SUPPLEMENTARY MATERIALS

Anti-inflammatory Activity-Guided Isolation and in Silico Validation of Turmeric (*Curcuma longa* L.) Phytochemicals

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Abstract

Turmeric (*Curcuma longa* L., Zingiberaceae) is a widely consumed spice and functional food valued for its bioactive constituents. Using an activity-guided strategy, this study identified the dichloromethane fraction as the most potent anti-inflammatory fraction, exhibiting markedly stronger inhibition of reactive oxygen species (ROS) production than ibuprofen ($IC_{50} \leq 0.4$ *vs.* 11.2 µg/mL). Bioassay-guided purification yielded bisacurone (**1**), didemethoxycurcumin (**2**), and β-turmerone (**3**), with compounds **1** and **2** reported here for the first time in this fraction. Among them, β-turmerone displayed the strongest anti-inflammatory activity (IC_{50} = 4.7 µg/mL), consistent with *in silico* docking and molecular dynamics analyses revealing greater binding affinity and complex stability with myeloperoxidase (ΔG_{bind} = –20.90 *vs.* –18.89 kcal/mol for ibuprofen). Gas chromatography-mass spectrometry (GC-MS) profiling revealed a phytochemical profile dominated by turmerones and curlone, correlating with the observed bioactivity. None of the fractions exhibited acute toxicity in brine shrimp lethality assays, indicating a favorable preliminary safety profile. Our findings demonstrate the value of activity-guided isolation combined with computational validation for identifying turmeric-derived bioactives with promising nutraceutical potential, warranting further *in vivo* evaluation.

Keywords: *Curcuma longa* L.; β-Turmerone; Bioassay-guided isolation; Anti-inflammatory activity; Dynamic simulations.

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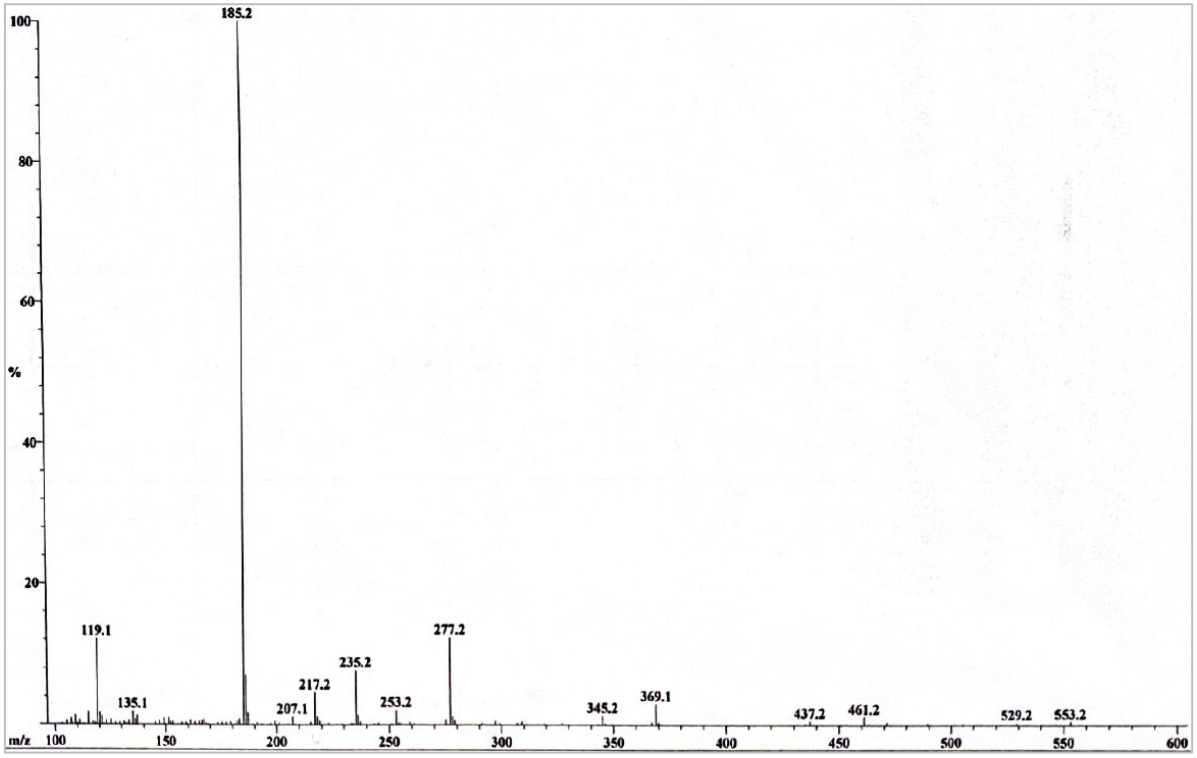


Figure S1. (+)-FAB-MS spectrum of bisacurone (**1**).

