SUPPLEMANTARY MATERIAL

Fungal Biotransformation of Carvone and Camphor by *Aspergillus flavus* and Investigation of Cytotoxic Activities of Naturally Obtained Essential Oils

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ABSTRACT

In this study, the biotransformation of carvone and camphor by *Aspergillus flavus* and the products were investigated. The biotransformation reaction of carvone by *A. flavus* resulted in the production of neodihydrocarveol, dihydrocarvone, 2-cyclohexene-1-one,2-methyl-5-(1-methylethenyl), limonene-1,2-diol, trans-p-mentha-1(7),8-dien-2-ol, p-menth-8(10)-ene-2,9-diol, and the biotransformation reaction of camphor resulted in the production of 2 -campholenic acid, 2-cyclohexene-1-one,2-hydroxy-4,4,6,6-tetramethyl, α-campholene aldehyde. The naturally produced essential oils by biotransformation of carvone and camphor were observed to be cytotoxic to breast cancer cells while no significant inhibition was seen in the healthy cell line. Additionally, biotransformation products had the highest inhibition (74%) against aflatoxin B1. The bioactivities of biotransformation products are promising, and they can be further investigated for their therapeutic potential as active agents.

Keywords: *Aspergillus flavus*, biotransformation, carvone, camphor, cytotoxicity, monoterpene

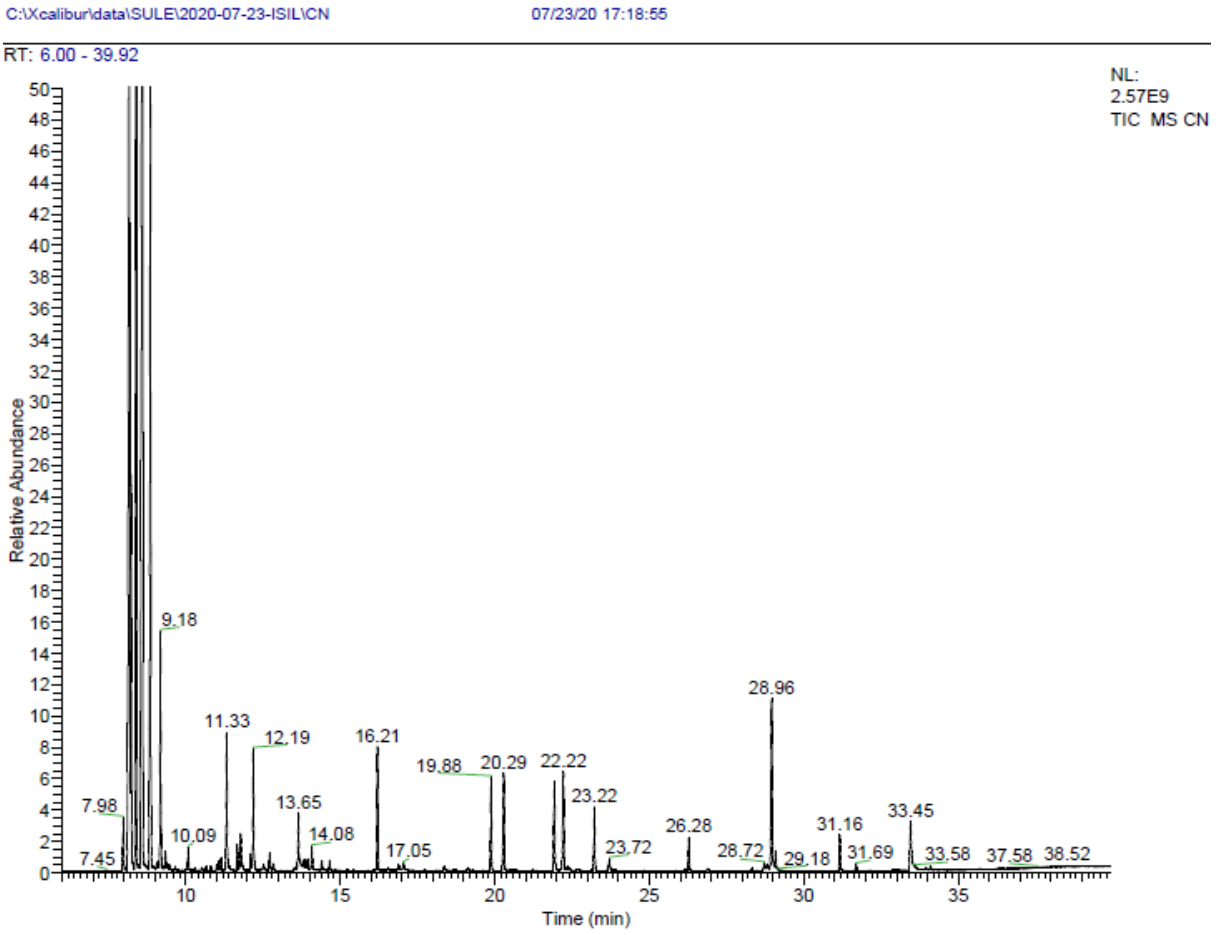


Figure S1. GC-MS chromatogram of naturally obttained essential oils by carvone biotransformation

