Supplementary Material

Antimicrobial and antibiofilm activity and machine learning classification analysis of essential oils from different Mediterranean plants against *Pseudomonas aeruginosa*

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Table S1. GB parameters used in the grid search for optimal hyperparameterization

N estimators	100	250	500	750	1000	1250	1500	1750
Max depth	3	4	5	6	7	8	9	10
Min samples leaf	1	3	5	7	9	11	13	15
Max features	0.7	0.6	0.5	0.4	0.3	0.2	log2[a]	sqrt[b]

[a] sqrt: max features = sqrt(n features). [b] log2: max features = log2(n features)

Table S2. Relative yields % of EOs over time.

Plant species	**h** [1]	**1**	**2**	**3**	**6**	**12**	**24**
	m [2]						
CG	*Jul.*	0.300	0.350	0.360	0.366	0.370	0.373
	Aug.	0.300	0.360	0.400	0.420	0.426	0.432
	Sep.	0.190	0.250	0.300	0.360	0.376	0.381
	Oct.	0.180	0.260	0.290	0.320	0.328	0.328
FV	*Aug.*	0.070	0.110	0.140	0.180	0.196	0.213
	Sep.	0.090	0.140	0.170	0.200	0.218	0.240
	Oct.	0.360	0.640	0.830	1.090	1.210	1.250
RS	*na*	0.200	0.300	0.440	0.640	0.740	0.800

[1] Extraction hour, [2] Month of harvesting, na – not applicable.

Table S3. EO Yield % calculated on the dried (FV and RS) or fresh (CG) plant material.

Plant species	**h** [1]	**1**	**2**	**3**	**6**	**12**	**24**
	m [2]						
CG	*Jul.*	0.300	0.050	0.010	0.006	0.004	0.003
	Aug.	0.300	0.060	0.040	0.020	0.006	0.006
	Sep.	0.190	0.060	0.050	0.060	0.016	0.005
	Oct.	0.180	0.080	0.030	0.030	0.008	0.0004
FV	*Aug.*	0.070	0.040	0.030	0.040	0.016	0.017
	Sep.	0.090	0.050	0.030	0.030	0.018	0.022
	Oct.	0.360	0.280	0.190	0.260	0.120	0.040
RS	*na*	0.200	0.100	0.140	0.200	0.100	0.060

[1] Extraction hour, [2] Month of harvesting, na – not applicable.

Table S4. Chemical composition (%) of the most active FVEO samples.

# [1]	Name	Sample [2]										
		A1h	**A3h**	**A6h**	**A12h**	**AM1**	**AM2**	**AM3**	**AM 4**	**S1h**	**OM1**	**OM4**
1	*α*-pinene	-	2.9	4.9	1.1	1.0	7.0	3.9	3.3	1.9	4.3	19.6
2	*β*-pinene	-	-	-	-	-	-	-	-	-	3.3	1.4
3	*β*-terpinene	-	-	-	-	-	0.4	0.9	0.6	-	-	-
4	*β*-myrcene	1.6	1.4	1.5	0.8	0.2	0.4	1.0	1.1	1.4	3.0	2.0
5	*α*-phellandrene	18.3	6.1	20.5	3.9	0.4	0.7	1.0	10.5	13.0	2.7	7.7
6	d-limonene	7.5	7.0	8.2	3.7	1.8	3.4	6.8	6.0	12.4	1.0	4.4
7	*β*-phellandrene	6.6	4.1	4.6	2.2	1.2	2.2	5.0	4.4	6.8	1.1	1.6
8	*γ*-terpinene	1.1	1.1	2.6	0.8	0.1	0.2	1.0	1.1	1.5	3.9	1.6
9	*o*-cymene	35.5	25.2	19.1	14.0	16.0	23.5	35.3	28.7	52.5	1.5	2.9
10	terpinolene	-	-	-	-	-	-	-	-	-	0.1	0.5
11	fenchone	2.4	1.3	0.4	0.2	4.8	2.6	2.0	1.7	-	4.8	8.8
12	dehydro-p-cymene	0.2	0.5	0.6	1.8	-	-	-	-	-	-	-
13	isomenthone	0.5	1.1	1.0	2.2	-	-	-	-	-	-	-
15	pulegone	2.3	4.4	0.3	9.3	4.5	2.2	2.8	3.7	0.4	-	-
16	estragole	18.5	14.5	6.1	1.7	22.5	14.8	14.6	13.2	-	70.6	47.1
17	*p*-ment-en-2-one	-	3.4	3.5	9.7	3.8	1.1	2.0	2.7	0.6	-	-
18	phellandral	-	1.1	2.3	4.1	1.4	1.6	1.1	1.3	0.2	-	-
20	*cis*-sabinol	4.7	8.6	6.3	9.5	7.8	5.6	6.1	6.5	2.2	-	-
21	*p*-cymen-8-ol	-	3.0	1.7	3.5	5.2	2.0	1.8	1.8	0.8	-	1.1
22	2,3-pinanediol	-	-	-	-	7.8	3.3	2.8	5.7	1.3	-	-
26	thymol	-	5.9	9.1	14.7	4.8	6.7	3.7	4.6	1.2	-	-
27	myristicin	-	-	2.2	5.6	1.6	0.8	1.0	-	2.7	1.2	1.1
28	piperitenone oxide	-	-	2.5	5.8	4.9	19.5	4.0	-	-	0.7	-
	Unidentified compounds	**0.8**	**8.4**	**2.6**	**5.4**	**10.2**	**2.0**	**3.2**	**3.1**	**1.1**	**1.8**	**0.2**

