marine drugs

MDPI

Supplementary Materials

Therapeutic Potential of (−)-Agelamide D, a Diterpene Alkaloid from the Marine Sponge *Agelas* sp., as a Natural Radiosensitizer in Hepatocellular Carcinoma Models

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Table S1. Serum biochemical parameters in two groups of mice that received radiation therapy (RT) or RT plus (−)-agelamide

Parameters	Unit	RT	RT plus (−)-agelamide
Aspartate transaminase	U/L	81.0 ± 26.3	81.3 ± 7.1
Alanine transaminase	U/L	28.7 ± 2.1	26.0 ± 4.4
Alkaline phosphatase	U/L	99.3 ± 20.2	91.0 ± 11.5
Total protein	g/dL	4.8 ± 0.5	4.9 ± 0.2
Albumin	g/dL	2.6 ± 0.3	2.7 ± 0.2
Total bilirubin	mg/dL	0.1	0.1
Blood urea nitrogen	mg/dL	22.2 ± 4.0	25.6 ± 1.8
Creatine	mg/dL	0.5 ± 0.1	0.5
Glucose	mg/dL	247.7 ± 74.7	224.7 ± 64.1
Cholesterol	mg/dL	69.0 ± 3.6	74.7 ± 7.6
Triglyceride	mg/dL	65.0 ± 4.6	70.3 ± 14.6
Phosphorus	U/L	7.4 ± 1.6	5.6 ± 1.0
Ca^{2+}	mg/dl	8.8 ± 0.7	8.5 ± 0.3
Creatine phosphokinase†	U/L	232.1 ± 69.0	485.4 ± 147.3
A/G ratio		1.2 ± 0.2	1.3 ± 0.3

†Statistically not significant

Figure S1. Underwater (**A**) and *in situ* (**B**) photographs of *Agelas* sp. used in this study