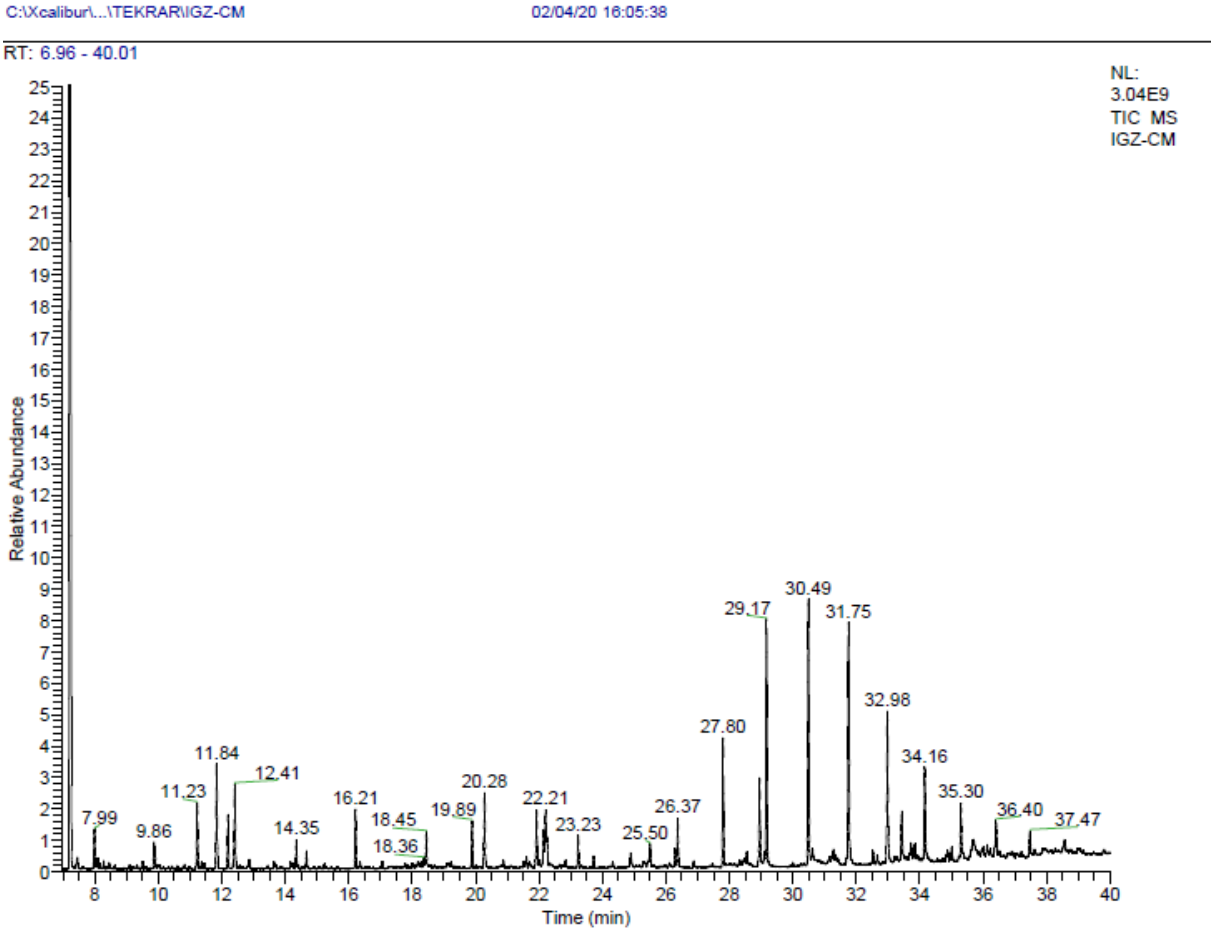


Figure S2. GC-MS chromatogram of naturally obtained essential oils by camphor biotransformation

