Electronic Supplementary Material (ESI) for Materials Advances.

Keratin-cinnamon essential oil biocomposite fibrous patches for skin burns care

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Supporting Information

S1. Molecular weight analysis of poly(*N*-vinyl pyrrolidone) (PVP)

Gel Permeation Chromatography (GPC). PVP (17.20 mg) was dissolved in 9 mL of *N*,*N*-dimethylformamide (DMF) containing or not 0.1 % (w/v) lithium bromide (LiBr) and the solution filtered through a 0.22 µm PVDF filter. GPC was performed on an integrated OMNISEC system (Malvern Panalytical Ltd., Malvern, UK) equipped with a D6000M and a D3000 column (10 and 6 µm particle size respectively, 300 x 8 mm) using triple detection. DMF containing or not 0.1% LiBr was used as an eluent at a temperature of 50 °C and a flow rate of 1.0 mL/min. The system was calibrated with Poly(methyl methacrylate) (PMMA) (PolyCal standards, Malvern Panalytic, UK) 50 kDa narrow standard and verified with a 90 kDa broad standard of known dispersity, intrinsic viscosity and dn/dc. Data analysis was performed using OMNISEC software V10.

AF4. The protocol is described in the main text.

Table S1. Summary of PVP analysis by GPC in organic solvent and AF4 in water.

	GPC[a]	**AF4[b]**
Number average molecular weight ($\bar{M}_n$, kDa)	349	170
Weight average molecular weight ($\bar{M}_w$, kDa)	1'155	650
Z average molecular weight ($\bar{M}_z$, kDa)	2'630	1'412
Đ[c]	3.3	3.8
Intrinsic viscosity [η] (dL/g)	2.1	
Weight average hydrodynamic radius (nm)	30	
Weight average radius of gyration (nm)	25	29
Mark-Houwink parameter *a*	0.35	
Mark-Houwink parameter *logK* (dL/g)	-1.65	
dn/dc	0.071[d]	0.174[e]
Fractal dimension		1.78[f]

[a] Eluent: DMF + 0.1% (w/v) LiBr

[b] Eluent: 7 mM SDS, 0.02% (w/v) NaN_3 in water

[c] Dispersity index Đ = $\bar{M}_w$ / $\bar{M}_n$

[d] Calculated from the injection considering 100% mass recovery

[e] Literature value (DOI: 10.1016/j.ab.2004.06.010)

[f] This value lays in between the value expected for a random coil (i.e. Fractal dimension = 2) and that of and extended coil (i.e. Fractal dimension = 1.67)

