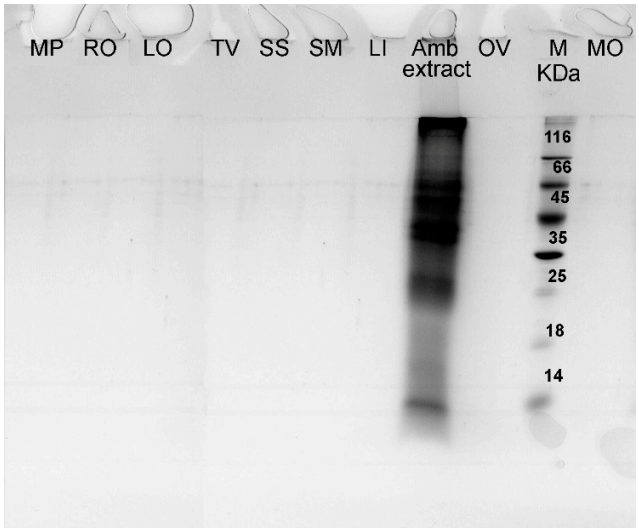

Figure S1. Polyacrylamide gel with samples obtained by TCA/acetone protein precipitation method from nine hydrolates. Electrophoretically resolved sodium dodecyl sulphate polyacrylamide gel (16%), with 10 mL of hydrolates precipitated with 40 mL 13% TCA/acetone overnight at -20 °C vacuum) per protocol described by Sheoran et al. [48]. Tentative pellet was mixed with 20 µL of 1 × Laemmli buffer in denaturing conditions, stained with CBB 250-R. Protein markers were the quantitative type of control, while protein extract obtained in house from the Ambrosia artemissfolia pollen, was used as a positive protein control (10 µL of 2,5 mg/mL). *M. piperita* (MP), *R. officinalis* (RO), *L. officinalis* (LO), *T. vulgaris* (TV), *S. sclarea* (SS), *S. montana* ssp. *variegata* (SM), *L. intermedia* (LI), *O. vulgare* (OV) and *M. officinalis* (MO), M – protein weight markers in kilo Daltons (kDa).

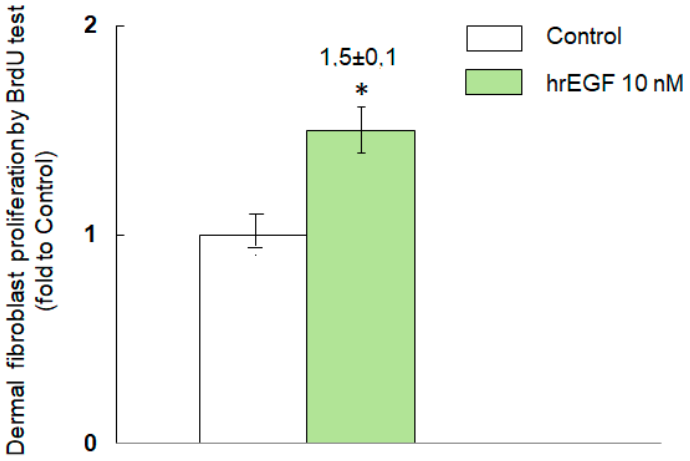

Figure S2. Proliferation of primary human dermal fibroblasts treated with 10 nM recombinant human epidermal growth factor (hrEGF) during 48 h, assessed through DNA synthesis via BrdU testing. *denotes significantly different proliferation at $p < 0.05$, compared to the control group.