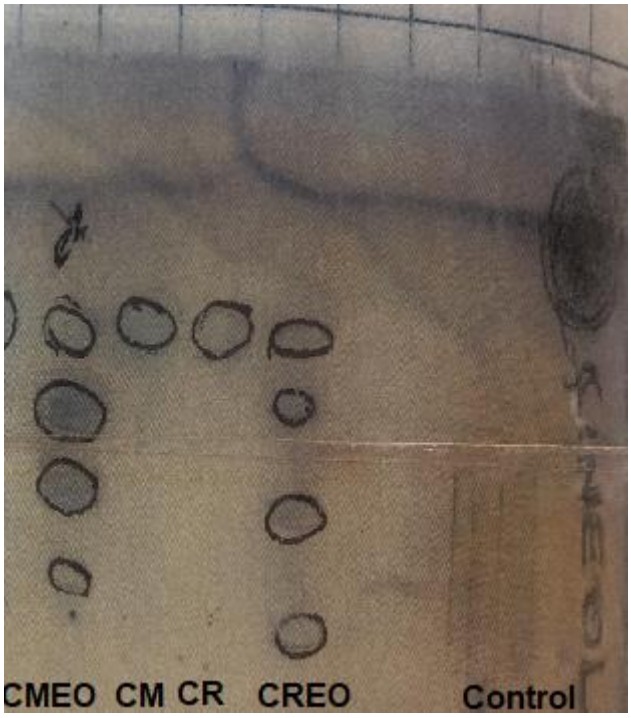


Figure S3. TLC chromatogram of naturally obtained essential oils by camphor (CMEO), carvone (CREO), camphor standard (CM), carvone standard (CR), and control (Sprayed with a 1% solution of vanillin in conc. sulfuric acid and at 120° C until giving blue color)

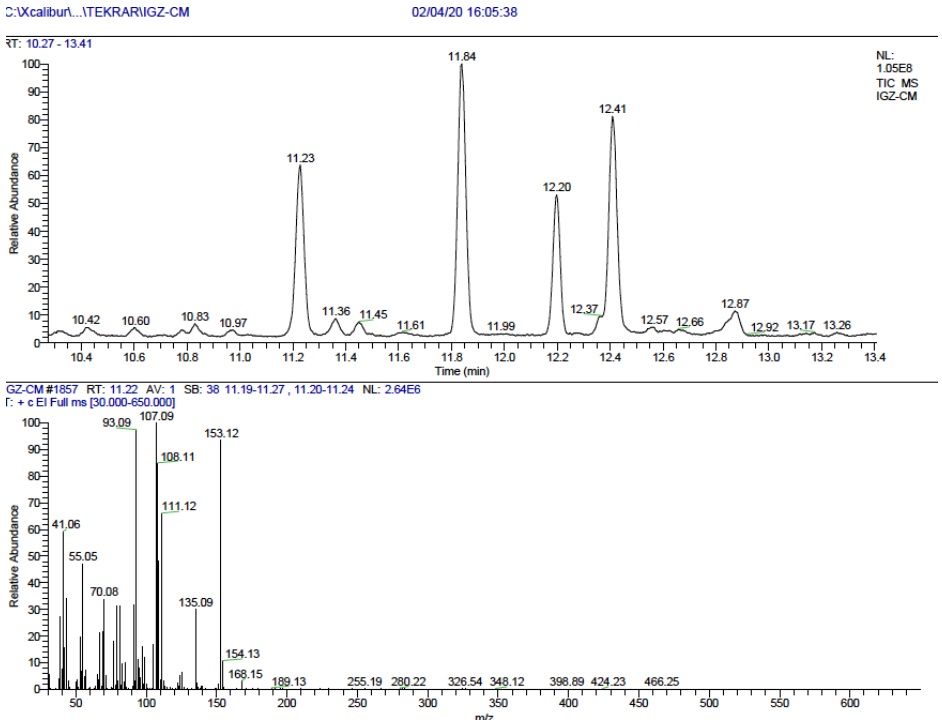


Figure S4. GC chromatogram of the campholenic acid (RT:11.22)

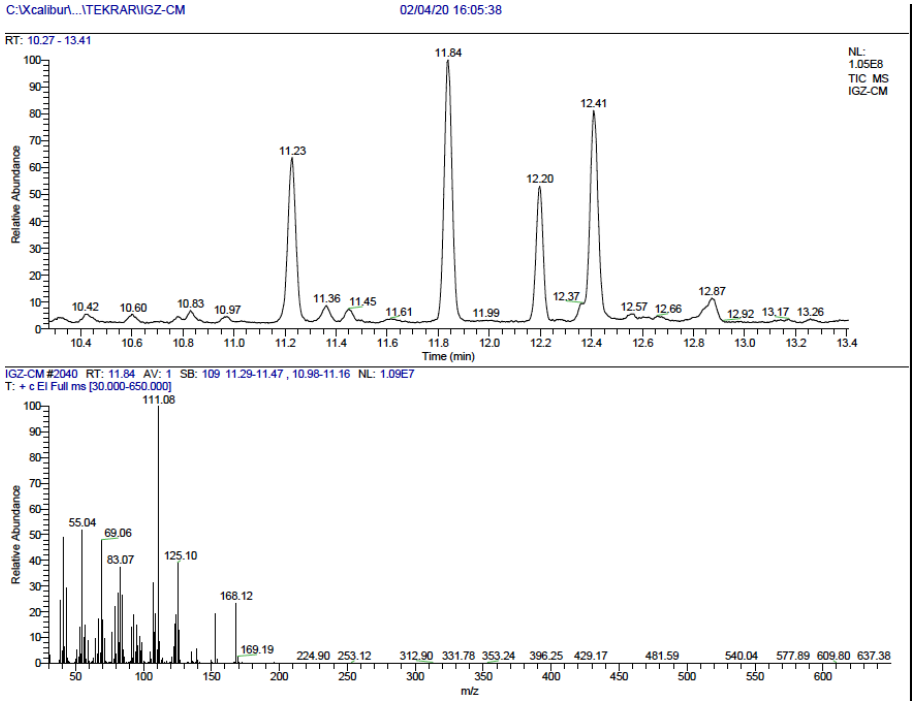


Figure S5. GC chromatogram of the 2-cyclohexen-1-one,2-hydroxy-4,4,6,6-tetramethyl (RT:11.84)

