Supporting Information

***Rec. Nat. Prod*. 15:6 (2021) 593-601**

Microbial Transformation of (–)-α-Bisabolol Towards Bioactive Metabolites

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S1. Materials and Methods: *Gas chromatography and Gas Chromatography-Mass Spectrometry (GC, GC-MS)*

The GC analysis was carried out using an Agilent 6890N GC system. FID temperature was set to 300 °C. In order to obtain the same elution order with GC-MS, simultaneous auto-injection was done by using same column and operational conditions. The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV, and mass range was from *m/z* 35 to 450.

Identification of the (–)-α-bisabolol and biotransformation products was carried out using the in-house library "Baser Library of Essential Oil Constituents" and in comparison with authentic samples. Relative percentage amounts (%) were calculated from the Total Ion Chromatogram (TIC) by the computer.

S2. Materials and Methods: *In vitro Antimicrobial Activity*

The bacterial strains used were *Escherichia coli ATCC* 8739, *Propionibacterium acnes* ATCC 6919, *Staphylococcus epidermidis* ATCC 12228, *Salmonella typhimurium* ATCC 13311 and *Staphylococcus aureus* ATCC 6538. The yeast used were *Candida albicans* ATCC 10231 *and C. glabrata* (clinical isolate, Eskisehir Osmangazi University, Faculty of Medicine) and *C. utilis* NRRL Y-900, respectively. The minimum inhibitory concentration (MIC) of (–)-*α*-bisabolol and biotransformation extracts (**E1-4**) were evaluated by broth microdilution method using u-shaped 96-well microtiter plates (Sigma, Germany). All microorganisms were stored at -85 °C in 15% glycerol. *Candida* cultures were inoculated on potato dextrose agar (PDA, Fluka) while bacteria were inoculated onto Mueller Hinton agar (MHA, Fluka). All cultures were checked for their purity before antimicrobial evaluations.

Overnight grown bacterial and *Candida* strains in sterile saline (0.85%) were standardized using McFarland No: 0.5 (1 x 10^8 CFU/ per well in Mueller-Hinton broth for bacteria and 5 x 10^3 CFU/ per well in RPMI medium for *Candida* sp.), according to their turbidity. All samples were prepared initially using 10% dimethylsulfoxide (DMSO). Dilution series were prepared from 9.375 to 300 μg/mL, respectively. Mueller Hinton Broth (MHB, Merck) was used for the bacteria, and RPMI 1640 medium was used for the *Candida* strains in 96-well microtiter plates. Each bacterial and *Candida* sp. suspensions (100 µL) were then transferred to each well. The last row containing medium with microorganism was used as negative control, and medium only served as a positive growth control. After incubation at 37 °C for 24 h, 0.01% resazurin (20 µL) was added to all wells for staining of viable microorganisms. The first blue colored well was determined as the minimal inhibitory concentration (MIC, μg/mL) for the tested bacterial, whereas the minimum fungicidal concentrations (MFCs) were determined for the yeast. Amoxicillin and chloramphenicol were used as standard antibacterial agents, whereas amphotericin B and nystatin were used as standard antifungal agents at a concentration range of 0.125-32 μg/mL. All tests were assayed in duplicate in two independent experiments and MICs/MFCs were reported as mean.

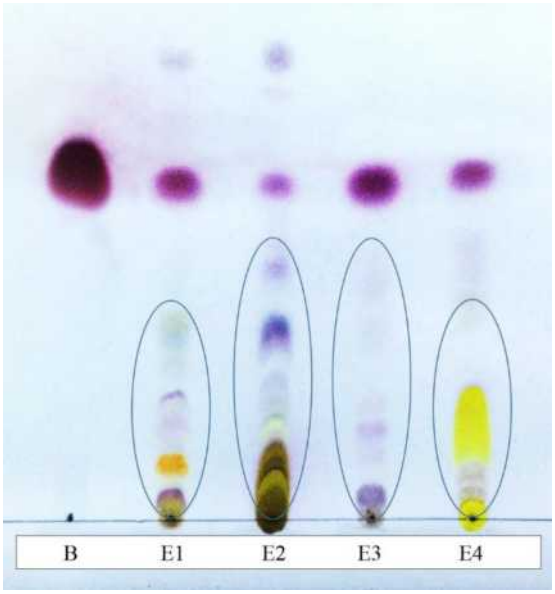


Figure S1: TLC Profile of (–)-*α*-Bisabolol and Biotransformed Product
[(–)-*α*-Bisabolol (**B**) and its biotransformation extracts by *T. elegans* (**E1**), *M. ramannianus* (**E2**), *T. harzianum* (**E3**), *P. neocrassum* (**E4**), solvent system: chloroform-toluene (3 : 1)]

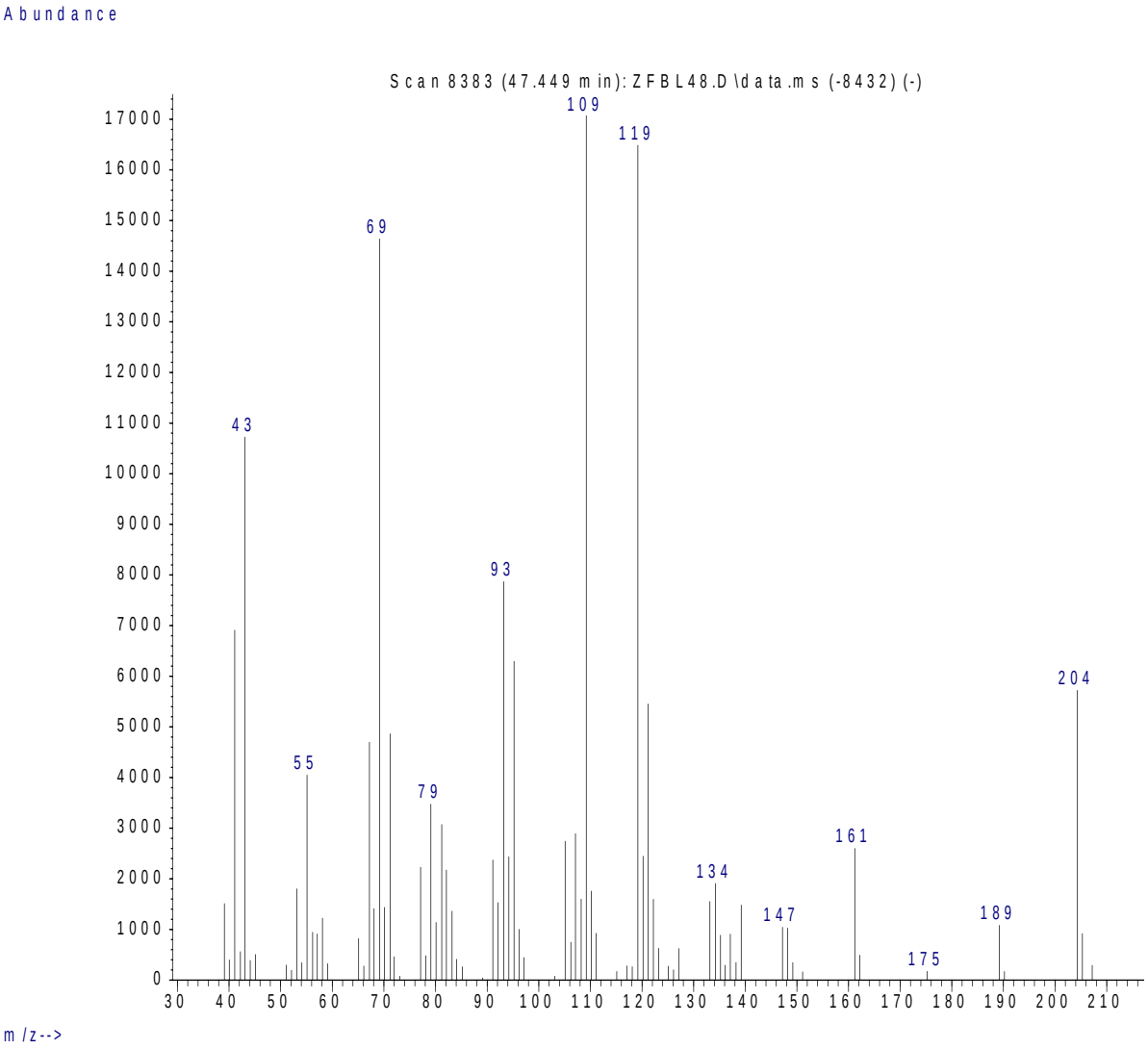


Figure S2: MS spectrum of (–)-*α*-Bisabolol (GC-MS data)

