

Supplemental material

The hempseed (*C. sativa*) peptides WVSPLAGRT and IGFLIIWV exert anti-inflammatory activity in LPS stimulated human hepatic cell line

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Reagents

Dulbecco's modified Eagle's medium (DMEM), L-glutamine, fetal bovine serum (FBS), phosphate buffered saline (PBS), penicillin/streptomycin, chemiluminescent reagent, and 24 or 96-well plates were purchased from Euroclone (Milan, Italy). Bovine serum albumin (BSA), RIPA buffer was bought from Sigma-Aldrich (St. Louis, MO, USA). Phenylmethanesulfonyl fluoride (PMSF), Na-orthovanadate inhibitors were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). The inhibitor cocktail Complete Midi from Roche (Basel, Swiss). Mini protean TGX pre-cast gel 7.5%, and Mini nitrocellulose Transfer Packs were purchased from BioRad (Hercules, CA, USA).

A

Sample Name :H2
 Sample ID :U7849GG260-7
 Time Processed :2:30:08 AM
 Month-Day-Year Processed :08/06/2021

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
 Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
 Total Flow: 1 ml/min
 Wavelength: 220 nm

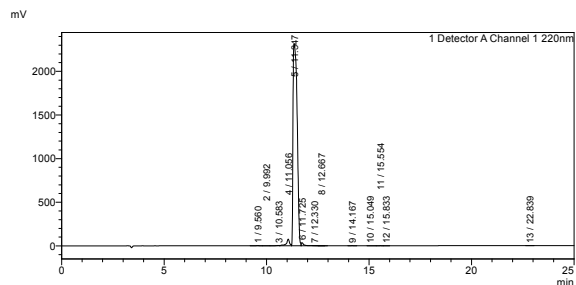
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	B Conc	5
25.00	Pumps	B Conc	65
25.01	Pumps	B Conc	95
27.00	Pumps	B Conc	95
27.01	Pumps	B Conc	5
35.00	Pumps	B Conc	5
35.01	Controller	Stop	

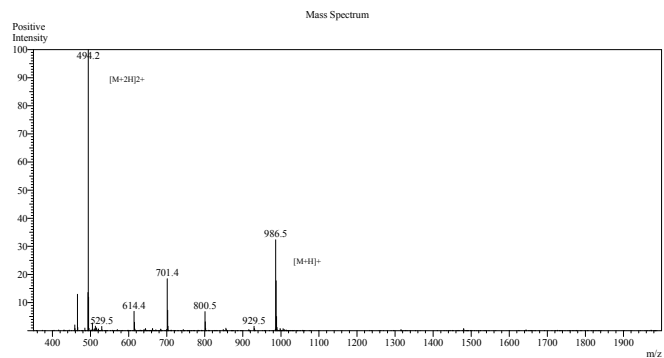
<<Column Performance>>

<Detector A>
 Column : Inertsil ODS-3 4.6 x 250 mm
 Equipment: ZJ21010026

<Chromatogram>



C



Sample Information	08/05/21	Interface	ESI	Equipment	:ZJ21010035
Month-Day Processed:	18:52:35	Nebulizing Gas Flow	:1.5L/min	Interface Bias	:+4.5 kV
Time Processed:	0.2	CDI Temp	:200	Drying Gas Flow	:3.4 L/min
Injection Volume:	0.2	Block Temp	:200	T Flow	:0.2 ml/min
Sample Name:	H2			B conc	:50%H2O/50%MeOH
Sample ID:	U7849GG260-7				
Theoretical MW:	986.13				
Observed MW:	986.4				

B

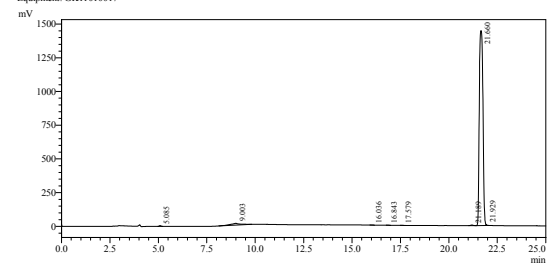
Sample Name :H3
 Sample ID :U7849GG260-4
 Time Processed :13:11:43
 Month-Day-Year Processed :08/15/2021