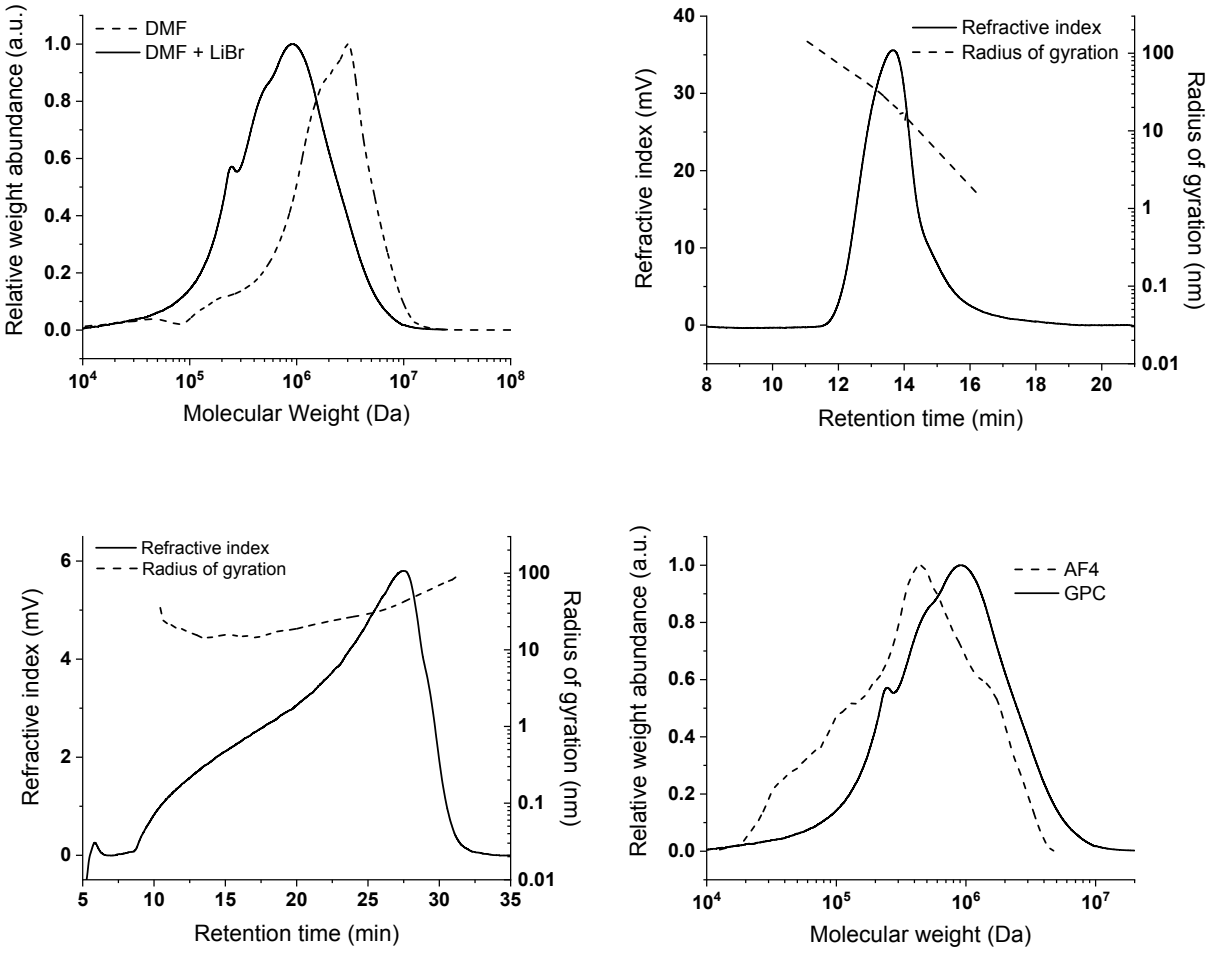

Figure S1: *Top left:* comparison of PVP molecular weight distribution obtained by GPC injecting the sample in DMF with (solid line) or without (dashed line) 0.1 % (w/v) LiBr. The shift of the distribution to lower molecular weights indicates that the salt minimised aggregation in solution, thus any further analysis was performed on samples eluted with DMF supplemented with 0.1% (w/v) LiBr. *Top right:* GPC chromatogram showing the signal of the concentration detector (refractive index) and the values of radius of gyration calculated from the light scattering detector (dashed line). *Bottom left:* AF4 elugram showing the signal of the concentration detector (refractive index) and the relative values of radius of gyration calculated from the light scattering detector (dashed line). *Bottom right:* comparison of the molecular weight distribution obtained by GPC (solid line) and AF4 (dashed line) analysis.

S2. AF4 analysis of fibre composition

An AF4 method for the simultaneous analysis of PVP and keratin was developed and applied first to the individual compounds, in order to build the respective calibration curves using the two concentration detectors, i.e. refractive index (RI) and UV-Vis at 280 nm. For PVP, the integral of the RI signal was calculated between 25 and 30 min and plotted against the sample concentration (Figure S2, *left*). For keratin, the integral of the UV signal was integrated between 7 and 35 min and plotted against the sample concentration (Figure S2, *right*). It is noteworthy to mention that PVP does not absorb significantly at 280 nm, see Figure S2, *top left*) thus allowing for a selective detection of the protein.