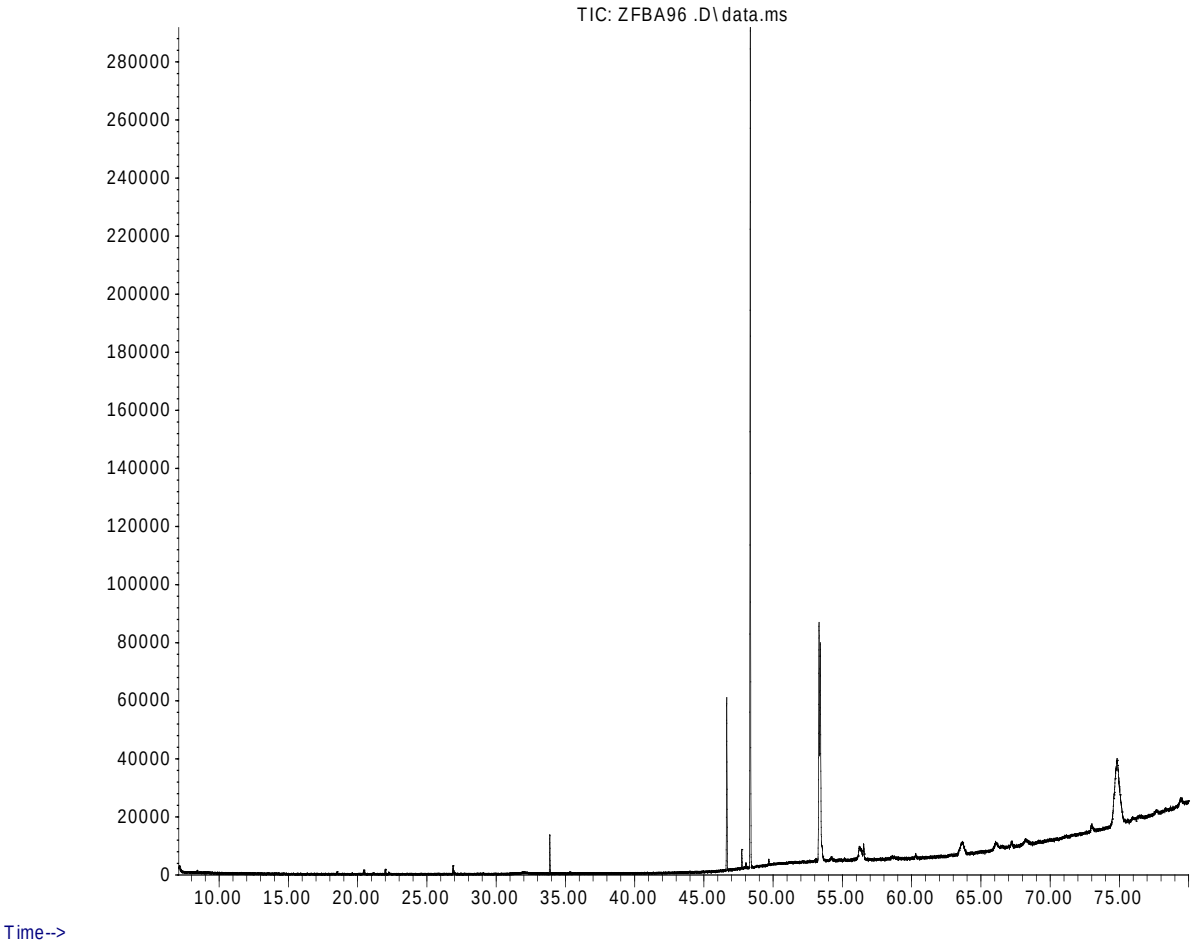


Figure S3: GC-MS analysis and chromatogram for **E1**

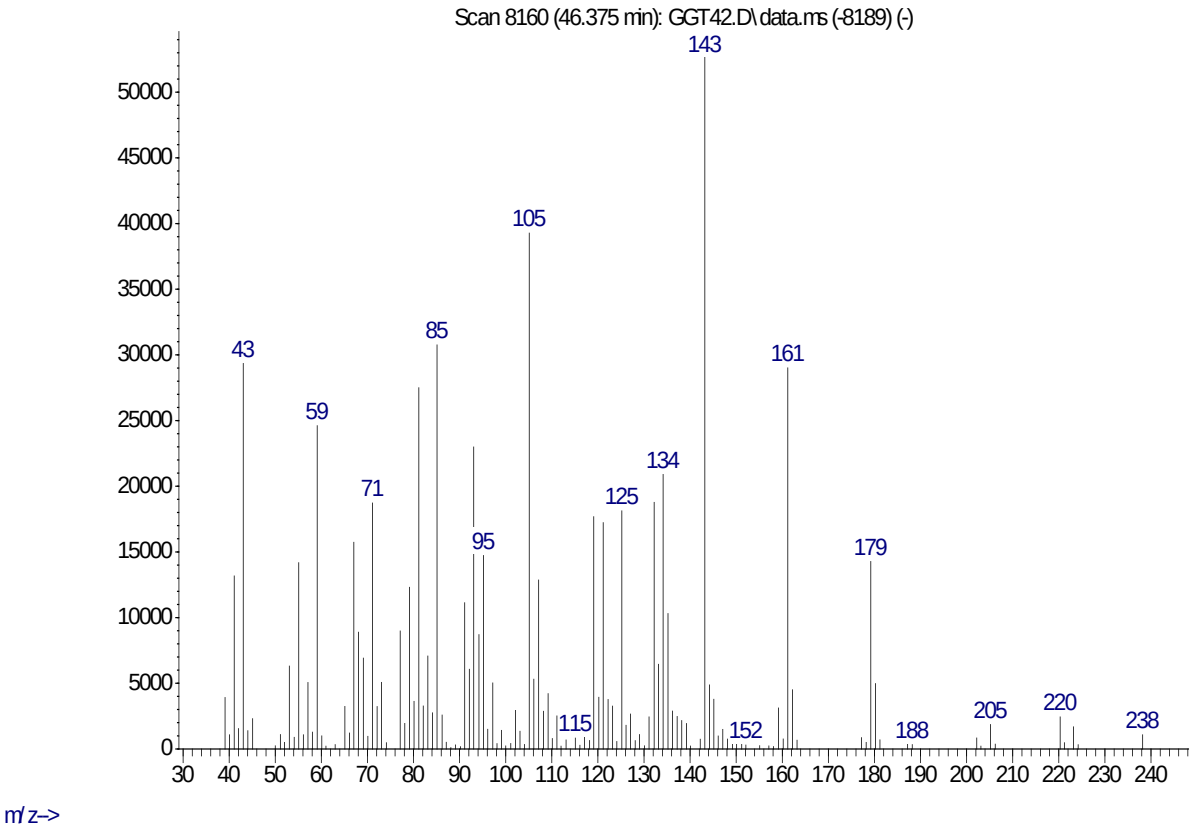


Figure S4: MS spectrum of **M1** (α-bisabolol oxide B) (GC-MS data)

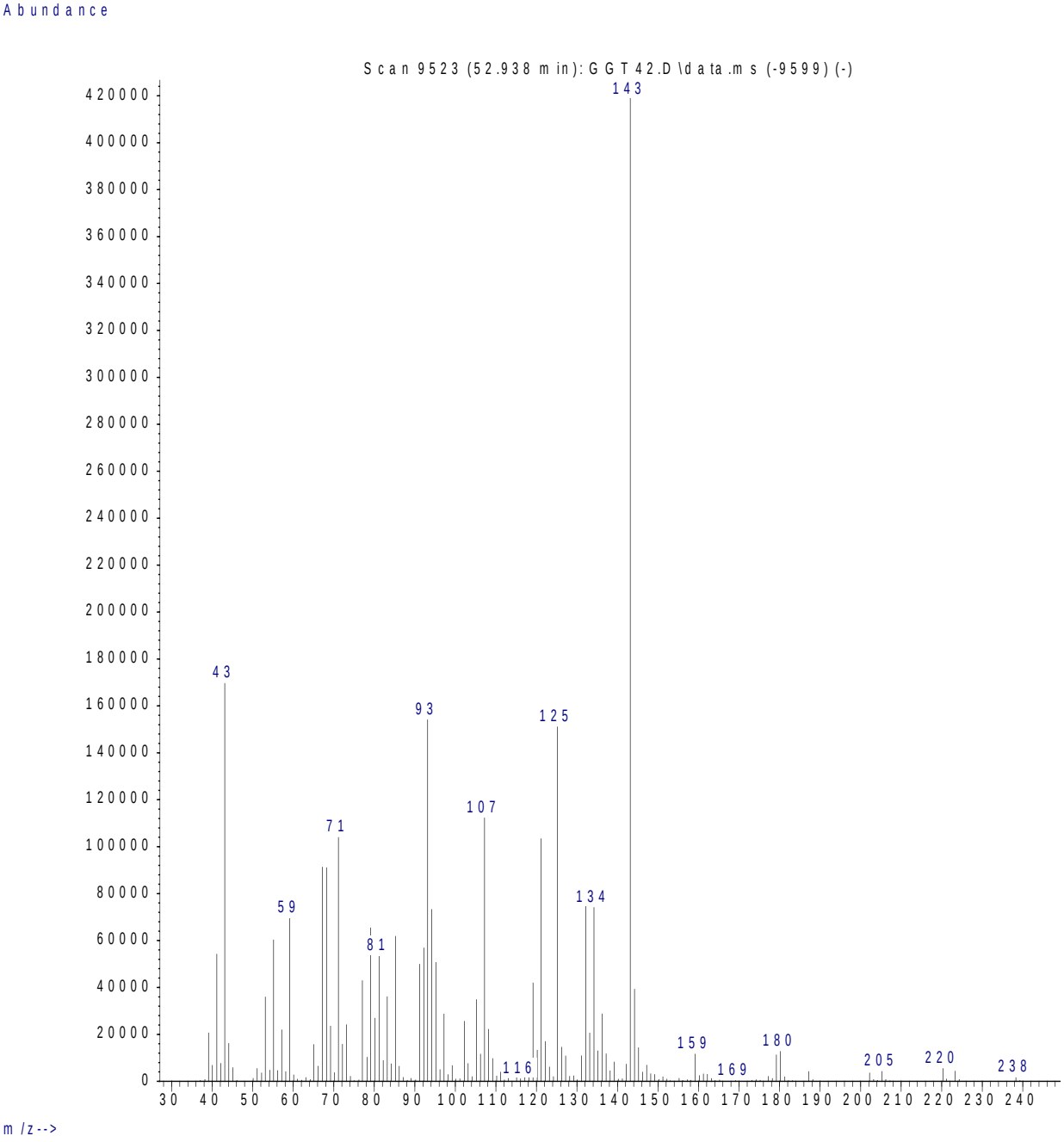


Figure S5: MS spectrum of **M2** (α-bisabolol oxide A) (GC-MS data)