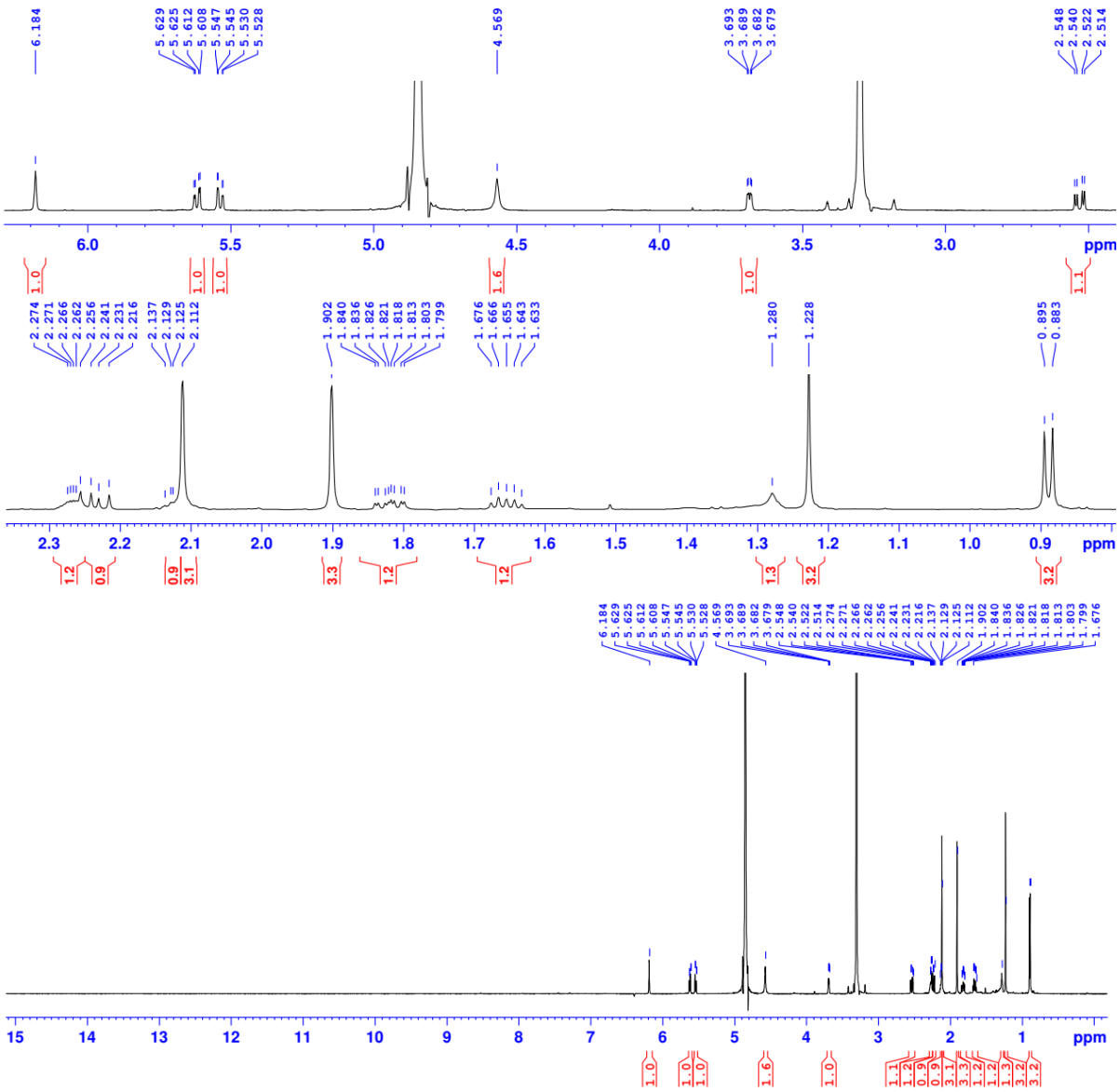


Figure S2. ^{1}H NMR spectrum of bisacurone (**1**) (CD_3OD, 600 MHz).