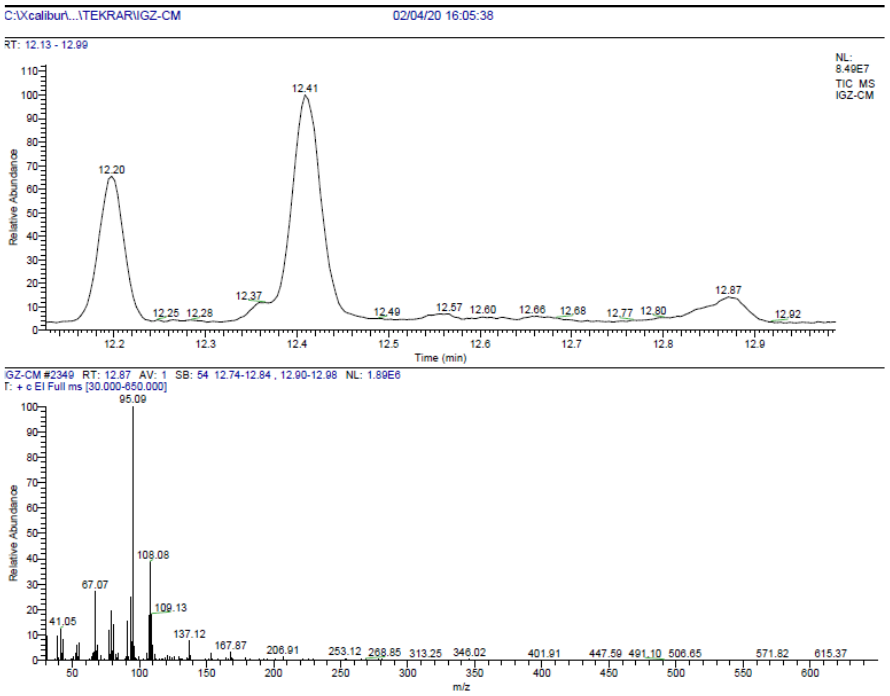


Figure S6. GC chromatogram of the campholene aldeyhyde (RT:12.87)

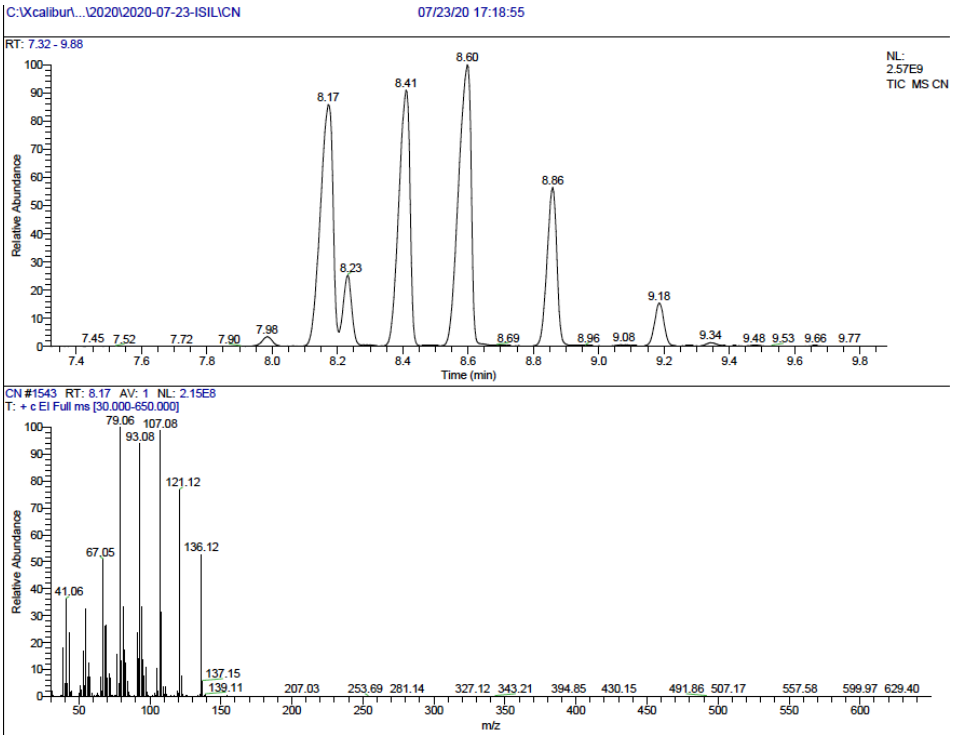


Figure S7. GC chromatogram of the neohydrocarveol (RT:8.17)