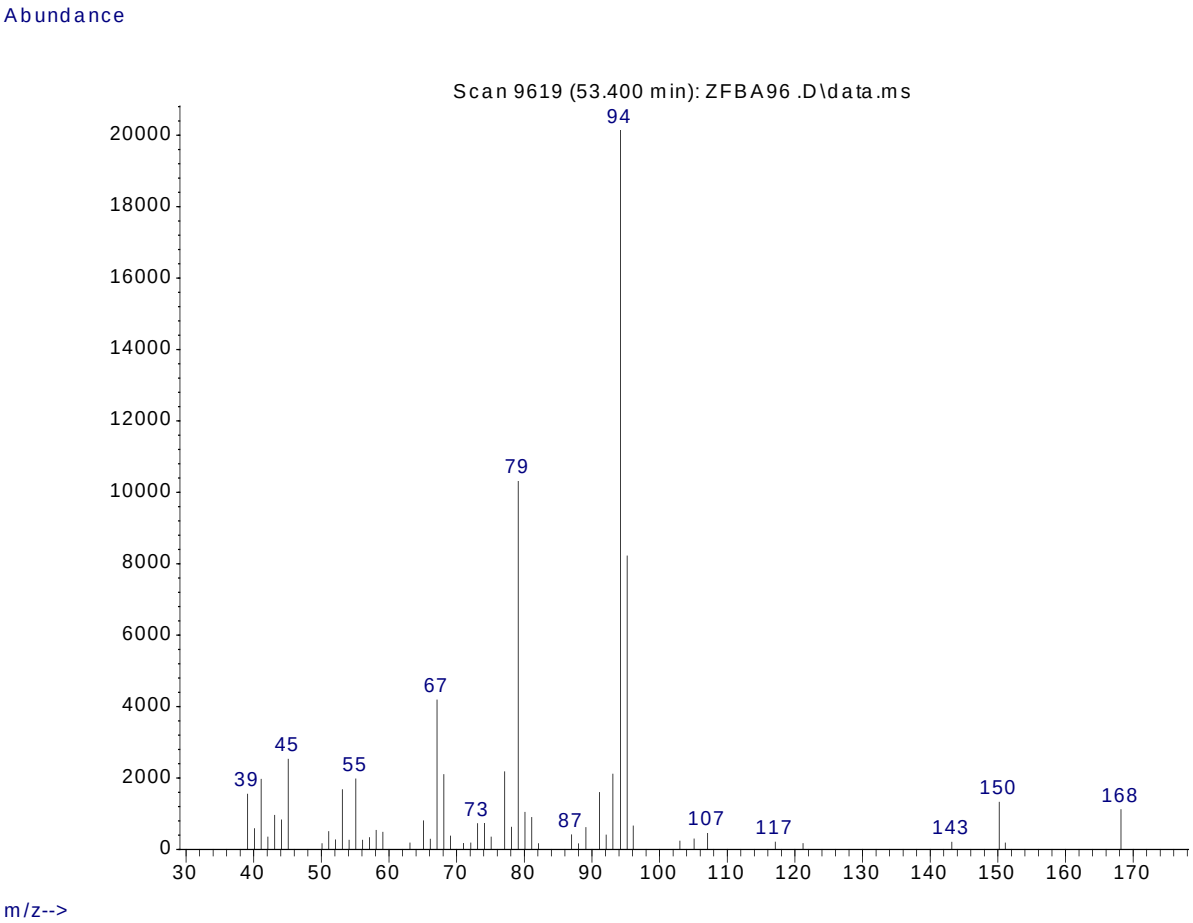


Figure S6: MS spectrum of **M3** (GC-MS data)

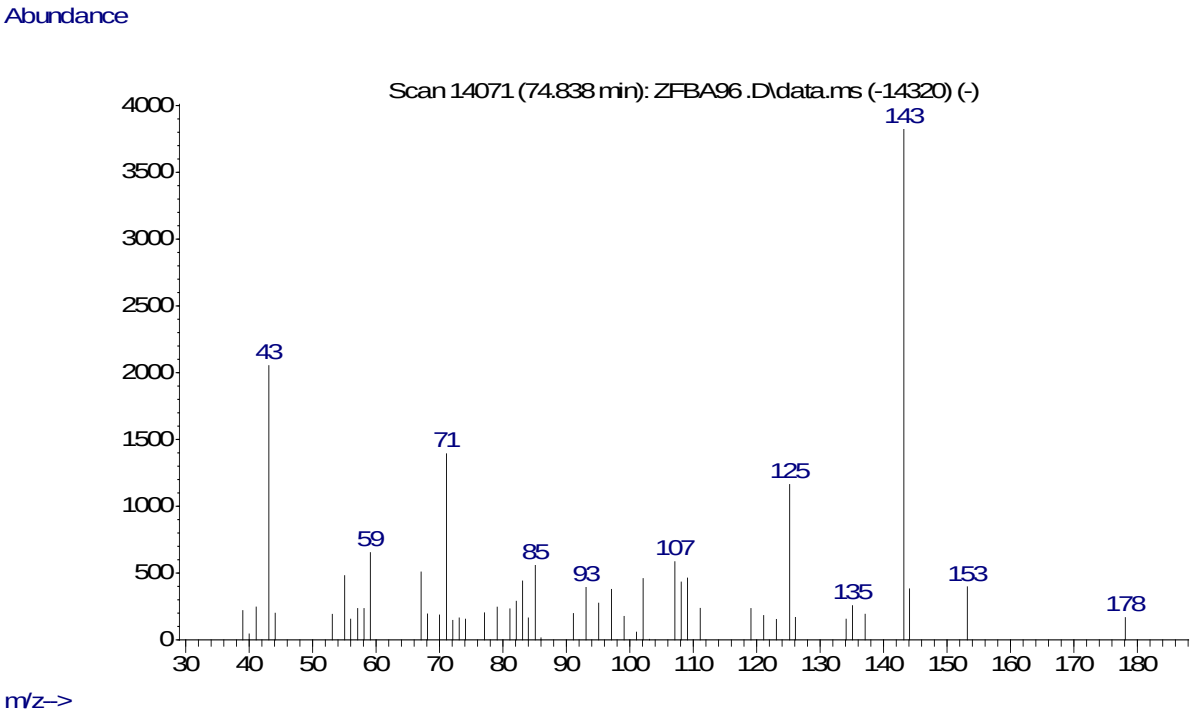


Figure S7: MS spectrum of **M4** (GC-MS data)