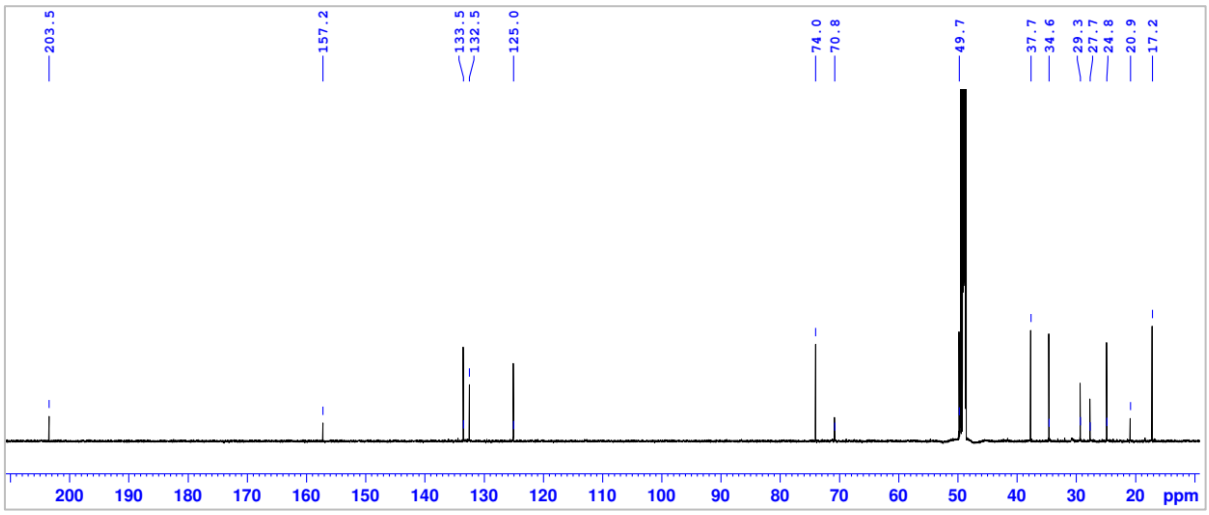


Figure S3. ^{13}C NMR spectrum of bisacurone (**1**) (CD_3OD, 150 MHz).

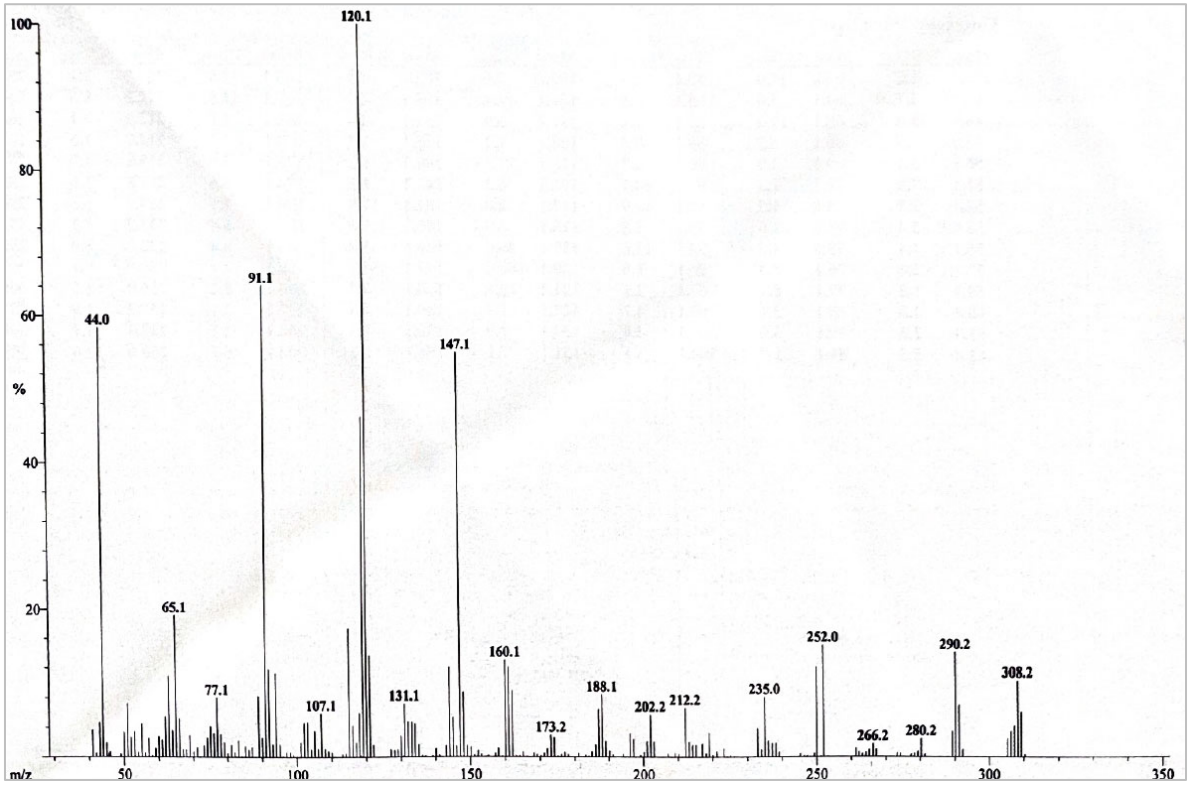


Figure S4. LR-EI-MS spectrum of didemethoxycurcumin (**2**).