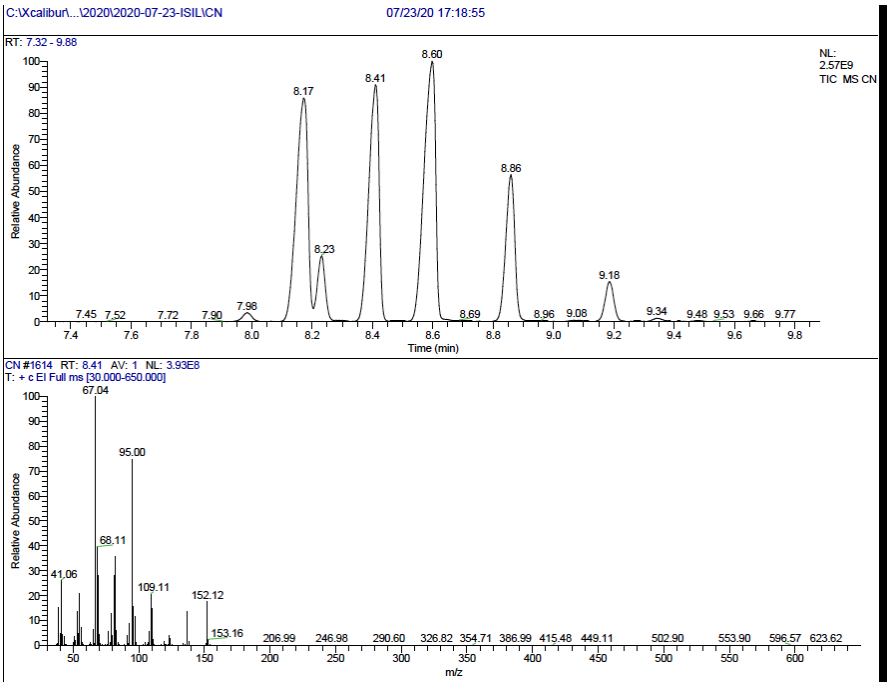


Figure S8. GC chromatogram of the dihydrocarvone (RT:8.41)

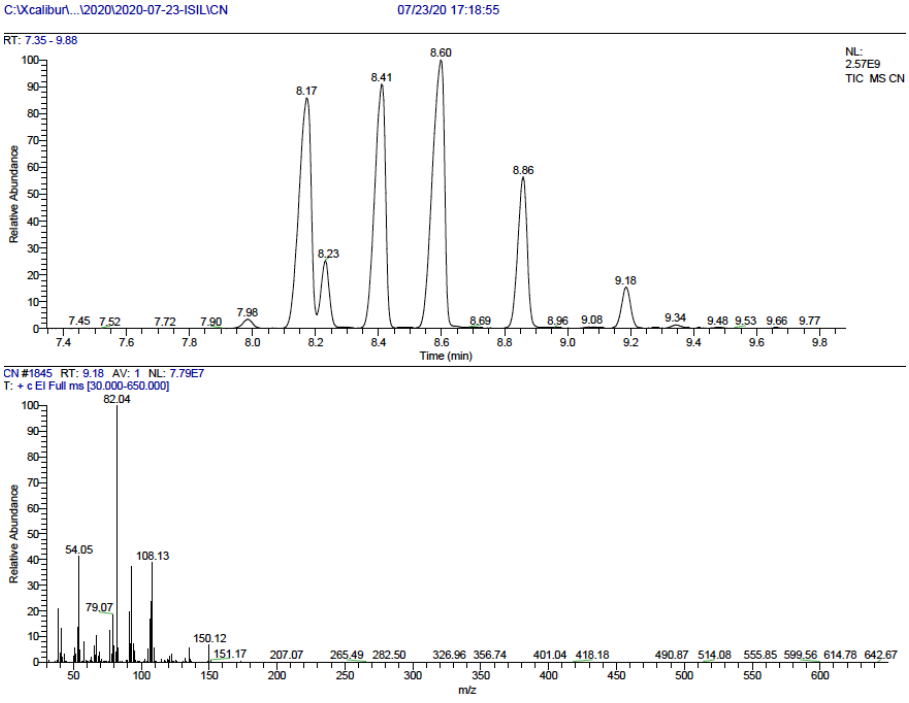


Figure S9. GC chromatogram of the 2-cyclohexen-1-one,2-methyl-5-(1-methylethenyl) (RT:9.18)