Table S2. PVP/keratin (KER) fibre analysis by AF4 from the curves shown in Figure S3

Theoretical PVP/KER mass ratio	Measured PVP/KER mass ratio	Theoretical concentration (mg/mL)		Measured concentration (mg/mL)		Measured/theoretical concentration (%)	
		PVP	KER	PVP	KER	PVP	KER
1.00	1.09 ± 0.04	5.00	5.00	5.67 ± 0.14	5.18 ± 0.11	113 ± 3	103 ± 2
2.00	2.24 ± 0.07	6.67	3.33	5.97 ± 0.14	2.67 ± 0.06	90 ± 2	80 ± 2
3.00	3.36 ± 0.11	7.50	2.50	9.66 ± 0.23	2.88 ± 0.06	129 ± 3	115 ± 2

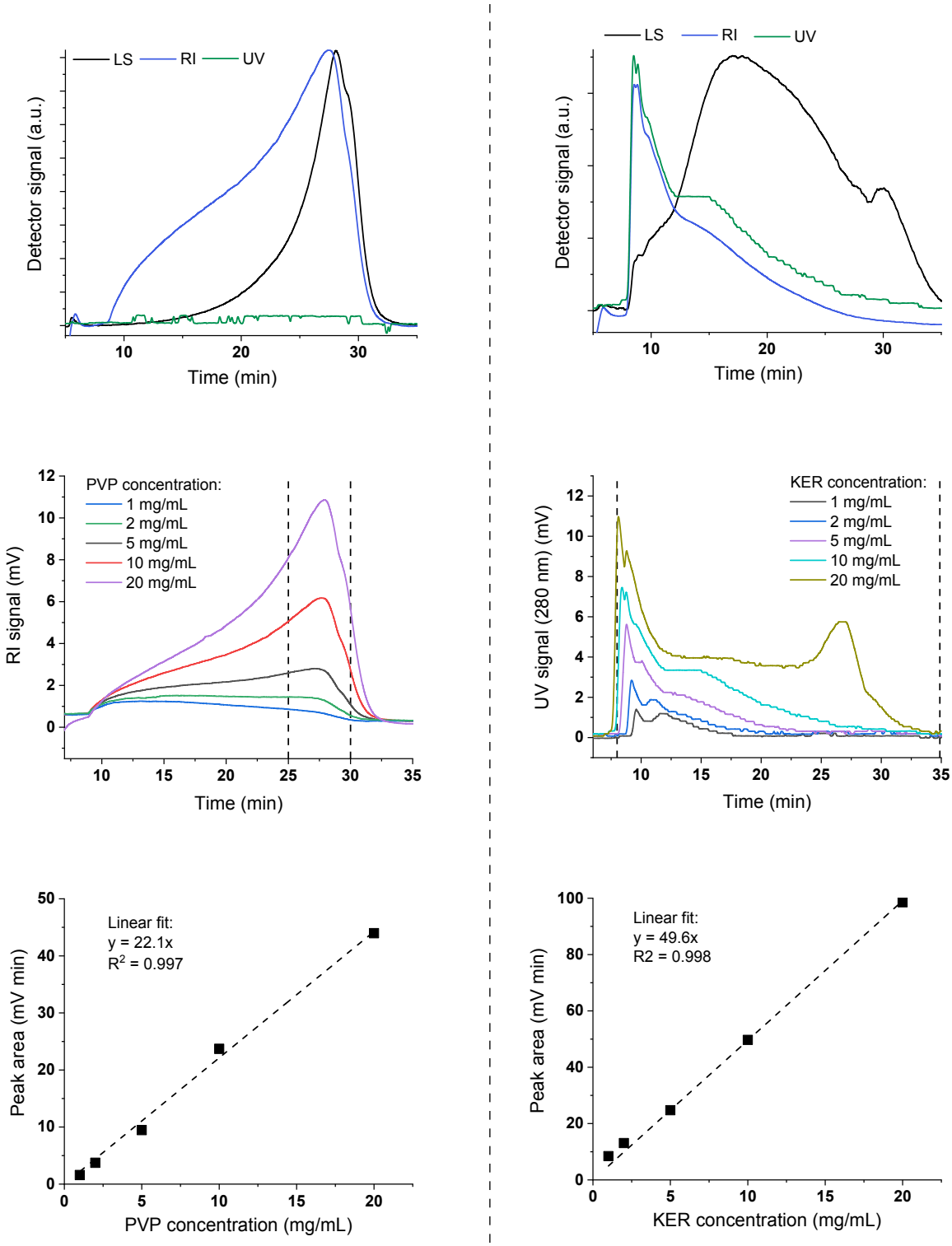

Figure S2. AF4 analysis of PVP (*left*) and keratin (*right*). *Top:* detector signals against elution time. Detectors: static light scattering at 90° (LS), refractive index (RI) and UV-Vis at 280 nm. *Centre:* Elugrams obtained injecting a series of concentration of PVP (*left*) and keratin (KER, *right*), the signals used for building the calibration curves are reported (i.e. RI for PVP and UV for

keratin). The dashed line marks the limits of the signal integration used for the calibration. *Bottom*: calibration for PVP (*left*) and keratin (*right*)