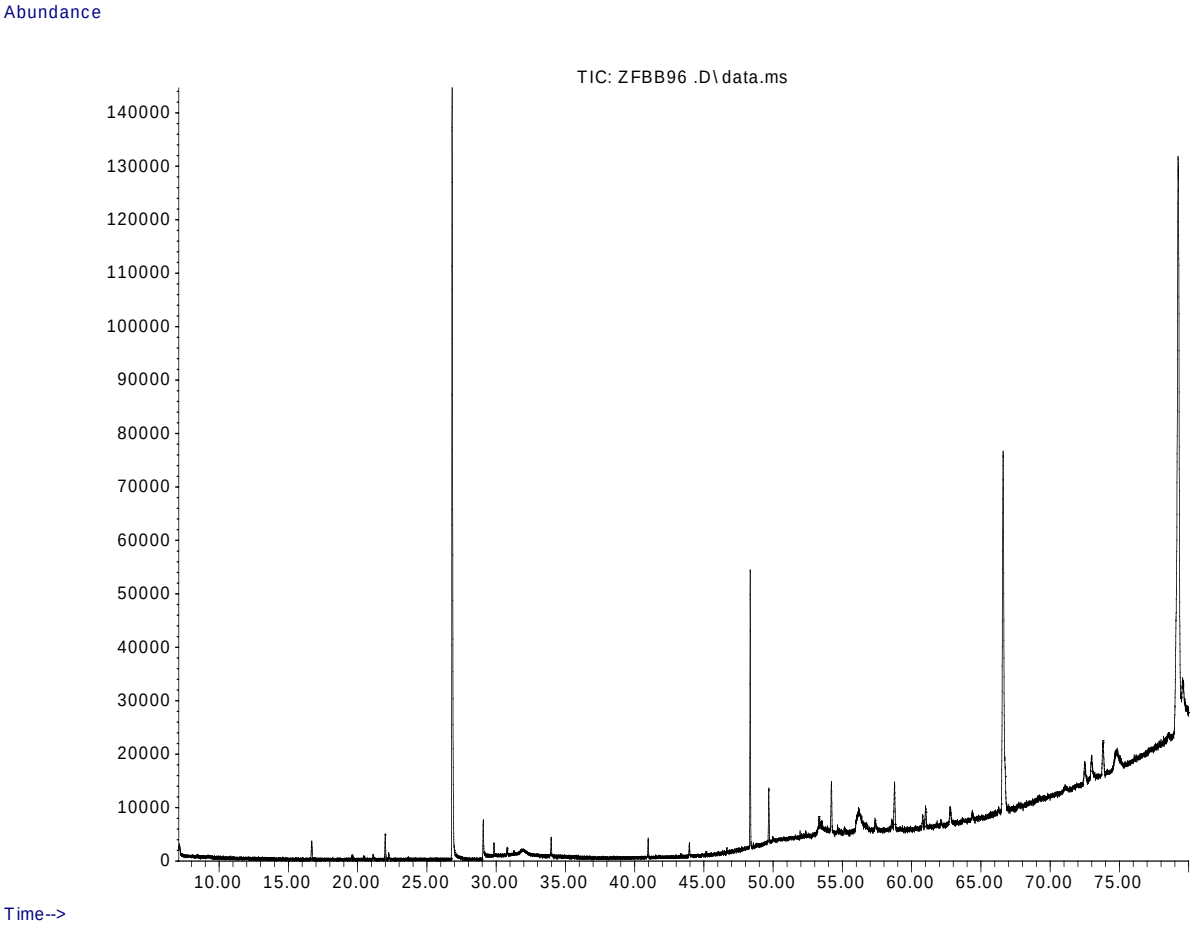


Figure S8: GC-MS analysis and chromatogram for **E2**

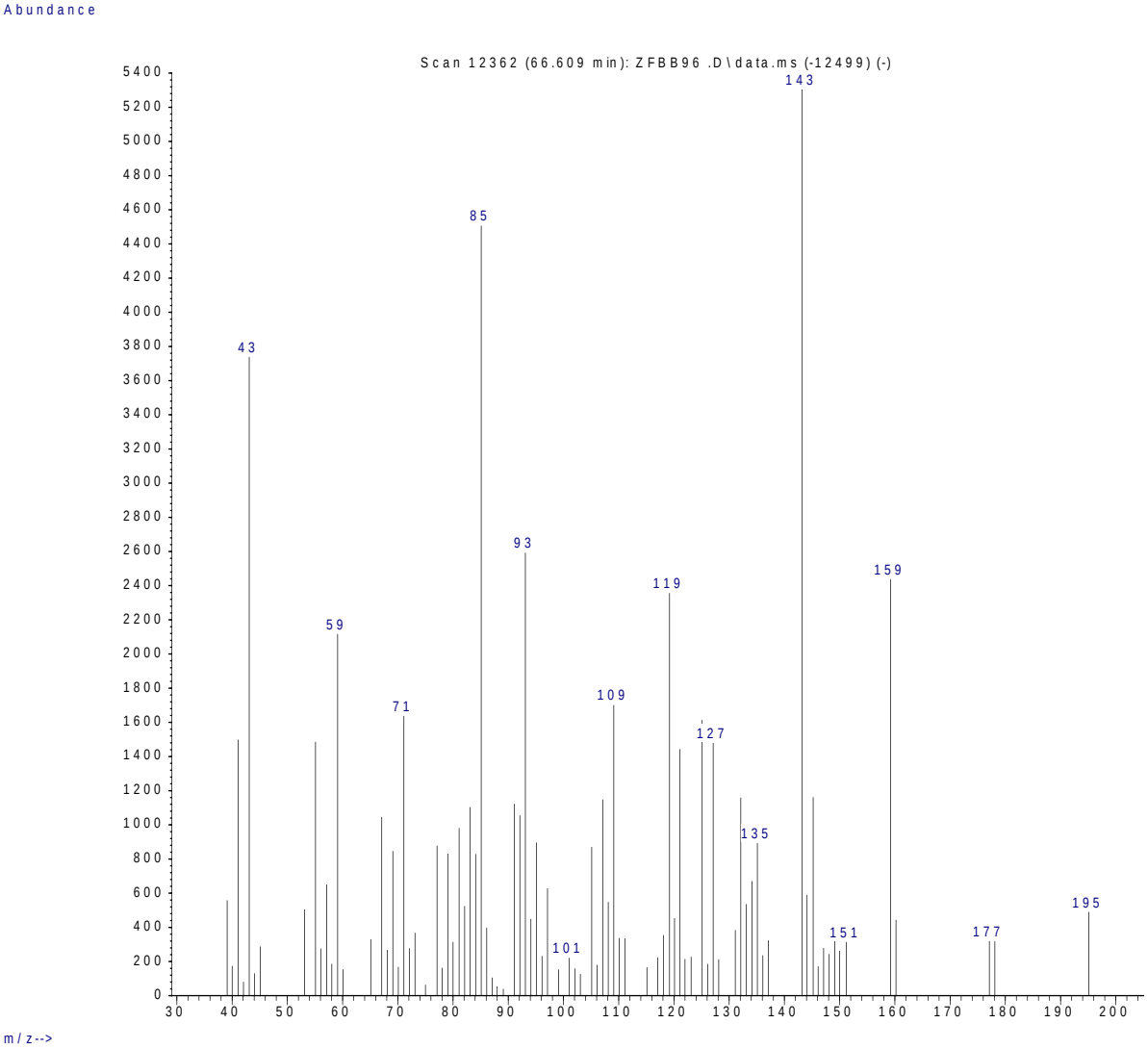


Figure S9: MS spectrum of **M5** (GC-MS data)

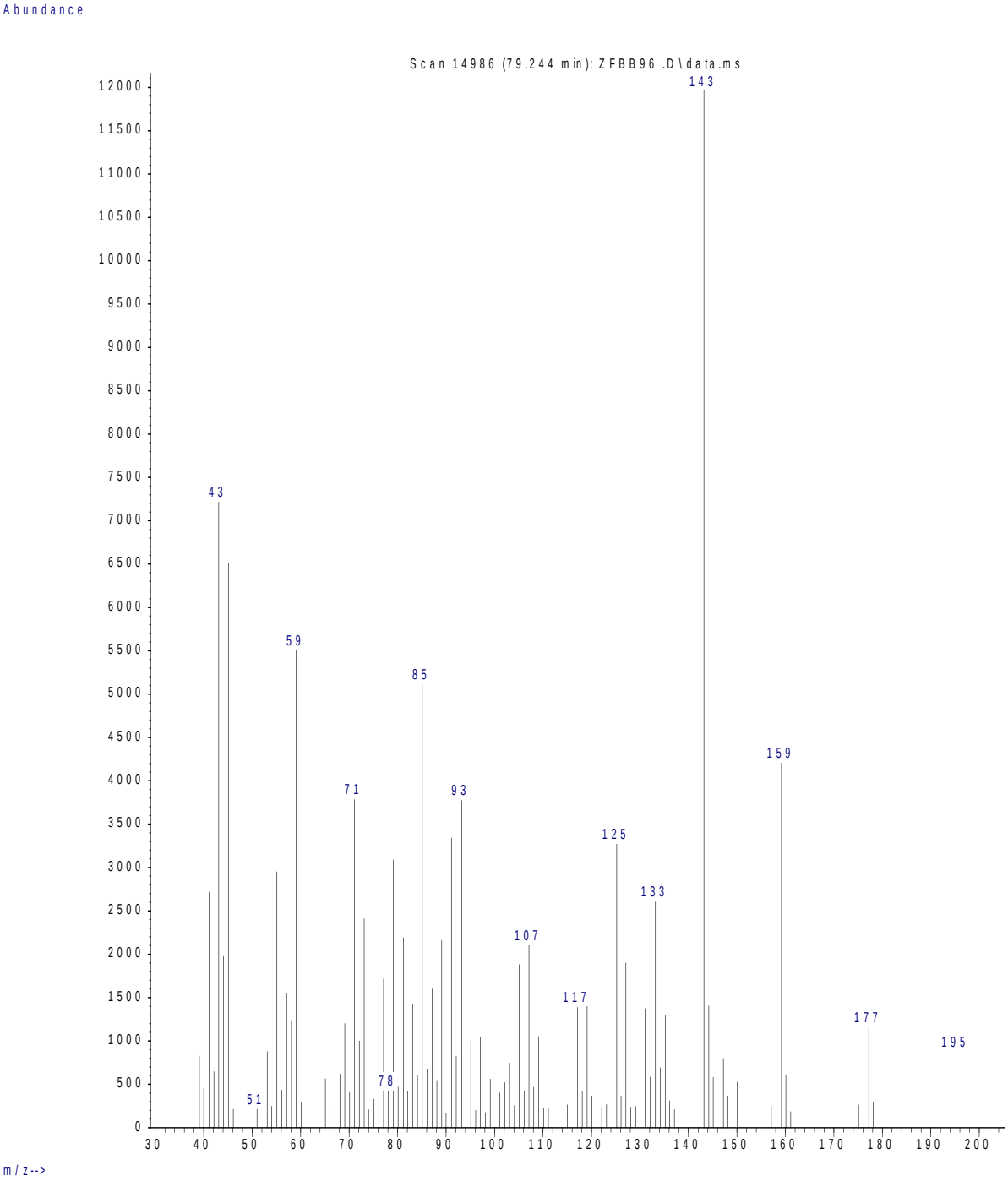


Figure S10: MS spectrum of **M6** (GC-MS data)