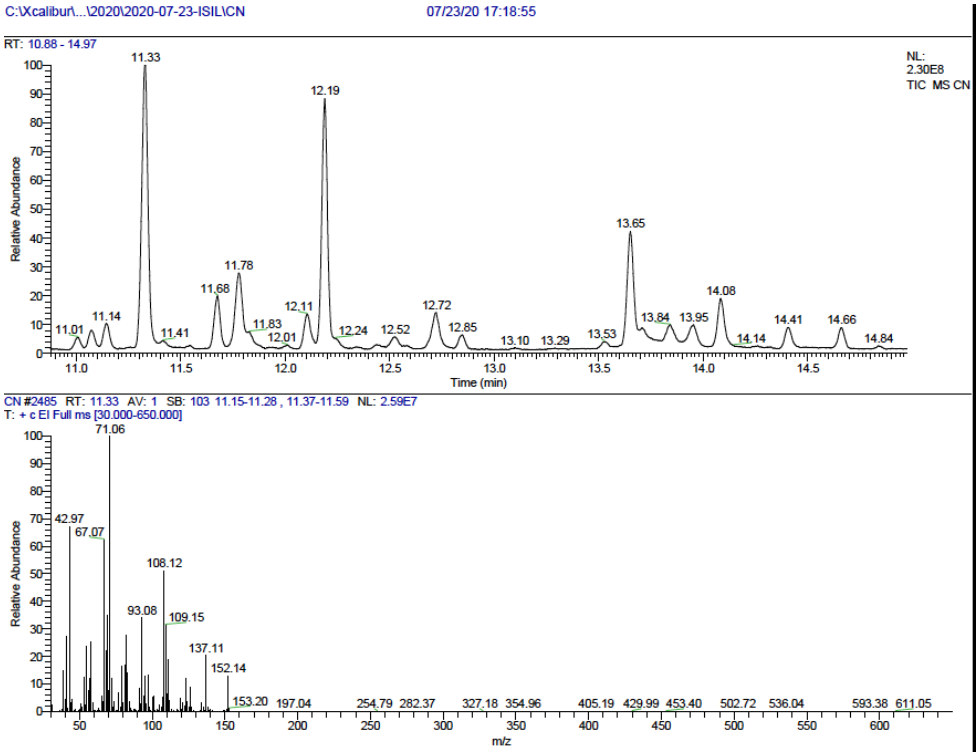


Figure S10. GC chromatogram of the limonene-1,2-diol (RT:11.33)

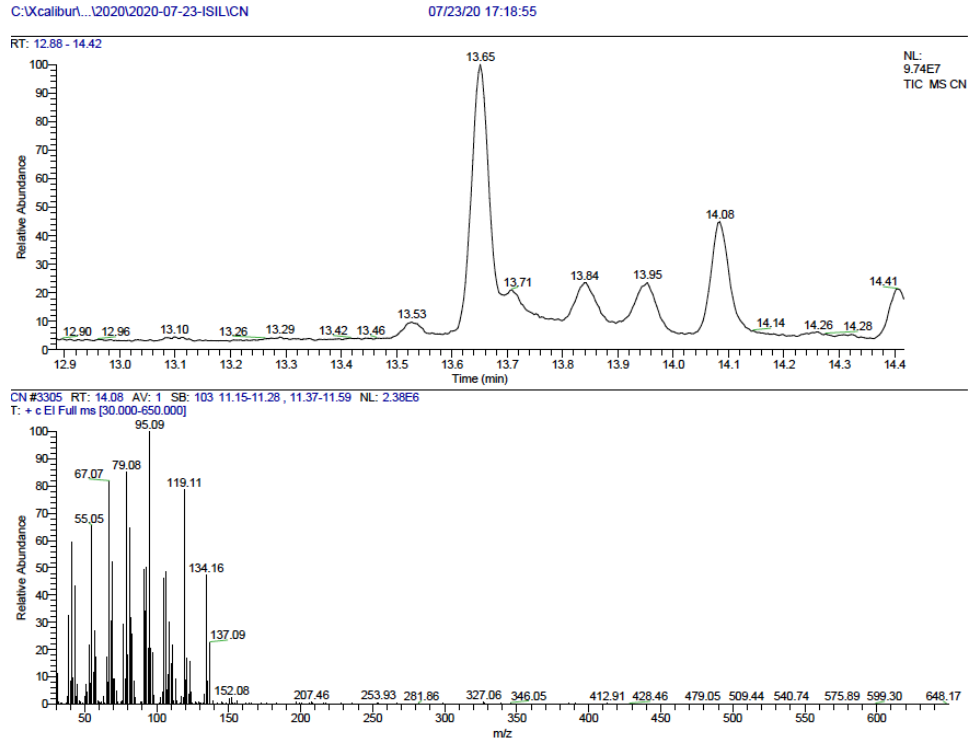


Figure S11. GC chromatogram of the trans-*p*-mentha-1(7),8-dien-2-ol (RT:14.08)