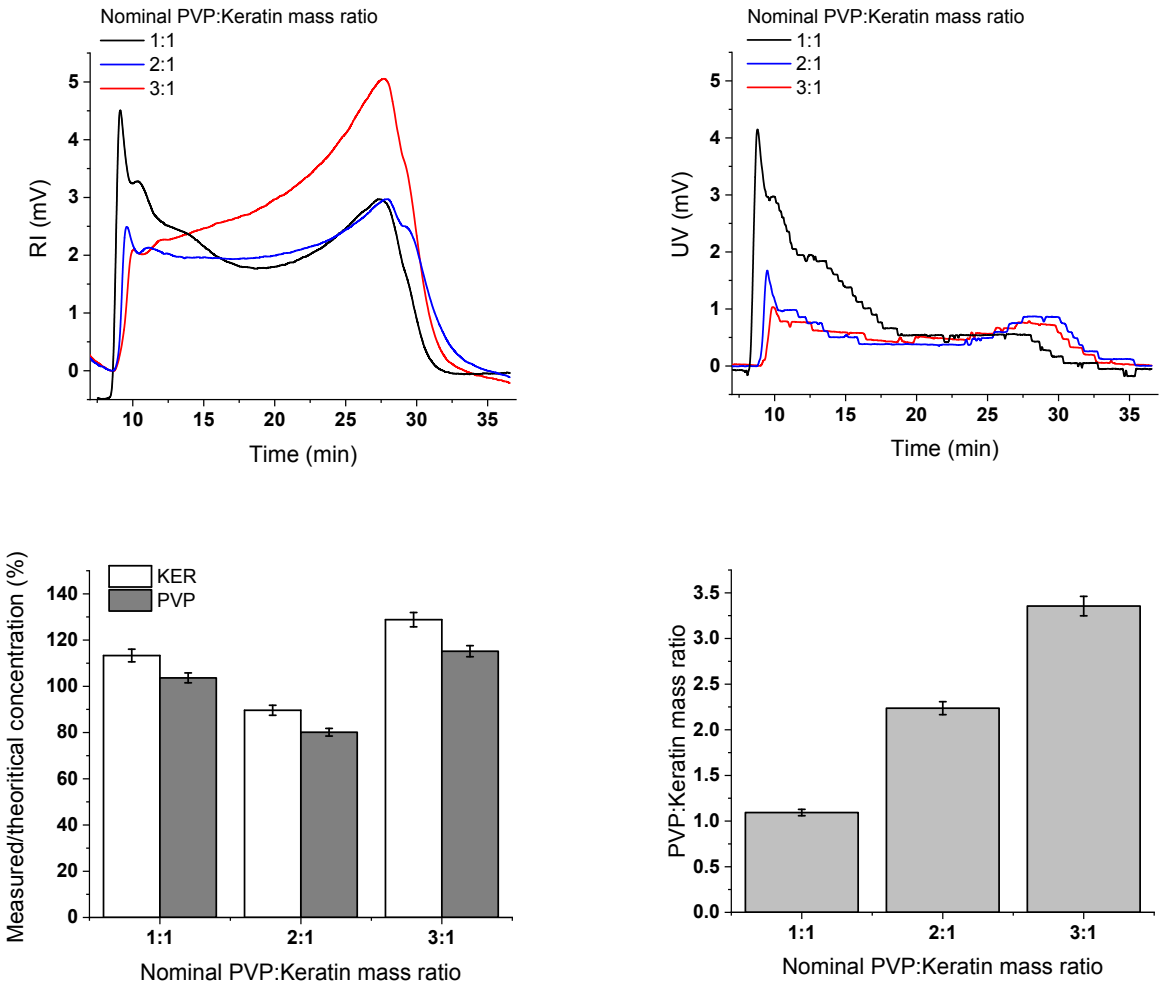

Figure S3. AF4 analysis of PVP/keratin electrospum fibres (K0). *Top:* RI and UV signals recorded injecting K0 fibres re-dissolved in water. *Bottom left:* the ratio between theoretical concentration of PVP and keratin in K0 fibres and that measured via AF4 is always close to 100%, thereby