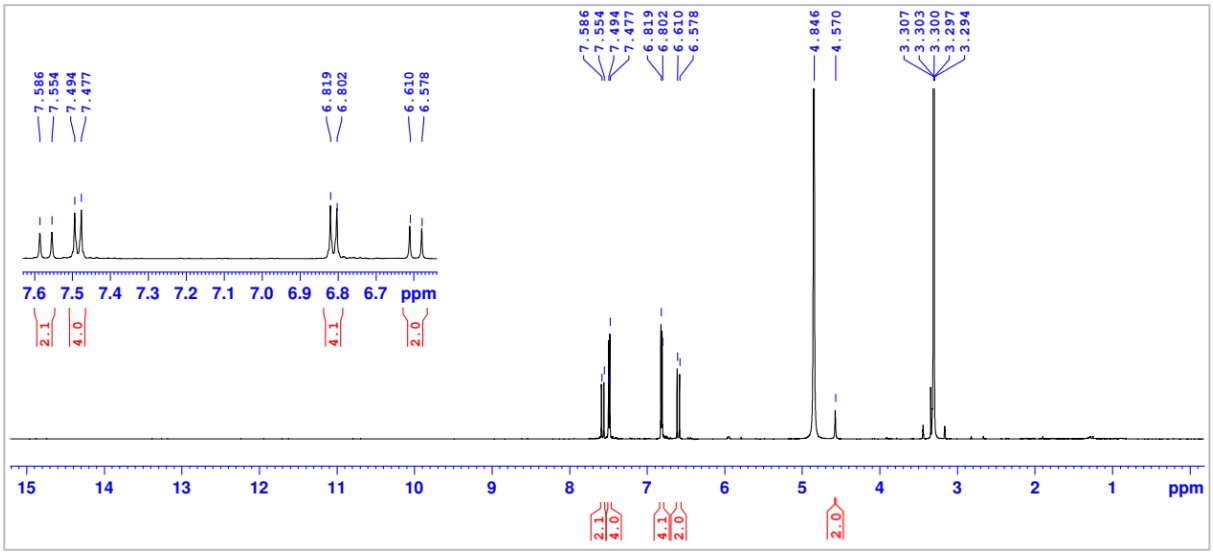


Figure S5. ^{1}H NMR spectrum of didemethoxycurcumin (**2**) (CD_3OD, 500 MHz).

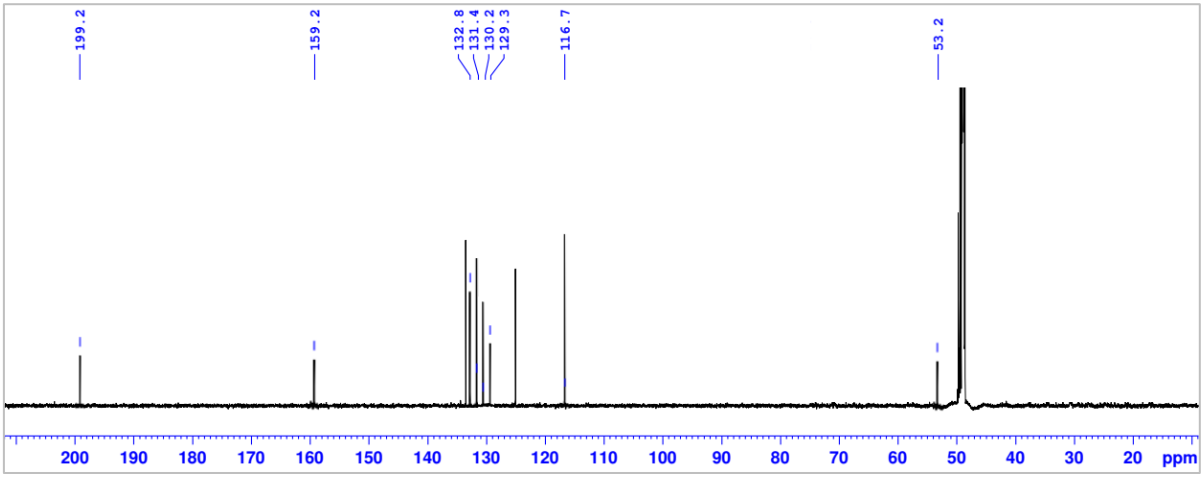


Figure S6. ^{13}C NMR spectrum of didemethoxycurcumin (**2**) (CD_3OD, 125 MHz).