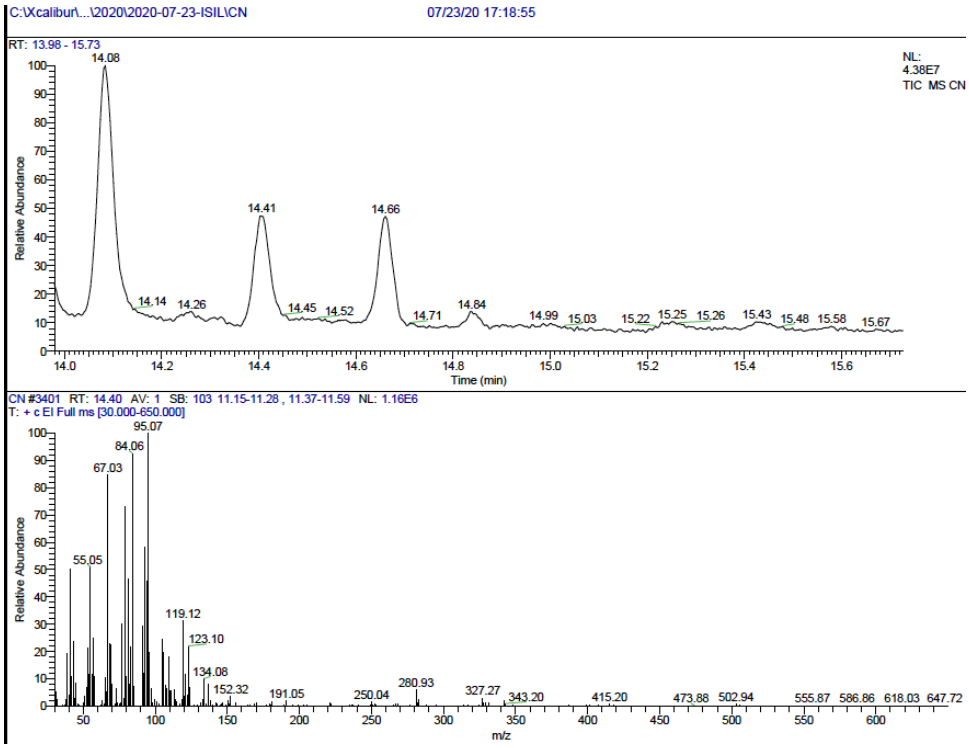


Figure S12. GC chromatogram of the *p*-menth-8(10)-ene-2,9-diol (RT:14.40)

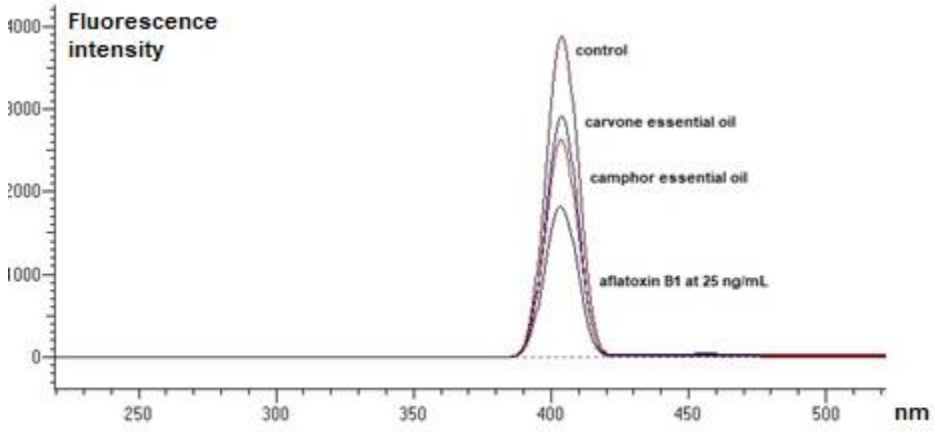


Figure S13. The fluorescence intensities of control, naturally obtained essential oils by camphor and carvone biotransformation and aflatoxin B1 standard at 25 ng/mL.