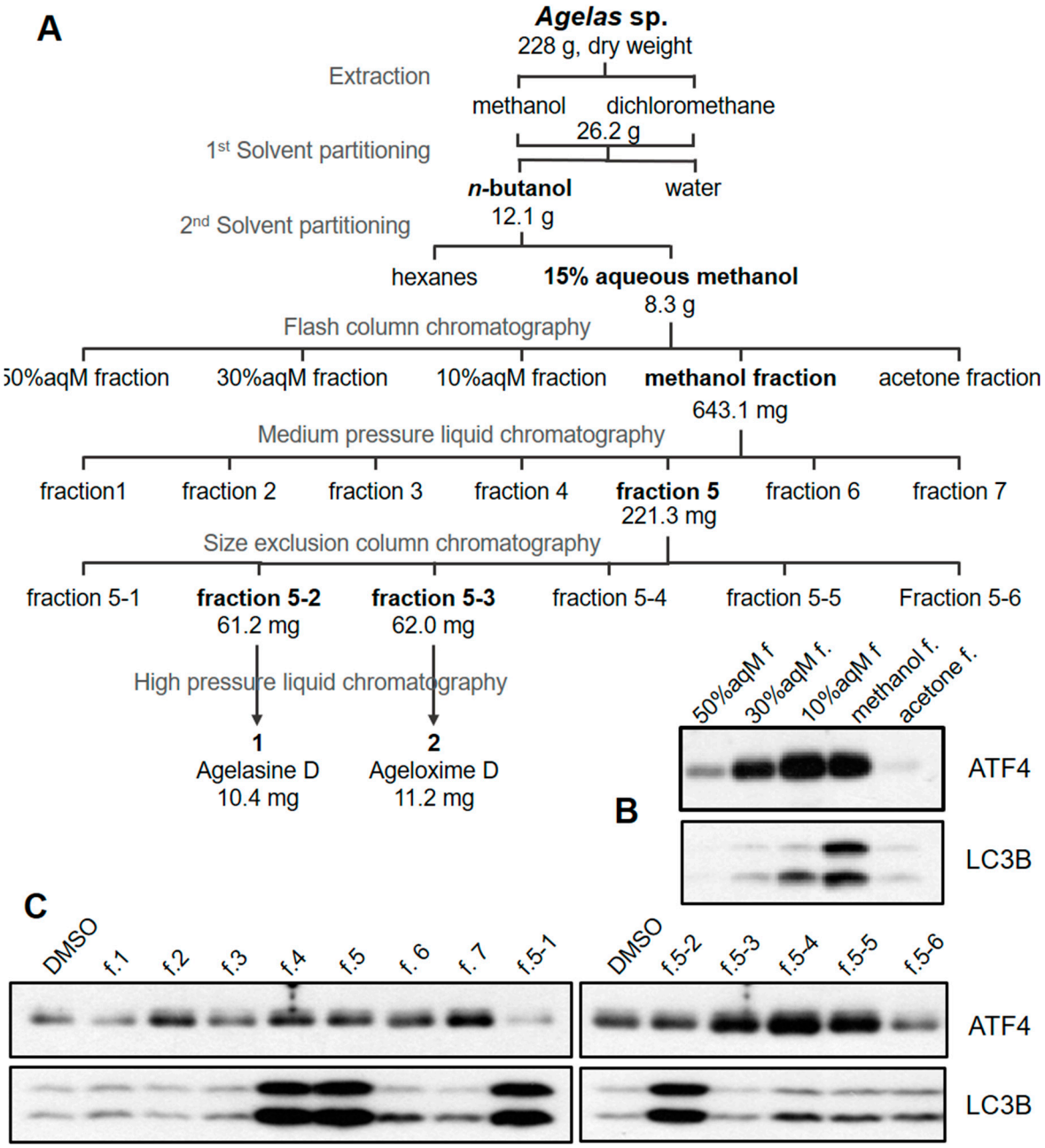

Figure S2. Bioassay-guided isolation of compounds 1 and 2 from *Agelas* sp. **A,** Extraction and isolation scheme for compounds 1 and 2 from *Agelas* sp. **B,** Levels of ATF4 and LC3B induced by the fractions obtained through flash column chromatography. **C,** Levels of ATF4 and LC3B induced by the fractions obtained through medium pressure liquid chromatography (MPLC) (fractions 1–7) and size exclusion column chromatography (fractions 5.1–5.6).

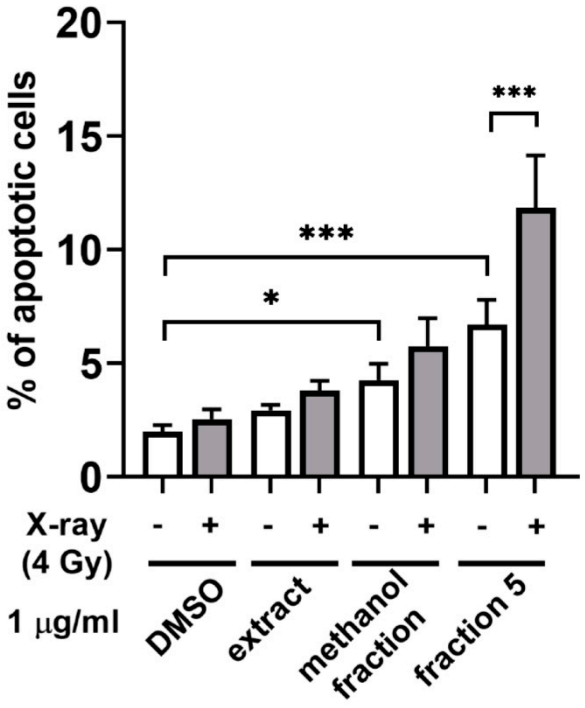

Figure S3. Enhancement of apoptosis by combined treatment with fractions from *Agelas* extract and radiation. Hep3B cells were pre-treated with the same amount (1 μg/mL) of the samples from each purification step for 3 h, followed by irradiation with 4 Gy of X-rays. After 48 h, the samples were collected, and the apoptotic cell population was analyzed through flow cytometry with Annexin V/propidium iodide co-staining. Data are mean ± standard deviation (SD) from two independent experiments (n = 6). * $p < 0.05$; *** $p < 0.001$.