[1] # indicates the compound identification number; [2] samples names were given by merging the month first letter and extraction time as reported in Table S2 or by merging the first letter of the month, the letter M (mixture) and serial number of the mix.

Table S5. Chemical composition (%) of the most active RSEO samples.

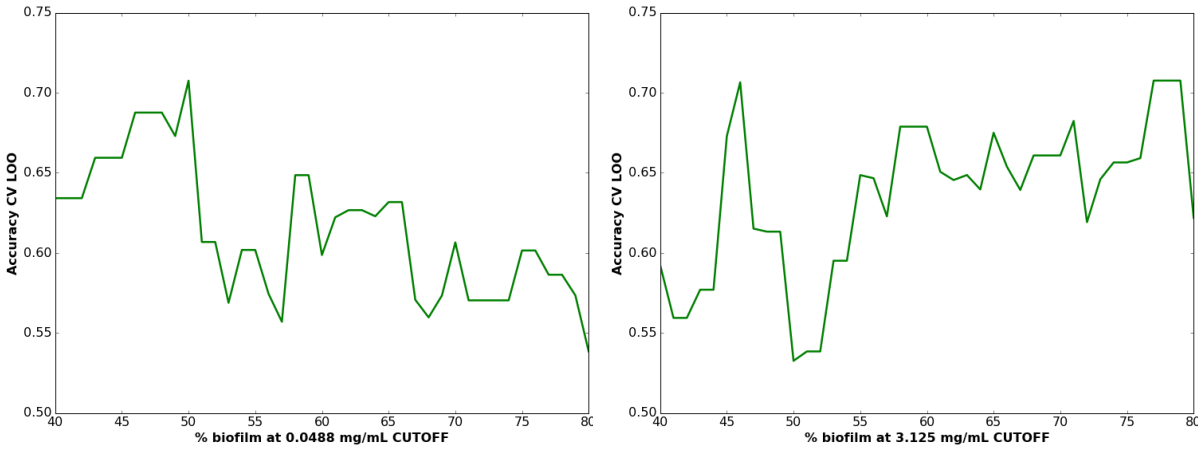
# [1]	Name	Sample [2]			
		1h	**3h**	**12h**	**30h**
1	*α*-pinene	3.9	3.6	1.2	-
2	*β*-pinene	4.6	3.4	1.5	0.1
3	*β*-myrcene	0.9	1.2	0.4	-
4	*α*-phellandrene	-	-	5.4	0.6
5	*d*-limonene	7.4	6.7	1.2	0.3
6	*β*-terpinene	3.0	4.9	1.8	0.3
7	*β*-ocimene	0.5	1.3	0.8	0.2
8	*o*-cymene	40.1	3.8	7.4	4.2
9	terpinolene	-	2.1	1.6	-
11	borneol	-	3.3	3.0	3.0
12	pulegone	-	-	0.8	2.6
13	citral	-	1.0	-	-
14	cryptone	-	2.5	-	-
15	*p*-menth-1-en-2-one	-	2.2	2.1	9.7
17	*cis*-sabinol	-	5.8	4.3	12.9
18	*p*-cymen-8-ol	9.2	13.4	3.0	6.4
19	piperitenone oxide	6.5	3.6	1.0	1.9
21	2,3-pinanediol	9.6	1.8	2.1	-
23	myristicin	-	-	3.2	1.7
24	apiol	6.5	21.1	59.2	56,1
	Unidentified compounds	**7.8**	**18.3**	**0.0**	**0.0**