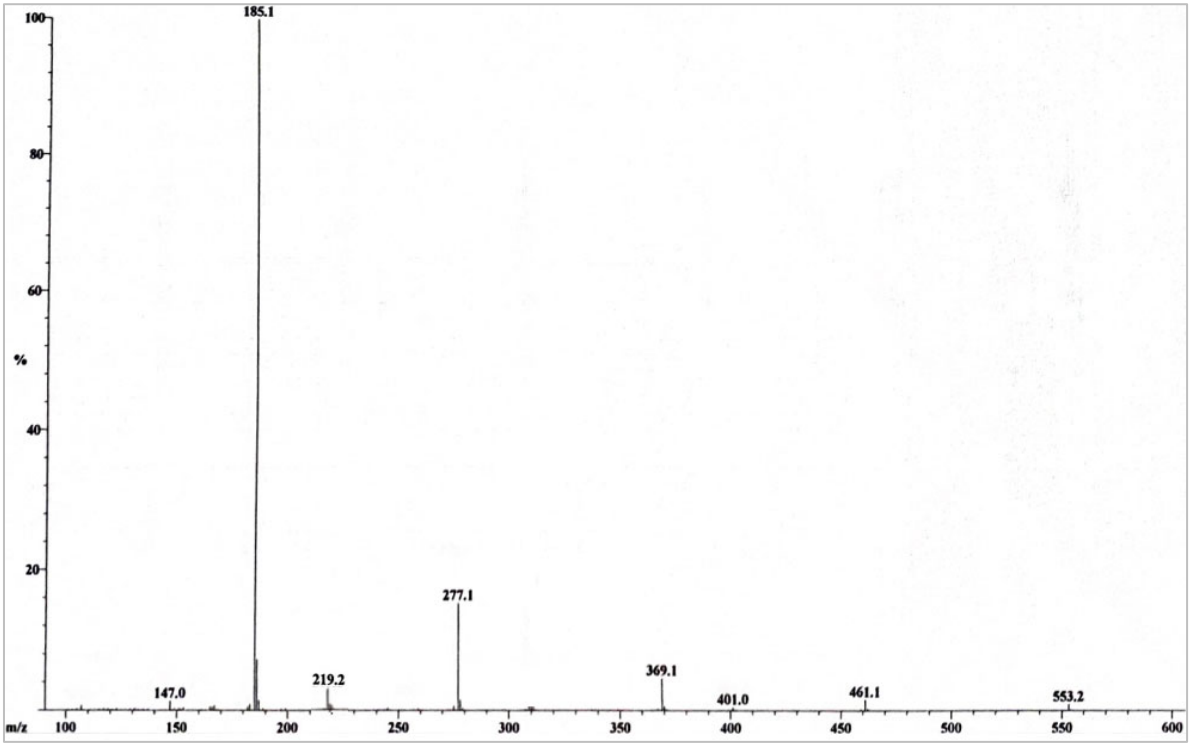


Figure S7. (+)-FAB-MS spectrum of β-turmerone (**3**).

