cancers

Supplementary Materials: Specific Compositions of *Cannabis sativa* Compounds Have Cytotoxic Activity and Inhibit Motility and Colony Formation of Human Glioblastoma Cells In Vitro

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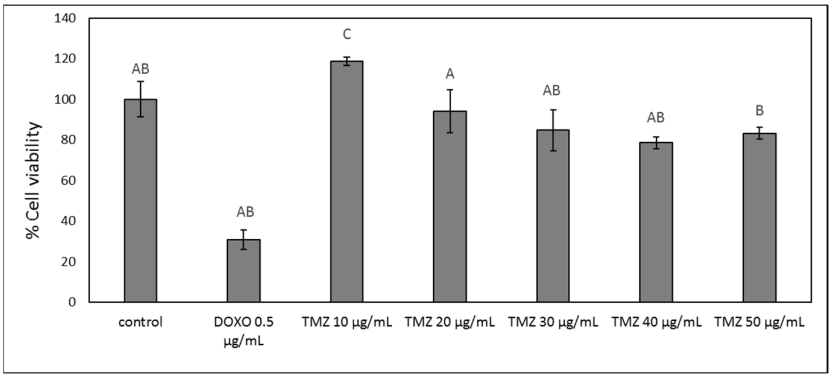

Figure S1. Cell viability of A172 cells following treatment with temozolomide (TMZ) at different concentrations for 48 h. Cell viability was determined by XTT assay as a function of live cell number. Doxorubicin (Doxo 0.5 µg/mL) served as positive controls; control is solvent (vehicle) control (0.5% *v*/*v* DMSO). Error bars indicate ± SE ($n = 3$). Levels with different letters are significantly different from all combinations of pairs by Tukey–Kramer honest significant difference (HSD; $p \leq 0.05$).

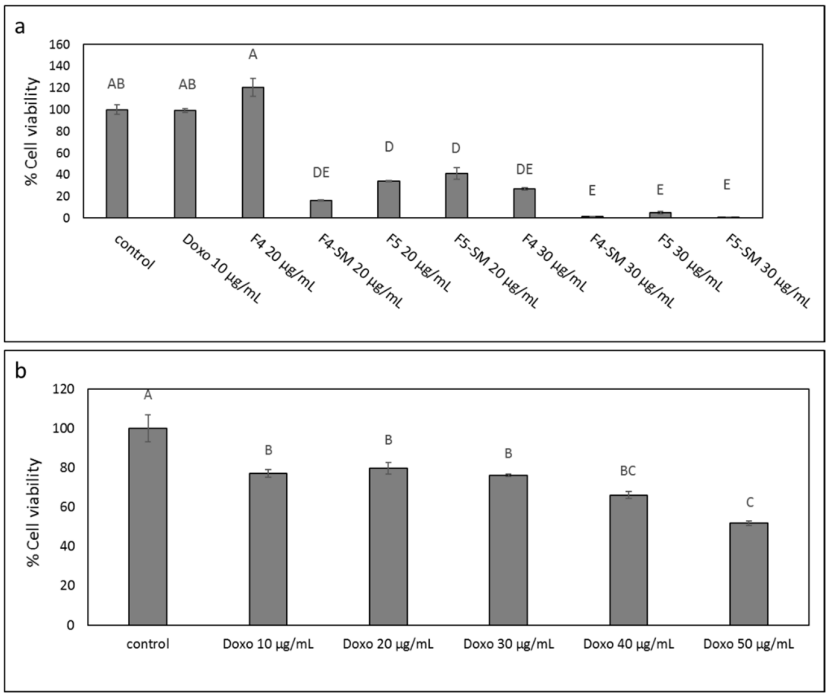

Figure S2. Cell viability of U87 cells following treatment with (**a**) F4, F5, F4-SM, or F5-SM and (**b**) doxorubicin (Doxo) at indicated concentrations for 48 h. Cell viability was determined by XTT

assay as a function of live cell number. Control is solvent (vehicle) control (1.5% *v*/*v* methanol). Error bars indicate ± SE (n = 3). Levels with different letters are significantly different from all combinations of pairs according to the Tukey–Kramer honest significant difference (HSD; $p \leq 0.05$).