[1] # indicates the compound identification number; [2] samples names indicate the extraction time as reported in Table S1.

Table S6. Arbitrary classification of 89 EOs samples in 4 different classes depending on their capability to impair biofilm formation.

Strong reduction (< 40% residual biofilm)			Medium reduction (40-80% residual biofilm)			No reduction (80 - 100% residual biofilm)			Enhancer effect (>100% residual biofilm)		
FV	**CG**	**RS**	**FV**	**CG**	**RS**	**FV**	**CG**	**RS**	**FV**	**CG**	**RS**
FA1	COM2		FA2	CJM3	R1	FO1	CA6	RM1		CA1	
FA3			FA24	CJM4	R2		CJ3	RM3		CA2	
FA6			FAM5	CO1	R3		CJM1	RM4		CA3	
FA12			FS2	CO2	R6		CJM5			CAM1	
FAM1			FS3	CO3	R12		COM1			CAM3	
FAM2			FS6	CO6	R24		COM3			CJ1	
FAM3			FS12	CO12	R30		COM5			CJ2	
FAM4			FS24	CO24	RM2		CS2			CJM2	
FS1			FSM2	CS1	RM5		CS3			COM4	
FOM1			FSM3	CS12	RM6		CSM1			CS6	
FOM4			FSM4	CS24						CSM3	
			FSM5	CSM2						CSM5	
			FO2	CSM4							
			FO3	CA12							
			FO6	CA24							
			FO12	CAM2							
			FO24	CAM4							
			FOM2	CAM5							
			FOM3	CJ6							
			FOM5	CJ12							
				CJ24							

FV: *Foeniculum vulgare*; CG: *Calamintha nepeta* subsp. *glandulosa*; RS: *Ridolfia segetum*.



A **B**

Figure S1. Analysis of best cutoff values for the GB classification models. A: at 48.8 μg/ml; B: at 3.125 mg/ml.