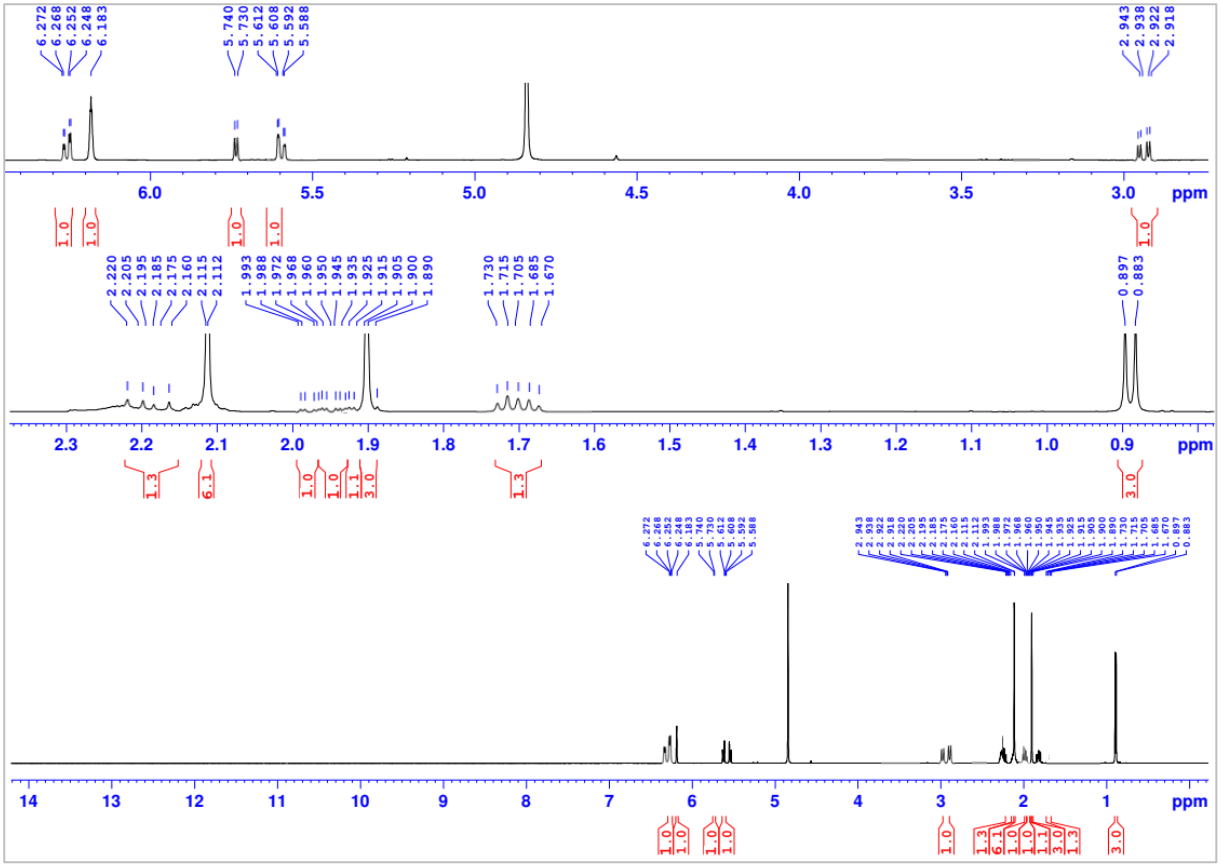


Figure S8. ^{1}H NMR spectrum of β-turmerone (**3**) (CD_3OD, 500 MHz).

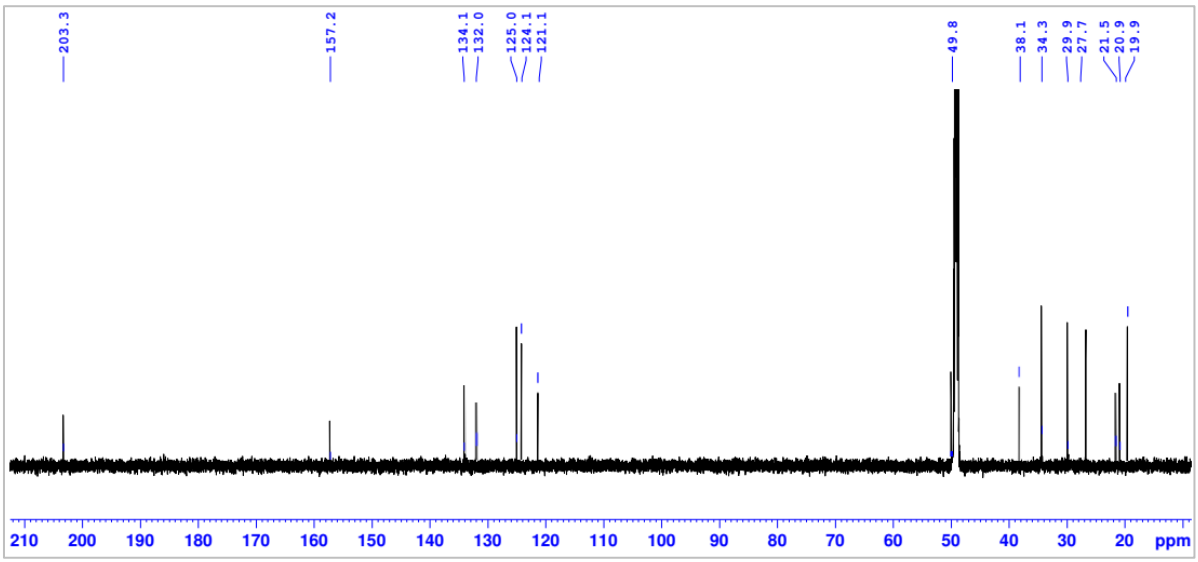


Figure S9. ^{13}C NMR spectrum of β-turmerone (**3**) (CD_3OD, 125 MHz).