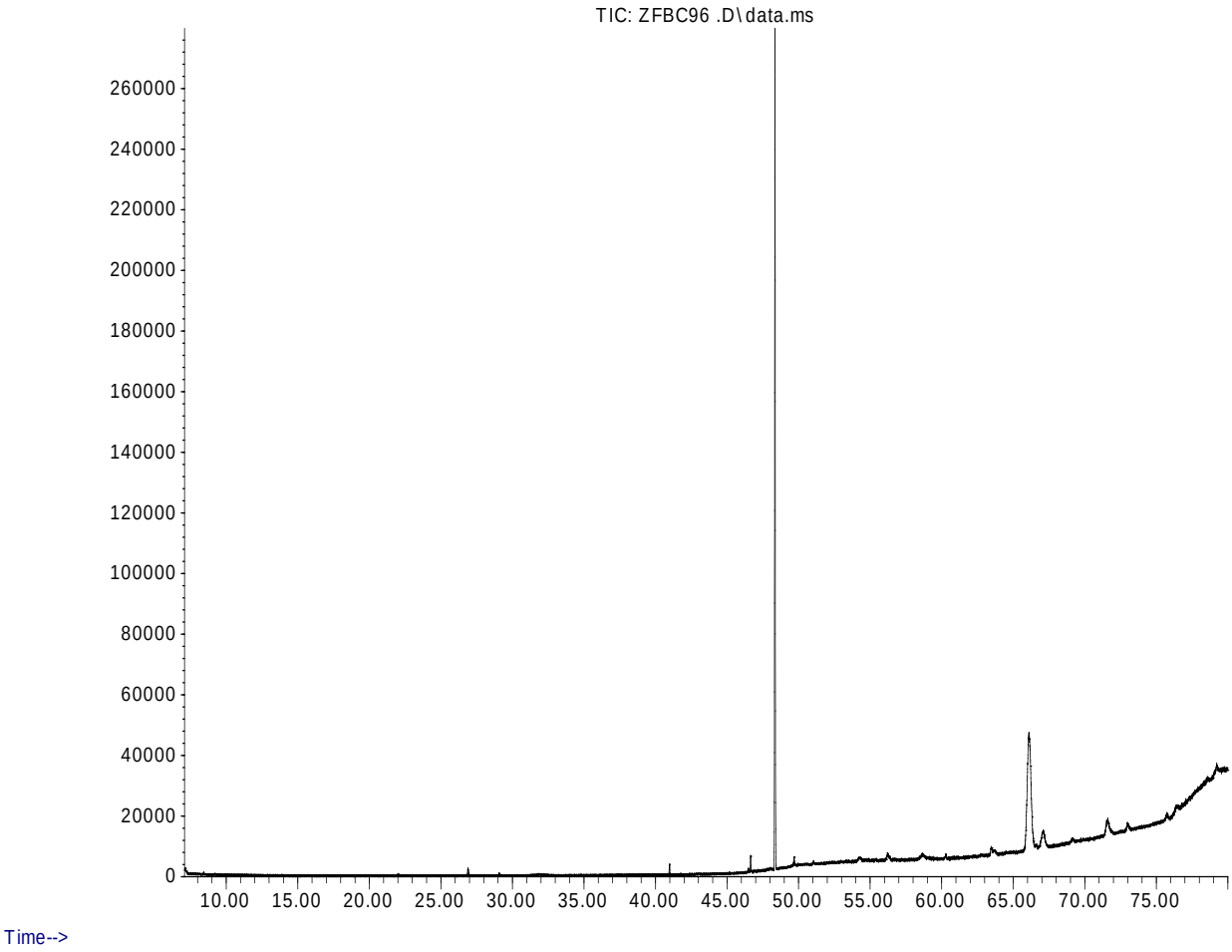


Figure S11: GC-MS analysis and chromatogram for **E3**

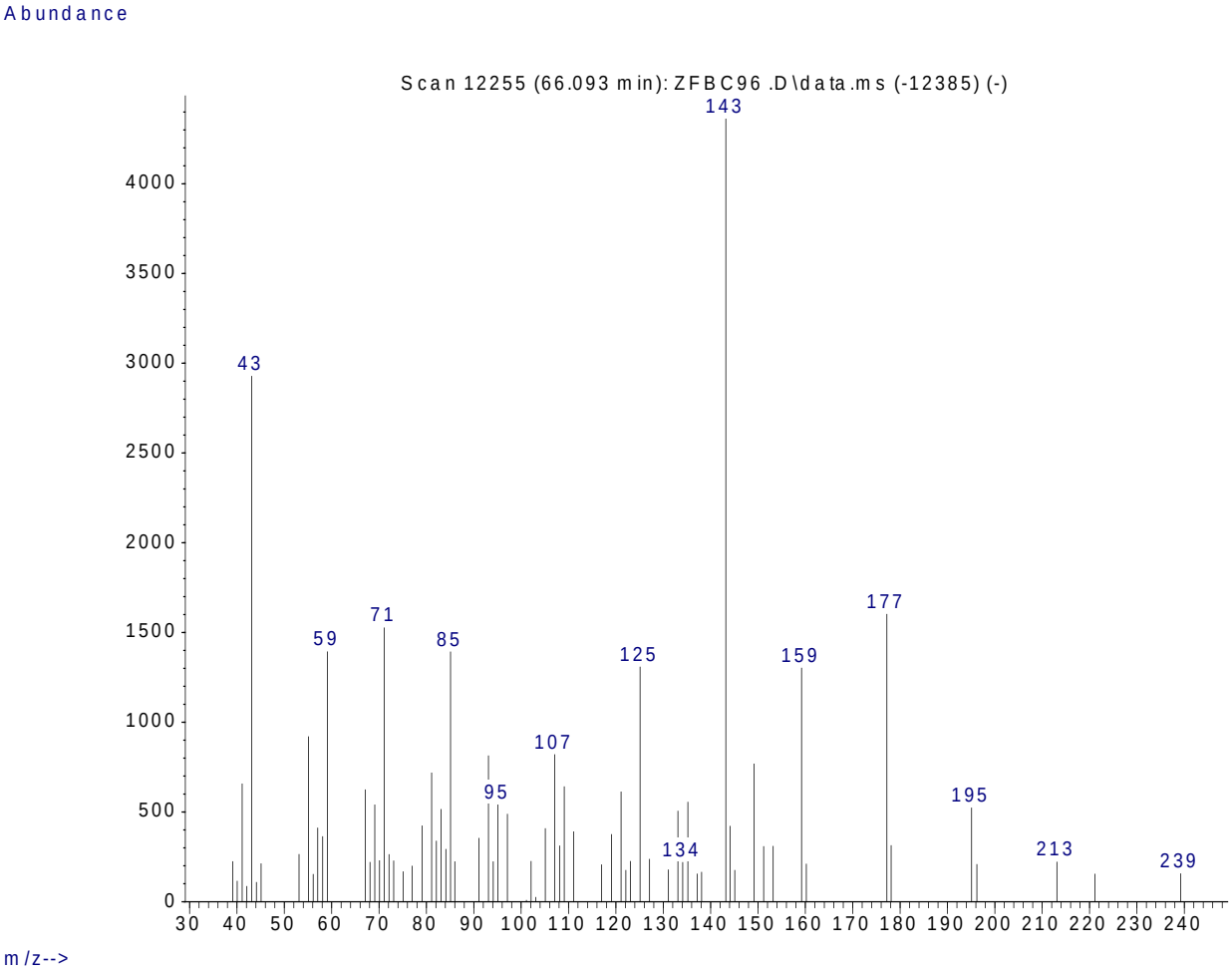


Figure S12: MS spectrum of **M7** (GC-MS data)