showing that the material is not significantly lost/degraded in the process. *Bottom right:* mass ratio between PVP and keratin in the different types of fibres.

S3. Further analyses of PVP:keratin fibres

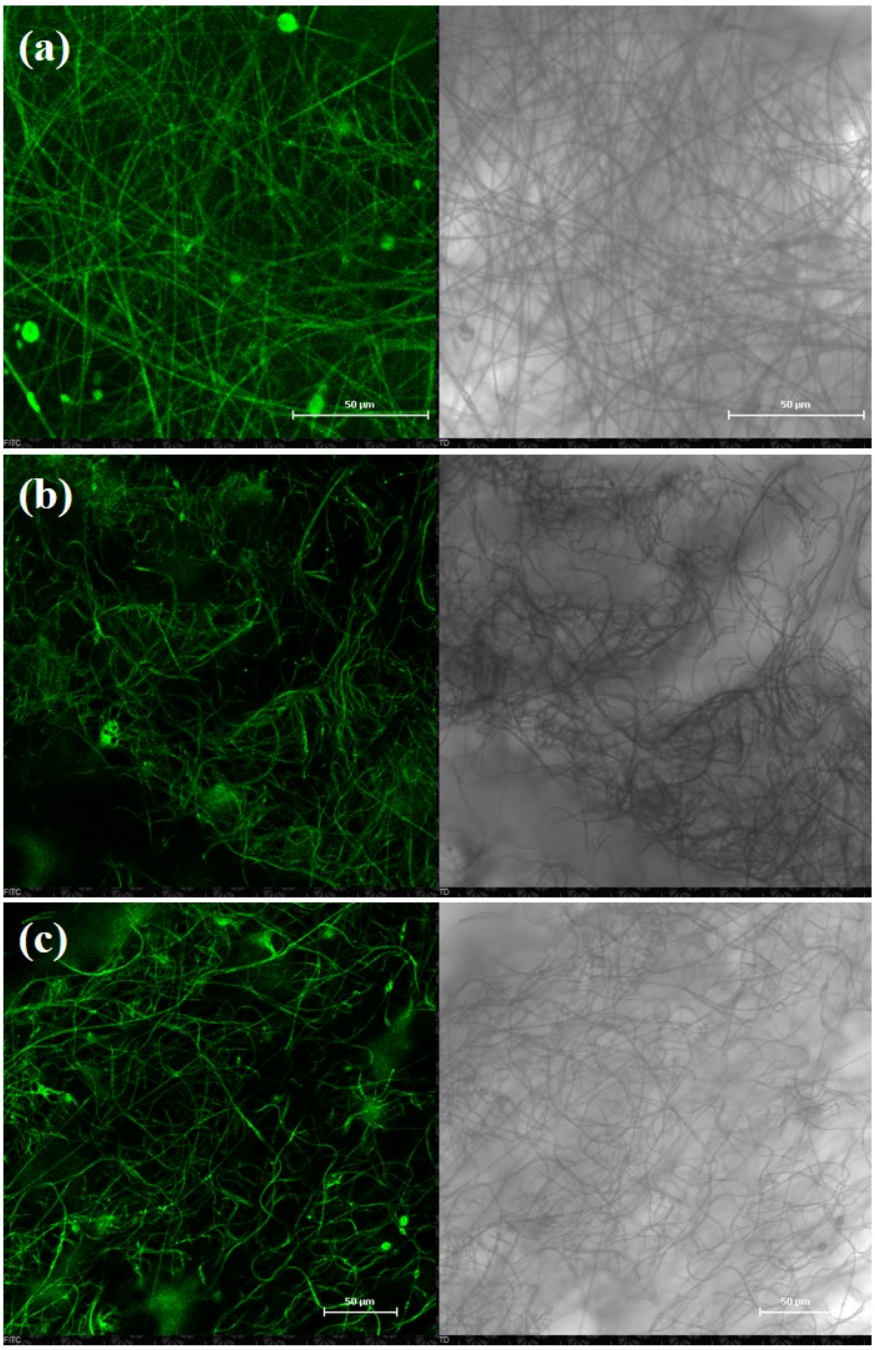

Figure S4: Confocal microscopy images of (a) KC5, (b) KC10 and (c) KC15 fibers.