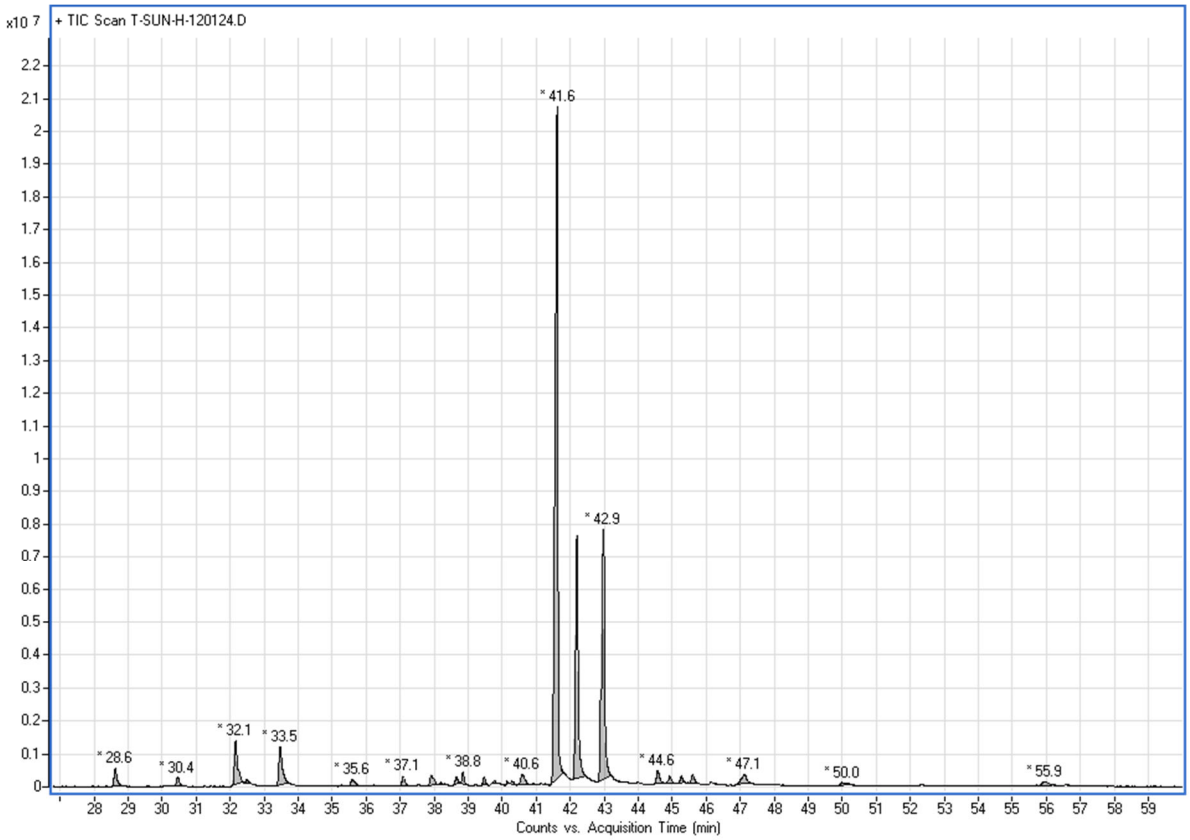


Figure S10. GC-MS chromatogram of *n*-hexane fraction of *Curcuma longa* L.

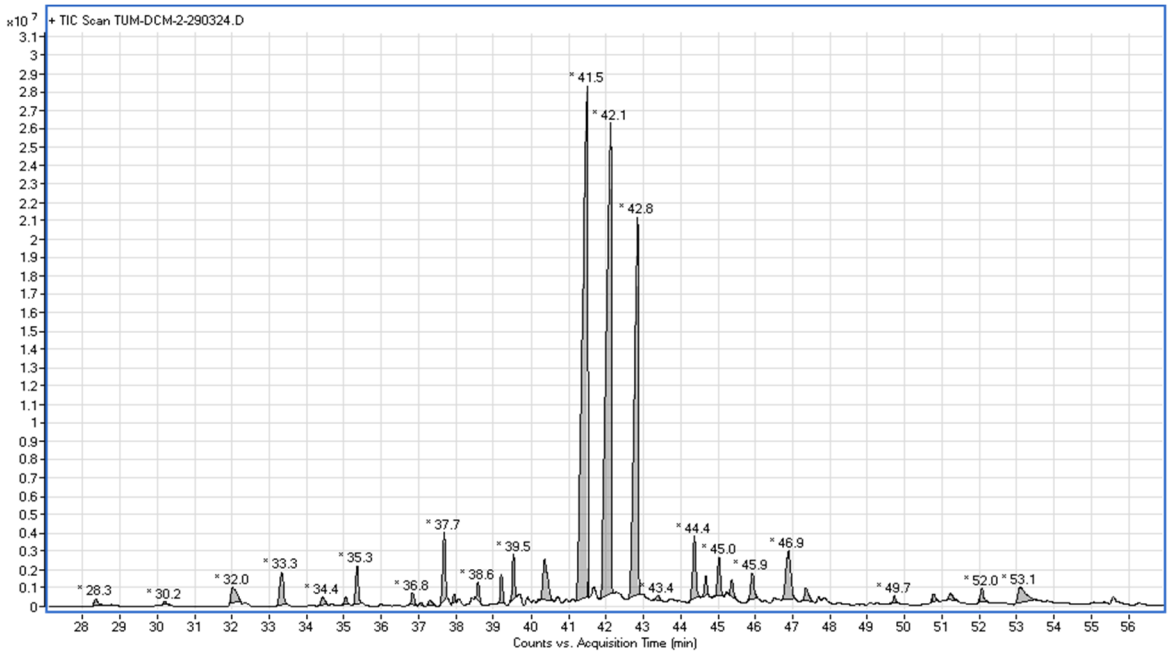


Figure S11. GC-MS chromatogram of dichloromethane fraction of *Curcuma longa* L.