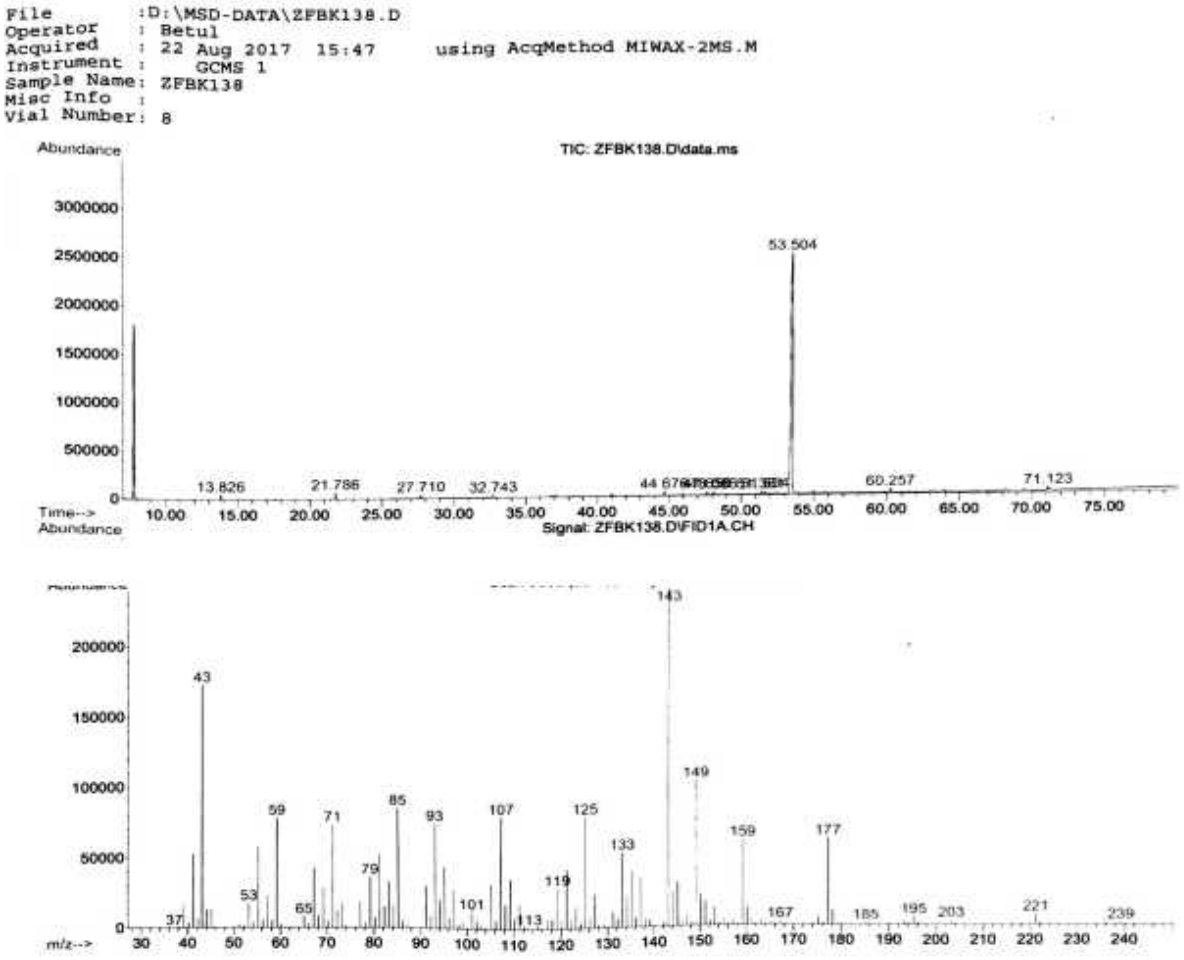


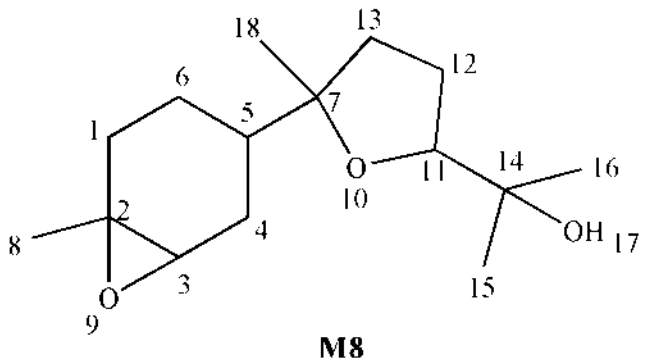
Figure S13: GC and GC-MS analysis for metabolite **M8**

Table S1: GC-MS analysis results of (–)-α-bisabolol and biotransformation metabolites

Substrate		**RT (min)**	***m/z* (Relative intensity, %)**	
(–)-*α*-Bisabolol		47.4	M^+ 222, 204 (33), 189 (6), 161 (15), 147 (6), 134 (11), 119 (97), **109 (100)**, 93 (46), 79 (20), 69 (85), 55 (23), 43 (63)	
Extracts	**Metabolites**	**RT (min)**	***m/z* (Relative intensity, %)**	**%***
E1	M1	46.6	M^+ 238 (2), 220 (5), 205 (3), 179 (27), 161 (55), **143 (100),** 134 (39), 125 (34), 107 (24), 105 (74), 95 (27), 85 (58), 71 (35), 59 (47), 43 (55)	10
	M2	53.3	M^+ 238 (1), 220 (2), 180 (3), 159 (3), **143 (100)**, 134 (18), 125 (36), 107 (26), 93 (37), 81 (12), 71 (24), 59 (17), 43 (40)	20
	M3	53.4	168 (5), 150 (6), 143 (1), 107 (2), **94 (100)**, 87 (2), 79 (51), 73 (4), 67 (21), 55 (10), 45 (12), 39 (8)	7
	M4	74.8	178 (6), 153 (9), **143 (100)**, 135 (8), 125 (30), 107 (15), 93 (9), 85 (15), 71 (36), 59 (17), 43 (53)	10
E2	M5	66.6	195 (9), 177 (5), 159 (46), **143 (100)**, 135 (17), 125 (29), 119 (46), 107 (20), 93 (49), 85 (85), 71 (31), 59 (40), 43 (71)	20
	M6	79.2	195 (8), 177 (9), 159 (35), **143 (100)**, 133(22), 125(28), 117 (12), 107 (17), 93 (31), 85 (35), 71 (32), 59 (46), 43 (60)	45
E3	M7	66.1	239 (4), 213 (5), 195 (12), 177 (36), 159 (30), **143 (100)**, 125 (30), 107 (18), 95 (12), 85 (32), 71 (35), 59 (32), 43 (66)	44
E4	M8	53.5	239 (1), 221 (3), 195 (2), 177 (26), 159 (25), 149 (43), **143 (100)**, 133 (22), 125 (32), 119 (12), 107 (32), 93 (31), 85 (35), 71 (30), 59 (32), 43 (72)	94

RT, retention time

%*, all figures are mean of triple analyses and reported as relative percentages.



M8

IUPAC Name: 2-(5-methyl-5-(6-methyl-7-oxabicyclo[4.1.0]heptan-3-yl)tetrahydrofuran-2-yl)propan-2-ol