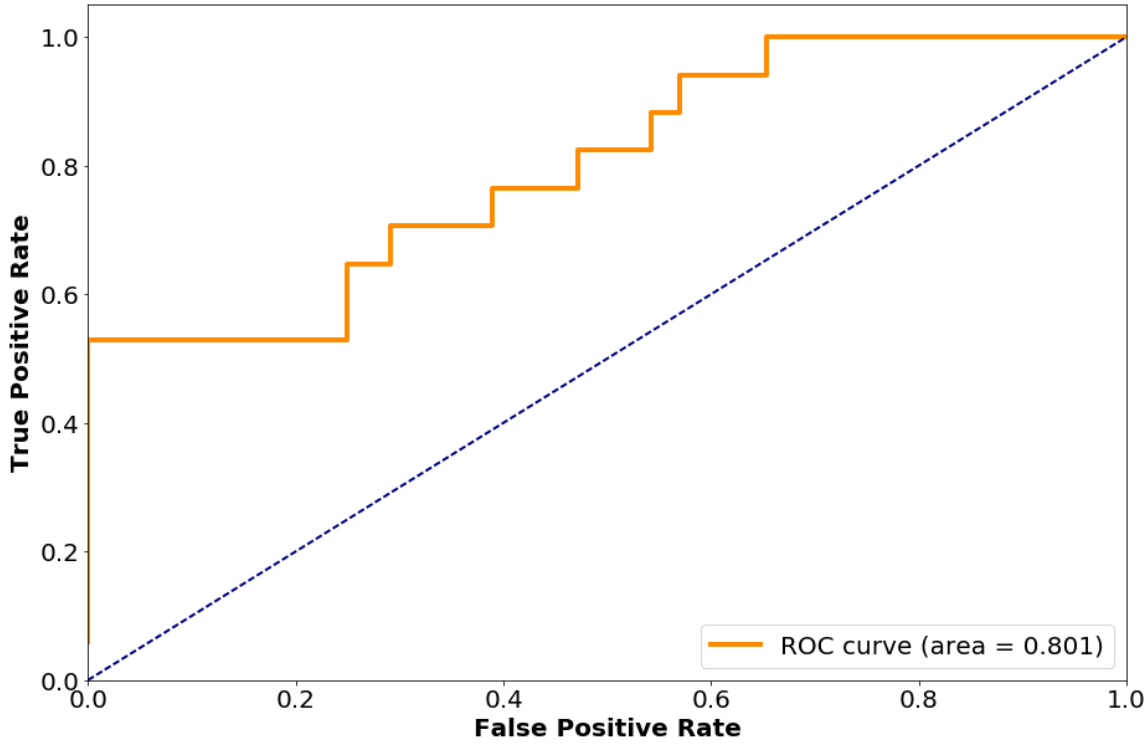


Figure S2. ROC curve for the GB classification model obtained for biofilm inhibition measured at 48.8 μg/ml.

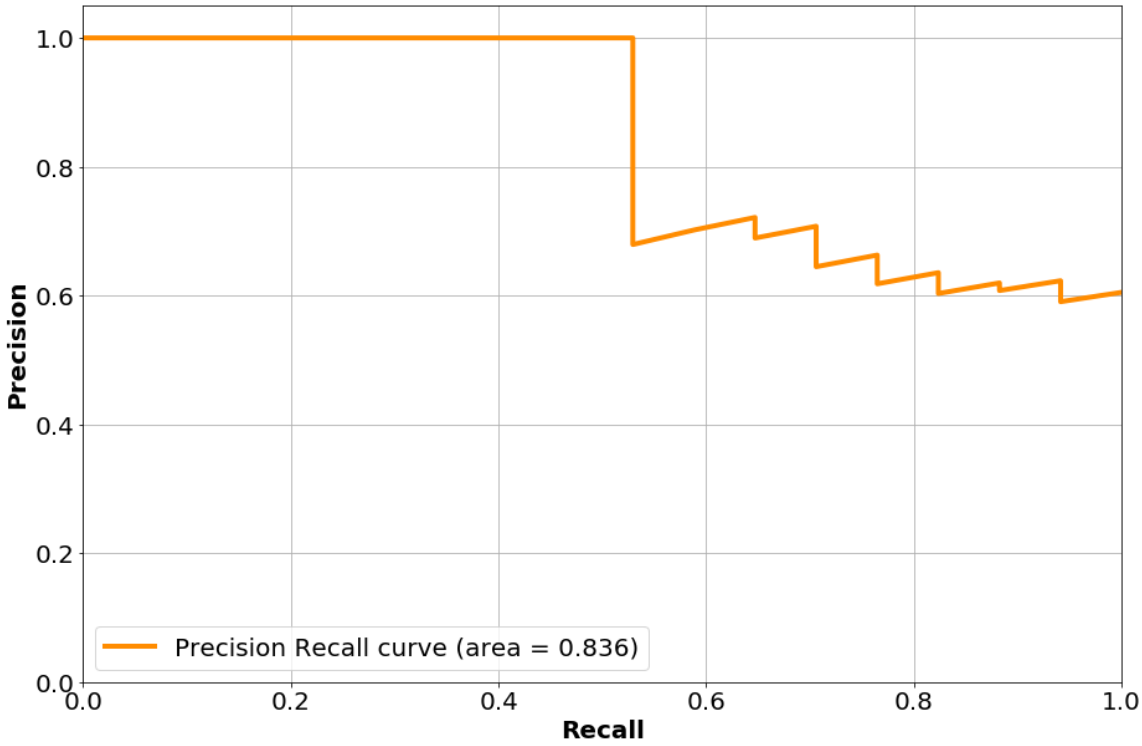


Figure S3. Precision Recall curve for the GB classification model obtained for biofilm inhibition measured at 48.8 μg/ml.