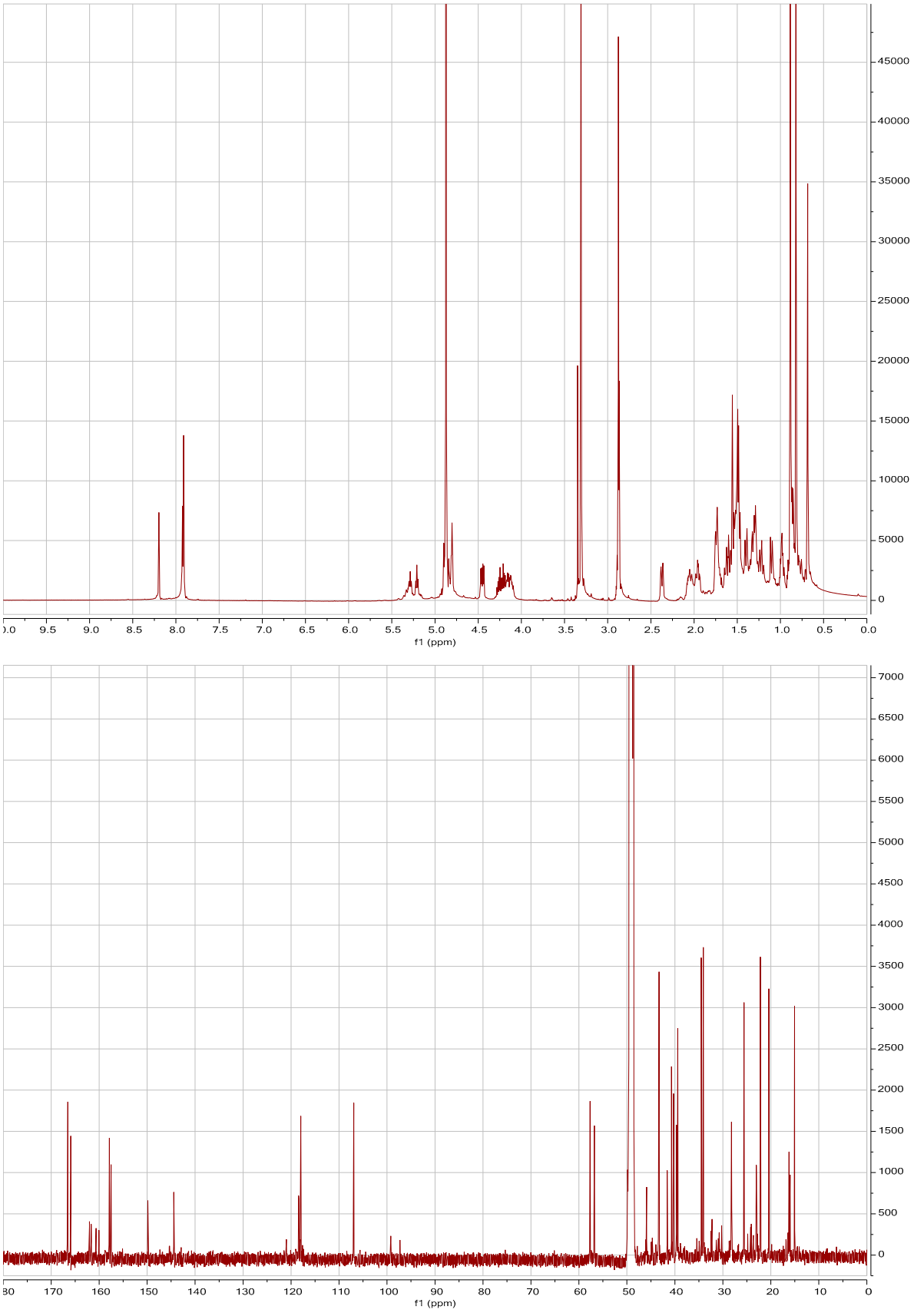

Figure S4. ^{1}H and ^{13}C NMR spectra (600 MHz and 150 MHz, methanol-d_4) of compound **2**