Acronym: bisafuranol

Figure S14: IUPAC name and numbering of **M8**

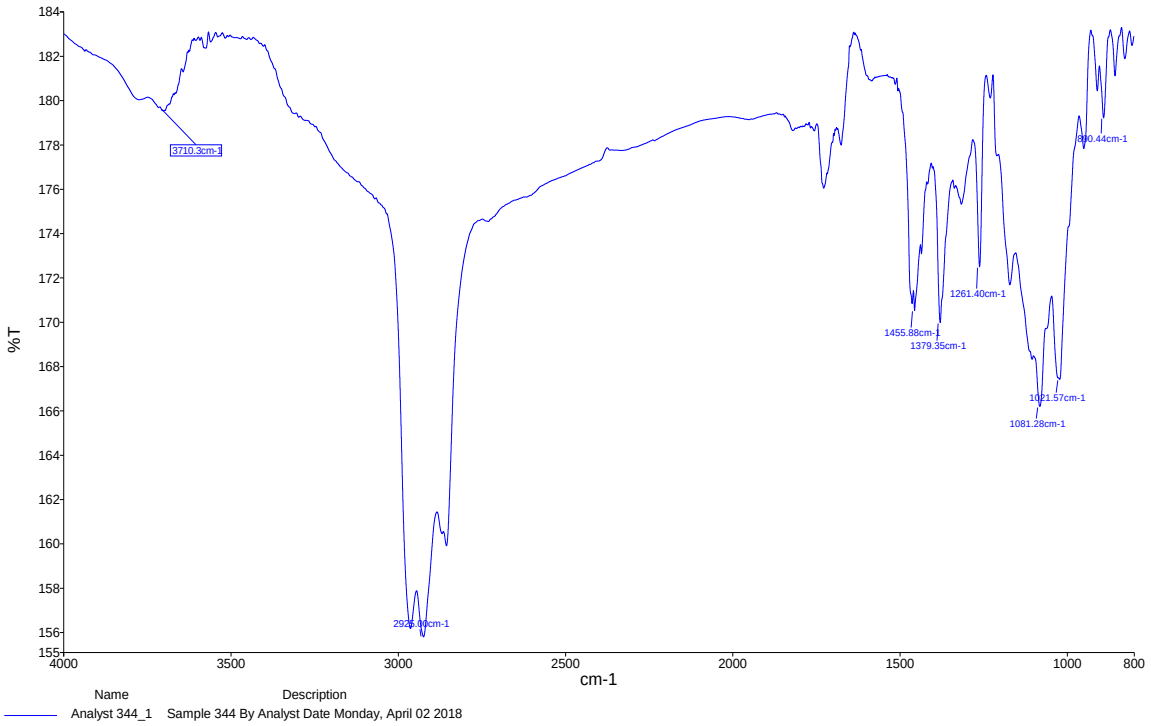


Figure S15: FT-IR analysis for metabolite **M8**

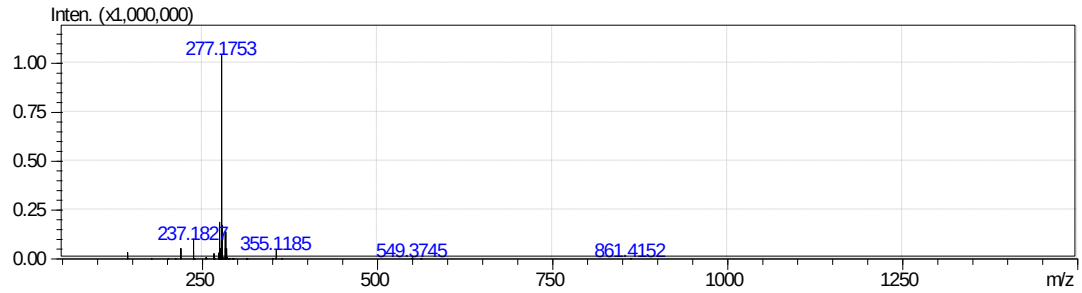


Figure S16: HR-ESI-MS for metabolite **M8**

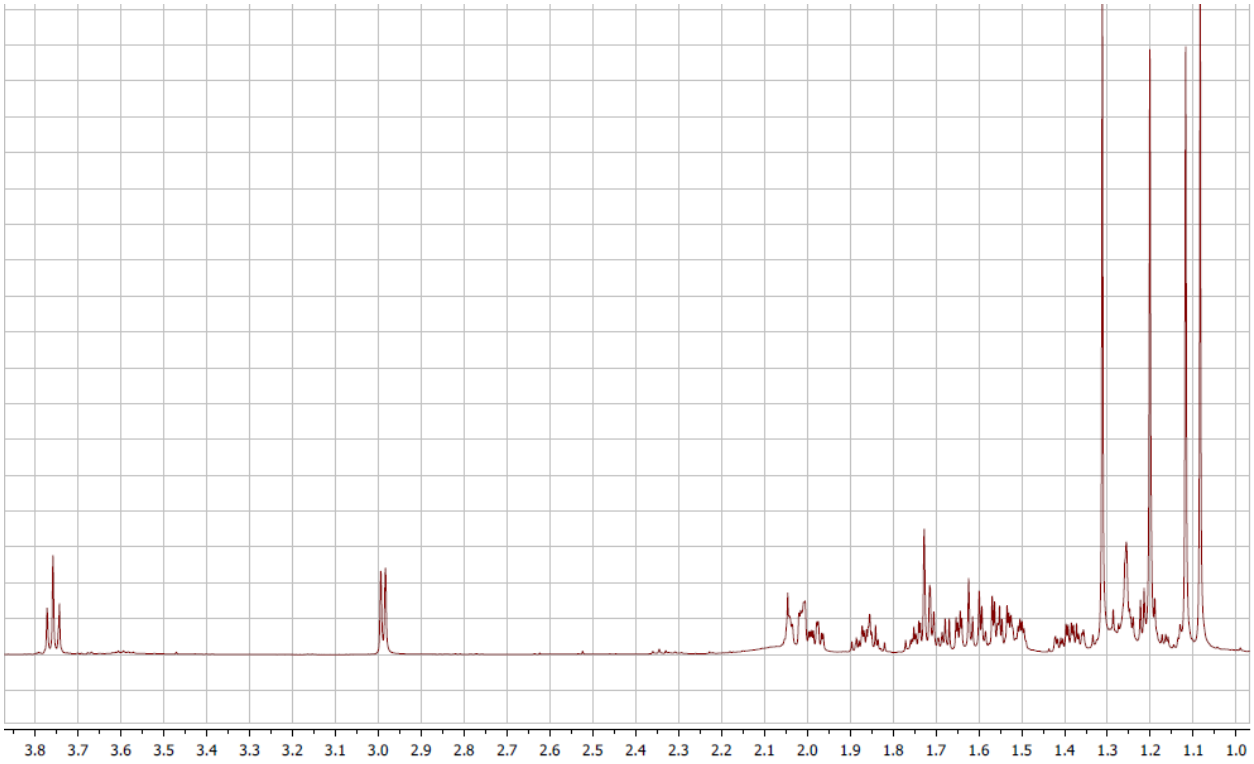


Figure S17: ^{1}H NMR (500 MHz) spectrum of **M8**

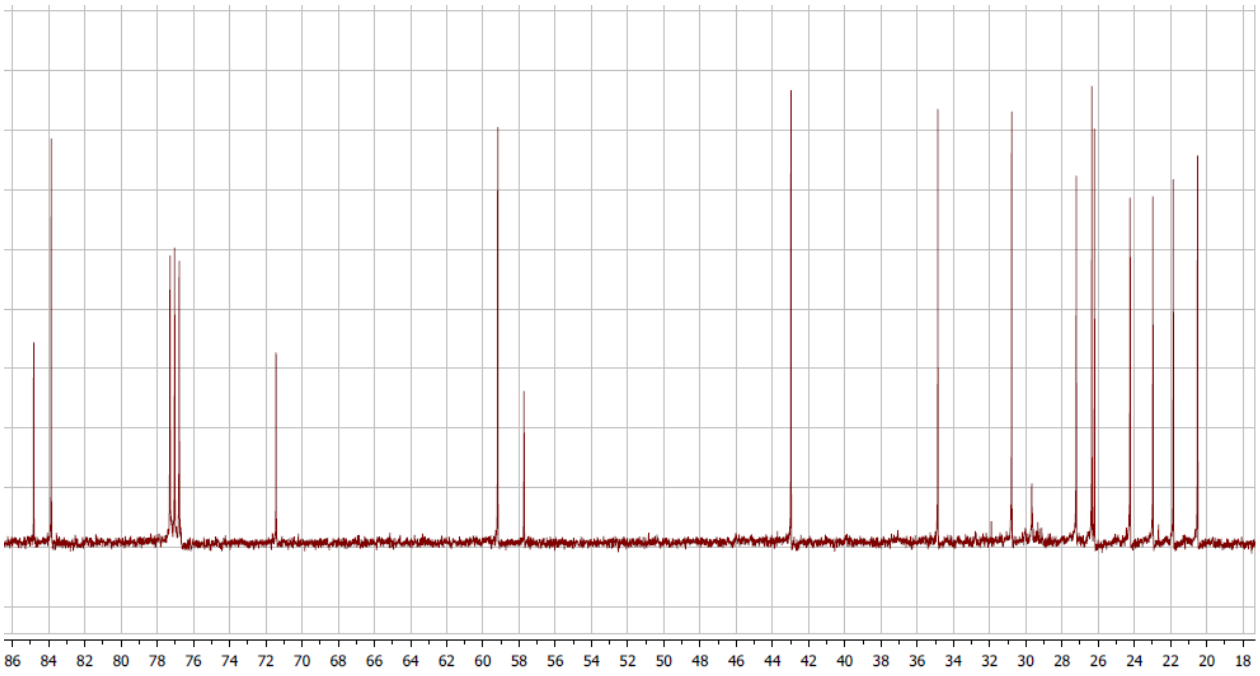


Figure S18: ^{13}C-NMR (125 MHz) spectrum of **M8**

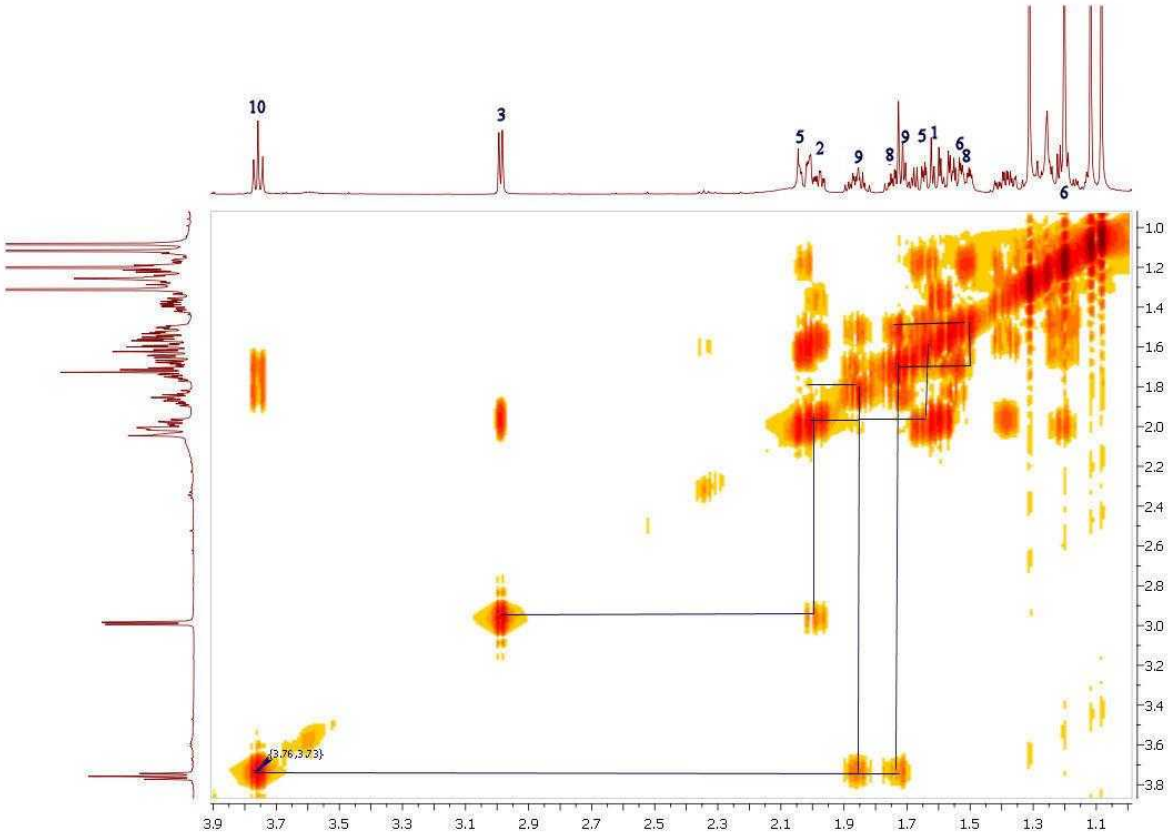


Figure S19: 2D ^{1}H-^{1}H COSY NMR spectrum of **M8**

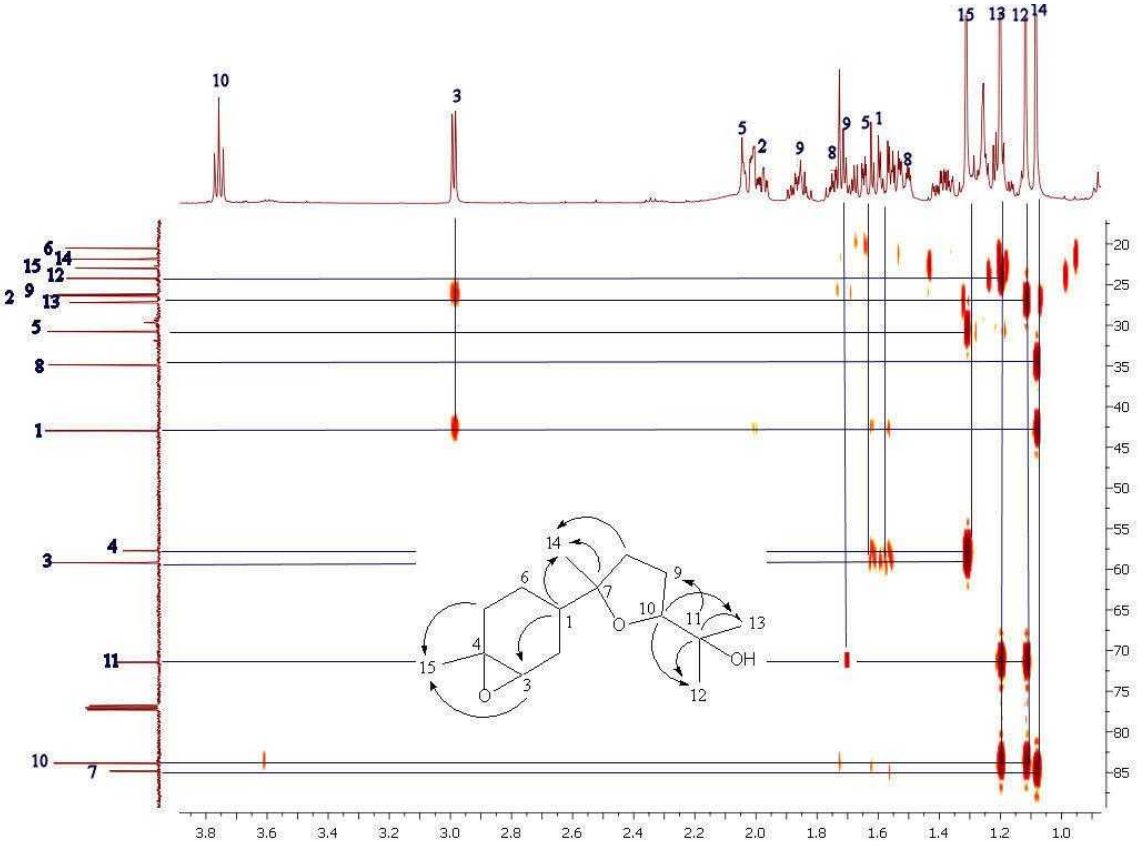