Table S3: Mechanical characterization: Young's modulus, elongation at break and tensile stress of the electrospun fibrous mats.

Sample	Young's modulus [MPa]	Elongation at break (%)	Tensile stress [MPa]
KC0	199.8 ± 62.2	1.8 ± 0.3	2.1 ± 0.6
KC5	67.2 ± 32.9	3.3 ± 0.9	1.1 ± 0.4
KC10	36.2 ± 7.9	5.8 ± 2.8	0.8 ± 0.1
KC15	48.1 ± 28.7	2.06 ± 1.5	0.4 ± 0.2

S4. Additional characterization

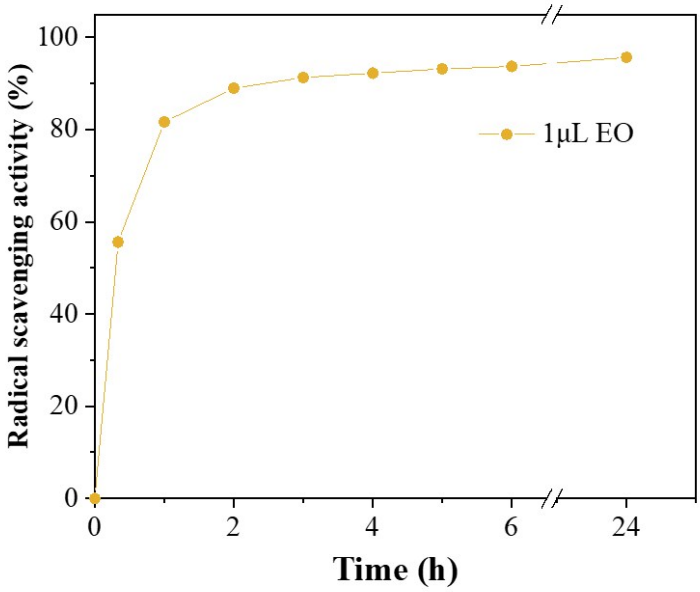

Figure S5: Results of the DPPH· scavenging assay for the free cinnamon essential oil as a function of time for 24h.

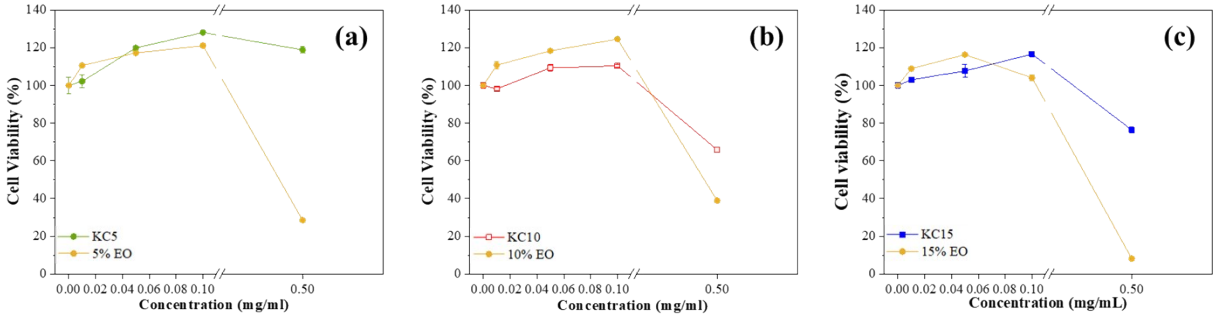

Figure S6: Viability of cells for equivalent concentrations of cinnamon essential oil and (a) KC5, (b) KC10 and (c) KC15, respectively.