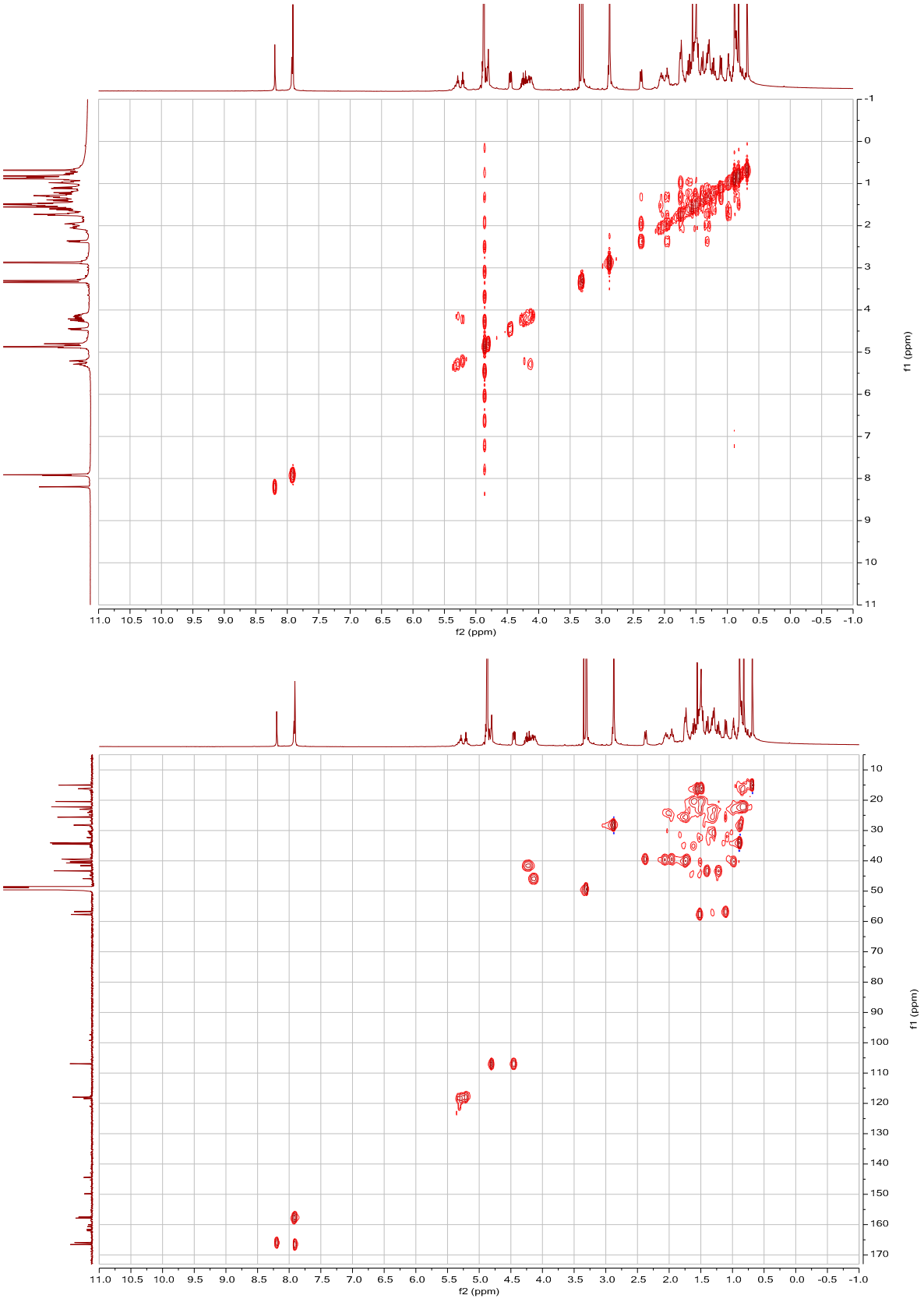

Figure S5. COSY and HSQC spectra of compound **2**

Figure S6. HMBC spectra of compound **2**

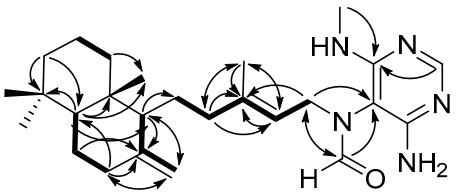

Figure S7. Selective COSY and HMBC correlations for compound **2**