Figure S20: 2D ^{1}H-^{13}C HMBC NMR spectrum of **M8**

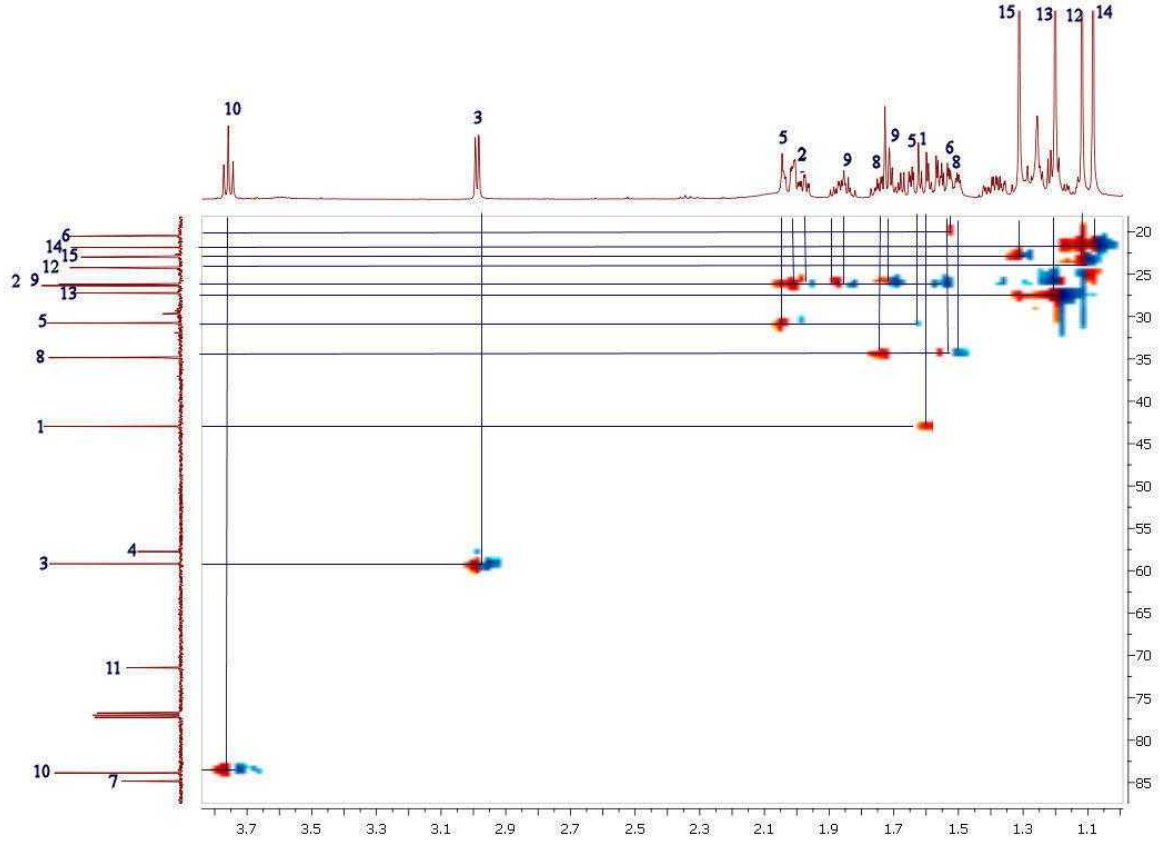


Figure S21: 2D ^{1}H - ^{13}C HSQC NMR spectrum of **M8**

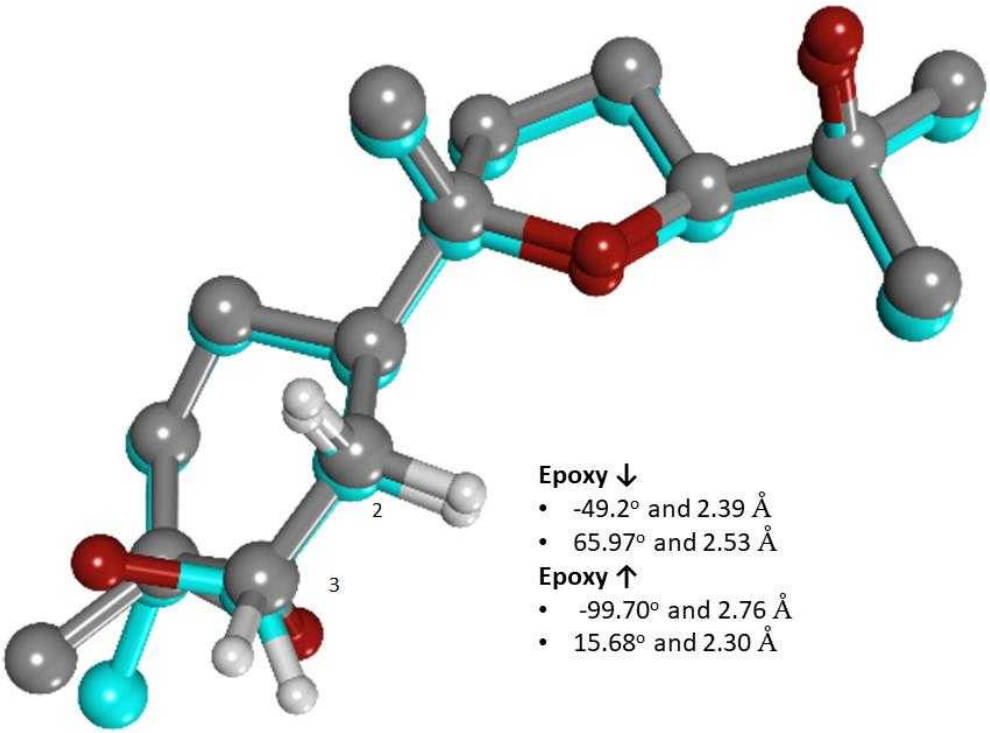


Figure S22: MOPAC analysis of **M8**

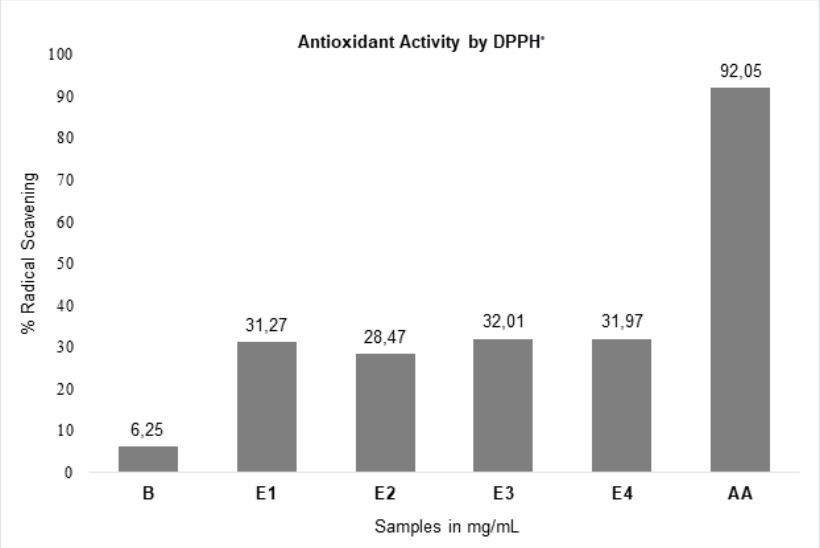


Figure S23. *In vitro* antioxidant activity of (–)-*α*-bisabolol and metabolite extracts by using DPPH• assay (**B**, (–)-*α*-bisabolol; biotransformation mixture by *T. elegans*, **E1**; *M. ramannianus*, **E2**; *T. harzianum*, **E3***; P. neocrassum*, **E4**; **AA**, Ascorbic acid)