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
 Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
 Total Flow: 1 ml/min
 Wavelength: 220 nm

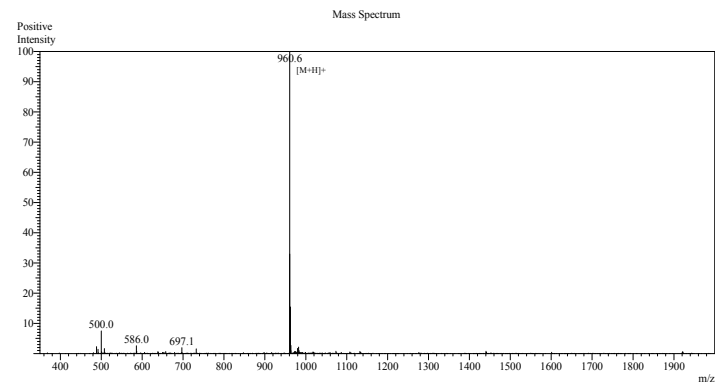
Time	Module	Command	Value
0.01	Pumps	Pump A.B Conc	0
25.00	Pumps	Pump A.B Conc	50
25.01	Pumps	Pump A.B Conc	95
32.00	Pumps	Pump A.B Conc	95
32.01	Pumps	Pump A.B Conc	0
40.00	Pumps	Pump A.B Conc	0
45.00	Controller	Stop	

<<Column Performance>>

<Detector A>
 Column : Inertsil ODS-3 4.6 x 250 mm
 Equipment: GK11010017



D



Sample Information	08/12/21	Interface	ESI	Equipment	:ZJ21010035
Month-Day Processed:	9:55:15	Nebulizing Gas Flow	:1.5L/min	Interface Bias	:+4.5 kV
Time Processed:	0.2	CDI Temp	:250	Drying Gas Flow	:3 L/min
Injection Volume:	0.2	Block Temp	:200	T Flow	:0.2 ml/min
Sample Name:	H3			B conc	:50%H2O/50%MeOH
Sample ID:	U7849GG260-4				
Theoretical MW:	960.22				
Observed MW:	959.6				

Figure S1: Chromatogram and Mass Spectrum of H2 (A and C) and H3 (B and D)

Table S1. Antibodies used in the Western Blot assays.

Name product	Dilution	Supplier
Anti-iNOS	1:1000	Cell Signaling Technology (D6B6S)
Anti-NF-kappaB, p65 subunit, active subunit, clone 12H11	1:1000	Sigma-Aldrich (MAB3026)
Anti-phospho-NF-kappaB p65 (pSer276)	1:1000	Sigma-Aldrich (SAB4504488)
Anti- β -actin	1:16000	Sigma-Aldrich (A5441)

Name of antibody, dilution used, and supplier. iNOS, inducible nitric oxide synthase; NF-kappaB, nuclear factor- κ B.

Table S2. Absolute values of cytokine production.

Cytokine	C	LPS	H2 [100 μM]	C	LPS	H3 [25 μM]
IFN- γ (pg/mL)	25.50 \pm 5.04	34.12 \pm 5.94	26.00 \pm 1.41	28.28 \pm 1.28	30.35 \pm 0.35	26.50 \pm 0.58
TNF (pg/mL)	33.71 \pm 1.21	38.00 \pm 2.94	32.00 \pm 1.41	33.89 \pm 0.75	36.68 \pm 0.43	29.83 \pm 1.17
IL-6 (ng/mL)	1329 \pm 8.60	1448 \pm 1.23	1470 \pm 5.02	603.8 \pm 1.71	672.5 \pm 2.38	584.8 \pm 19.16
IL-10 (pg/mL)	21.68 \pm 0.22	20.98 \pm 1.32	23.25 \pm 0.13	48.90 \pm 0.36	49.74 \pm 1.43	61.88 \pm 0.17

Mean \pm standard deviation (SD) of the cytokines production. C, unstimulated control group; LPS, lipopolysaccharide-stimulated cells; H, hempseed peptide; IFN- γ , interferon- γ ; TNF, tumor necrosis factor; IL, interleukin.