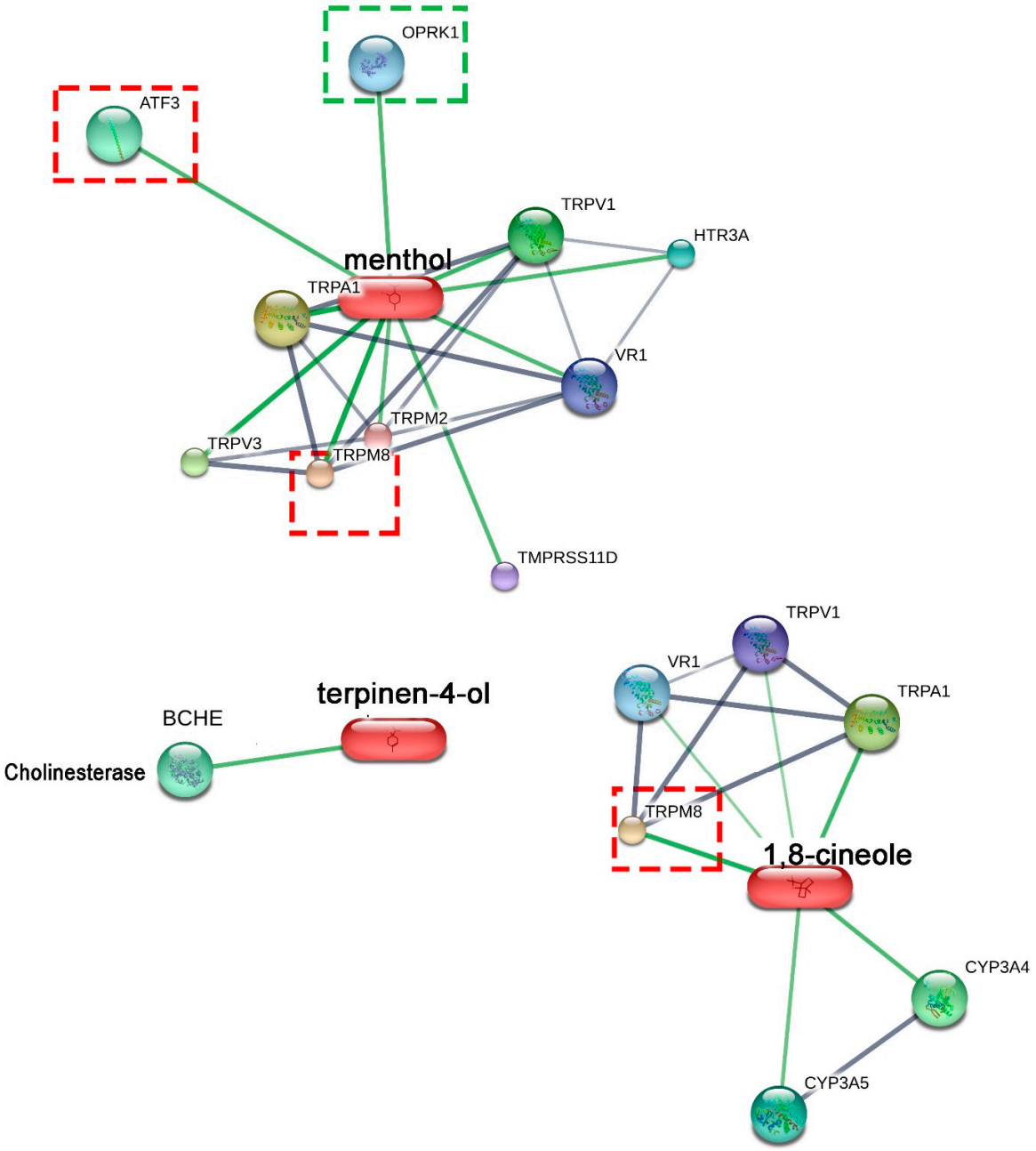

Figure S3. The confidence view of protein networking of menthol, terpinene-4-ol and 1,8-cineole (representatives of the major VOC from hydrolates that exert negative viability effect), done by STITCH database http://stitch.embl.de/. Only first-line protein targets of chemical compounds are shown with the host species of *Homo sapiens*.

References

Sheoran, I.; Ross, A.; Olson, D.; Sawhney, V. Compatibility of plant protein extraction methods with mass spectrometry for proteome analysis. *Plant Science* **2009**, *176*, 99-104, doi:10.1016/j.plantsci.2008.09.015.