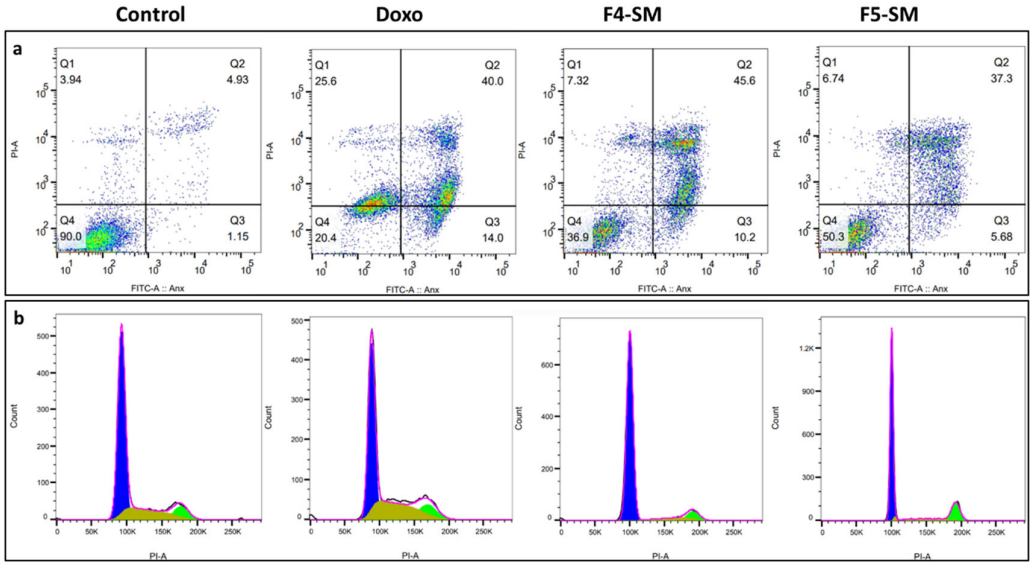

Figure S3. (**a**) Annexin V-FITC and PI staining for determining the proportion of viable (Q4), apoptotic (Q2 and Q3 for late and early apoptosis, respectively), or necrotic cells (Q1). (**b**) Example of FACS output following PI staining to determine the stages of cell cycle arrest. Treatments included F4-SM (10 µg/mL) or F5-SM (10 µg/mL) on the A172 cell line for 24 h for cell cycle and 48 h for apoptosis. Doxorubicin (Doxo, 0.5 µg/mL) served as a positive control. Methanol (control) treatment served as a solvent (vehicle) control.