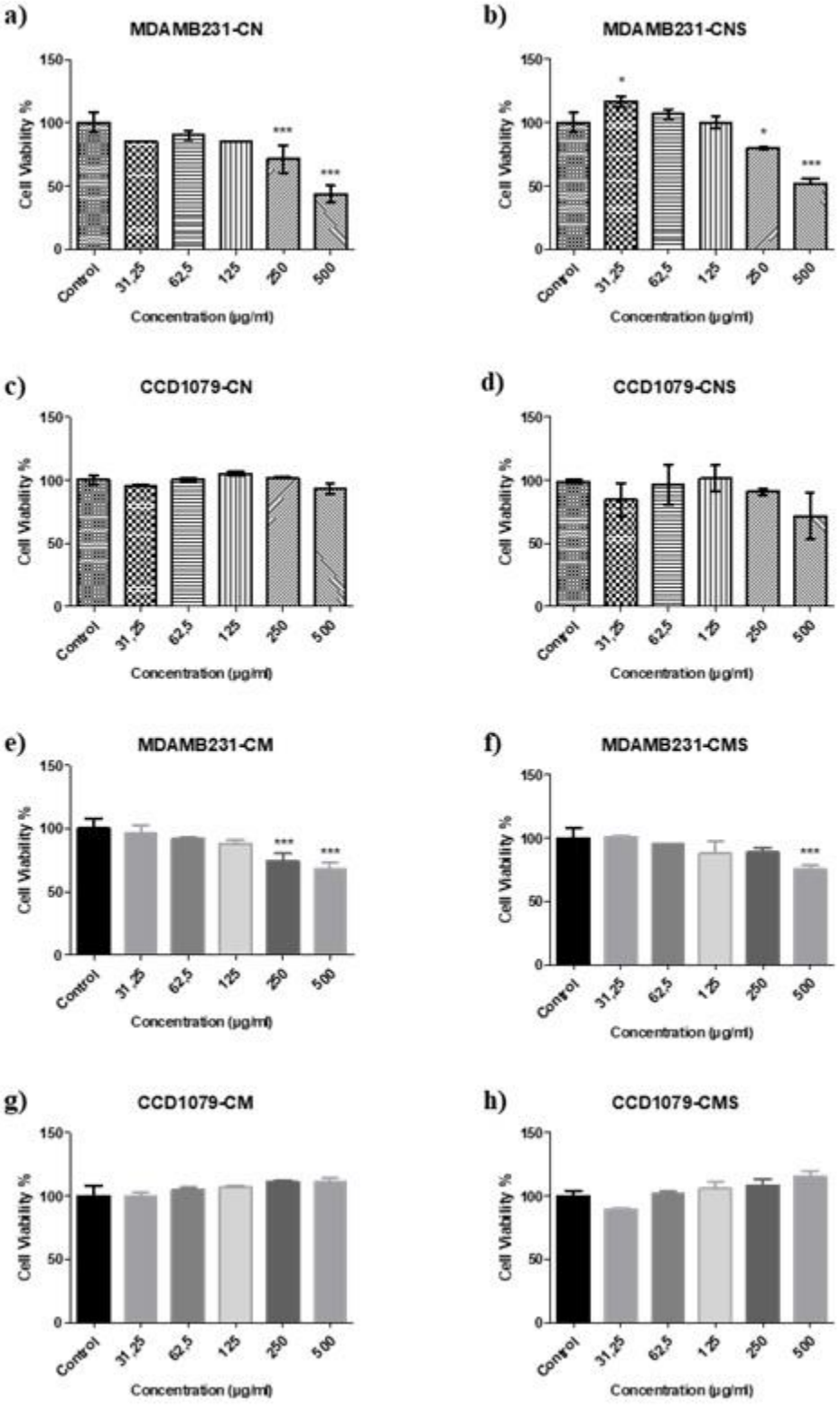


Figure S14. MTT assay results, effect of obtained essential oil from carvone biotransformation (CN), carvone standard (CNS), obtained essential oil from camphor biotransformation (CM), and camphor standard (CMS) on MDA-MB-231 and CCD-1079Sk cell viability following 24 h treatment. Treatment concentrations: 0-500 µg/ml. Data are given as mean= SD, *p<0.05, **p<0.01, ***p<0.001 versus control group.

Table S1. The composition of obtained essential oil by carvone biotransformation

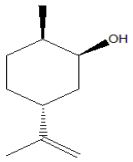
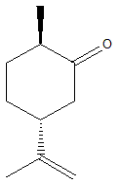
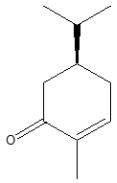
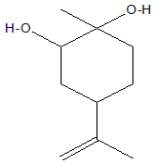
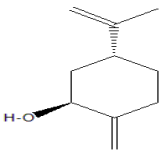
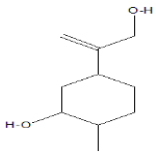
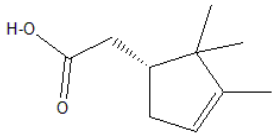
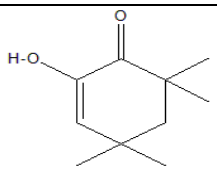
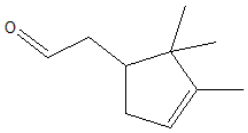
RT	Compound Name	Molecular Formula	Structural Formula	% Area	RSI
8.17	neodihydrocarveol	$C_{10}H_{18}O$		24.87	932
8.41	dihydrocarvone	$C_{10}H_{16}O$		18.86	956
9.18	2-cyclohexen-1-one,2-methyl-5-(1-methylethenyl)	$C_{10}H_{14}O$		2.76	948
11.33	limonene-1,2-diol	$C_{10}H_{18}O_2$		1.72	900
14.08	trans-*p*-mentha-1(7),8-dien-2-ol	$C_{10}H_{16}O$		1.32	832
14.40	*p*-menth-8(10)-ene-2,9-diol	$C_{10}H_{18}O_2$		4.89	781

Table S2. The composition of obtained essential oil by camphor biotransformation

RT	**Compound Name**	**Molecular Formula**	**Structural Formula**	**% Area**	**RSI**
11.23	(-)-α-campholenic acid	$C_{10}H_{16}O_2$		0.97	923
11.84	2-cyclohexen-1-one,2-hydroxy-4,4,6,6-tetramethyl	$C_{10}H_{16}O_2$		1.51	940
12.87	α-campholene aldehyde	$C_{10}H_{16}O$		0.25	798