Table S1. Cytotoxicity activity (brine shrimp lethality assay)

Sample	Dose (µg/mL)	No. of Shrimps	No. of Survivors	Mortality, %	STD Drug	Mortality, %
n-Hexane fr.	10	30	30	0	Etoposide	70
	100	30	30	0		
	1000	30	30	0		
DCM fr.	10	30	30	0		
	100	30	30	0		
	1000	30	30	0		
EtOAc fr.	10	30	30	0		
	100	30	30	0		
	1000	30	30	0		
n-Butanol fr.	10	30	30	0		
	100	30	30	0		
	1000	30	29	3.34		
Aqueous fr	10	30	30	0		
	100	30	30	0		
	1000	30	30	0		

Table S2. Identified compounds from the *n*-hexane fraction of *Curcuma longa* L. using GC-MS.

R_t (min)	%	Compound's name
28.6	1.26	Caryophyllene
30.4	0.64	*α*-Caryophyllene
32.1	3.5	Zingiberene
32.5	0.23	*β*-Bisabolene
33.5	3.4	*β*-Sesquiphellandrene
35.6	0.68	*trans*-Nerolidol
37.1	0.7	Epiglobulol
37.9	0.92	*α*-Bisabolol
38.2	0.19	*trans*-*α*-Bergamotol
38.6	0.46	Tumerone
38.8	0.71	*α*-Bisabolol
39.5	0.5	cis-*α*-Bergamotol
39.8	0.16	*E*-Nuciferol
39.9	0.07	cis,*α*-Santalol
40.1	0.15	Curlone
40.3	0.16	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl) methanol
40.6	1.02	*α*-Bergamotol
40.9	0.1	Bergamotol
41.6	46.74	Tumerone
42.2	16.02	*Ar*-tumerone
42.9	17.03	Curlone
44.6	0.99	1,8-dimethyl-4-(1-methylethyl)-spiro[4.5]dec-8-en-7-one
44.9	0.54	1-Methyl-6-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptane
45.2	0.44	Ethyl citral
45.6	0.6	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one
47.1	1.2	*α*-Santalol
50	0.47	Hexadecanoic acid ethyl ester
55.9	0.69	Linoleic acid ethyl ester
92	0.18	Ethyl *iso*-allocholate
97.4	0.25	Ethyl *iso*-allocholate

Table S3. Identified compounds from the dichloromethane fraction of *Curcuma longa* L. using GC-MS.

R_t (min)	%	Compound's name
28.3	0.32	Caryophyllene
30.2	0.14	*α*-Caryophyllene
32	1.13	Zingiberene
33.3	1.58	*β*-Sesquiphellandrene
34.4	0.41	Vanillin
35	0.28	Widdrol
35.3	1.19	(E)-3,7,11-trimethyl-1,6,10-Dodecatrien-3-ol
36.8	0.47	Thujopsene
37	0.08	Limonen-6-ol, pivalate
37.3	0.24	Caryophyllene oxide
37.7	2.07	Dihydrocurcumene
37.9	0.23	*trans*-*α*-Bergamotol
38.6	0.46	Widdrol
39.2	0.92	*trans*-*α*-Bergamotol
39.5	1.43	Tumerone
40.4	2.16	*trans*-*α*-Bergamotol
41.5	30.07	Tumerone
42.1	26.57	*Ar*-tumerone
42.8	16.47	Curlone
43.4	0.19	*E*-Nuciferol
44.4	2.25	(E)-1-(2,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-7-oxabicyclo[4.1.0]heptane
44.7	0.56	Caryophyllene oxide
45	1.28	(2-nitro-2-propenyl)-Cyclohexane
45.4	0.55	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one
45.9	1.15	5-methyl-2-(1-methyl-1-phenylethyl)-cyclohexanol
46.9	2.71	3,4-di[1-butenyl]-tetrahydrofuran-2-ol
47.3	0.7	7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]octane
49.7	0.3	Ethyl palmitate
50.8	0.35	2,5-Octadecadiynoic acid methyl ester
51.2	0.33	4,4aα,5α,8α-tetrahydro-5,8-dimethyl-5,8-epoxy-3H-2-benzopyran
52	0.57	6Z-2,5,5,10-Tetramethyl-undeca-2,6,9-trien-8-one
53.1	1.59	Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate
91.6	0.45	Sitostenone
96.6	0.77	Cholest-4-ene-3,6-dione