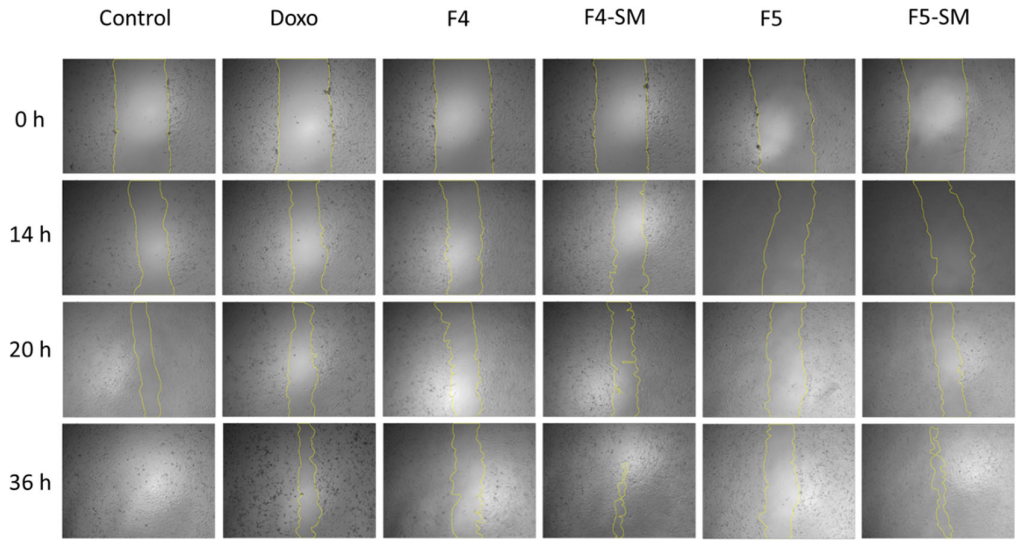

Figure S4. Examples of pictures taken for estimating the effectiveness of treatment F4 (20 µg/mL), F4-SM (11.5 µg/mL), F5 (20 µg/mL), F5-SM (11.5 µg/mL), or doxorubicin (Doxo, 0.5 µg/mL) on recovered area of confluent monolayers of the A172 cell line at 0, 14, 20, and 36 h. Methanol (control) treatment served as a solvent (vehicle) control.

Table 1. Percentage of terpenes out of total terpenes in F4 and F5 fractions of the DQ extract.

Terpenes (%)	F4	F5
α -Bisabolol	1.38	0.65
α-Eudesmol	62.35	19.29
γ-Eudesmol	18.13	23.78
9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo [5.4.0.0(3,8)]undecane		0.74
Agarospirol	1.80	
Azulemanthanol	5.43	
Bulnesol		15.46
Caryophyllene oxide		1.57
α -Cedrene		0.53
β-Selinene		2.32
Guaiol	10.92	26.90
Nerolidol		6.33
Selina-3,7(11)-diene		2.43

Table S2. Statistical analysis for quantitative PCR determination of the RNA steady state level in A172 cell line of *ATF4*, *TRIB3*, and *CHOP* (*DDIT3-3*) genes, after treatment with F4-SM or F5-SM (10 μg/mL) relative to control, presented in Figure 5. Control is vehicle control (1.2% *v*/*v* methanol). Letters with similar style (uppercase, lowercase, italic, and/or bold letters) were compared for each gene and time post treatment.

	Treatment/Time Post Treatment	4 h	12 h	24 h
ATF4	Control (for F4-SM)	**C**	*B*	C
	F4-SM	**A**	*A*	A
	F4-SM+CB2 IA	**B**	*A*	B
	Control (for F5-SM)	**b**	*c*	c
	F5-SM	**a**	*a*	a
	F5-SM+CB2 IA	**ab**	*b*	b
TRIB3	Control (for F4-SM)	**B**	*C*	C
	F4-SM	**A**	*A*	A
	F4-SM+CB2 IA	**B**	*B*	B
	Control (for F5-SM)	**a**	*b*	b
	F5-SM	**a**	*a*	a
	F5-SM+CB2 IA	**a**	*b*	b
CHOP	Control (for F4-SM)	**C**	*C*	C
	F4-SM	**A**	*A*	A
	F4-SM+CB2 IA	**B**	*B*	B
	Control (for F4-SM)	**b**	*b*	c
	F5-SM	**a**	*a*	a
	F5-SM+CB2 IA	**b**	*b*	b

Table S3. Cell viability of U87 cells from 3D structures following treatment with Figure 4. F5, F4-SM, and F5-SM at the indicated concentrations for 48 h. Cell viability was determined by 3D structure disintegration and cell dispersal following an Alamar Blue (resazurin) assay. Control is vehicle control (3% *v*/*v* methanol). Means (n = 3) with different letters are significantly different from all combinations of pairs according to the Tukey–Kramer honest significant difference (HSD; $p \leq 0.05$).

Treatment	Mean (% of Live Cells)
Control	98.52±0.08 [a]
Doxorubicin 2 μg/mL	37.22±3.08 [c]
F4 20 μg/mL	11.98±0.44 [d]
F4-SM 12.5 μg/mL	17.39±1.14 [d]
F5 16.5 μg/mL	12.32±0.02 [d]
F5-SM 10 μg/mL	72.81±0.05 [b]