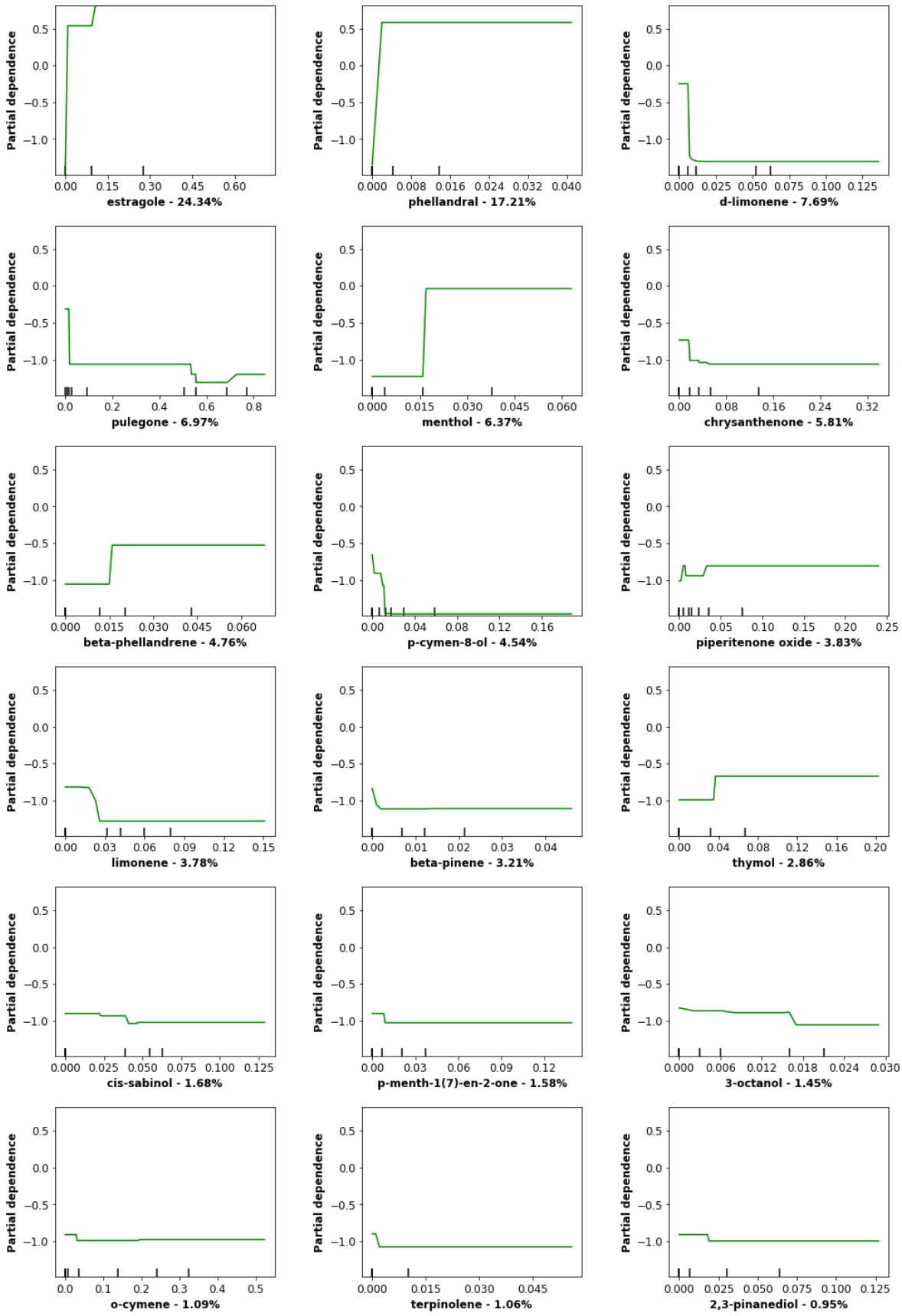
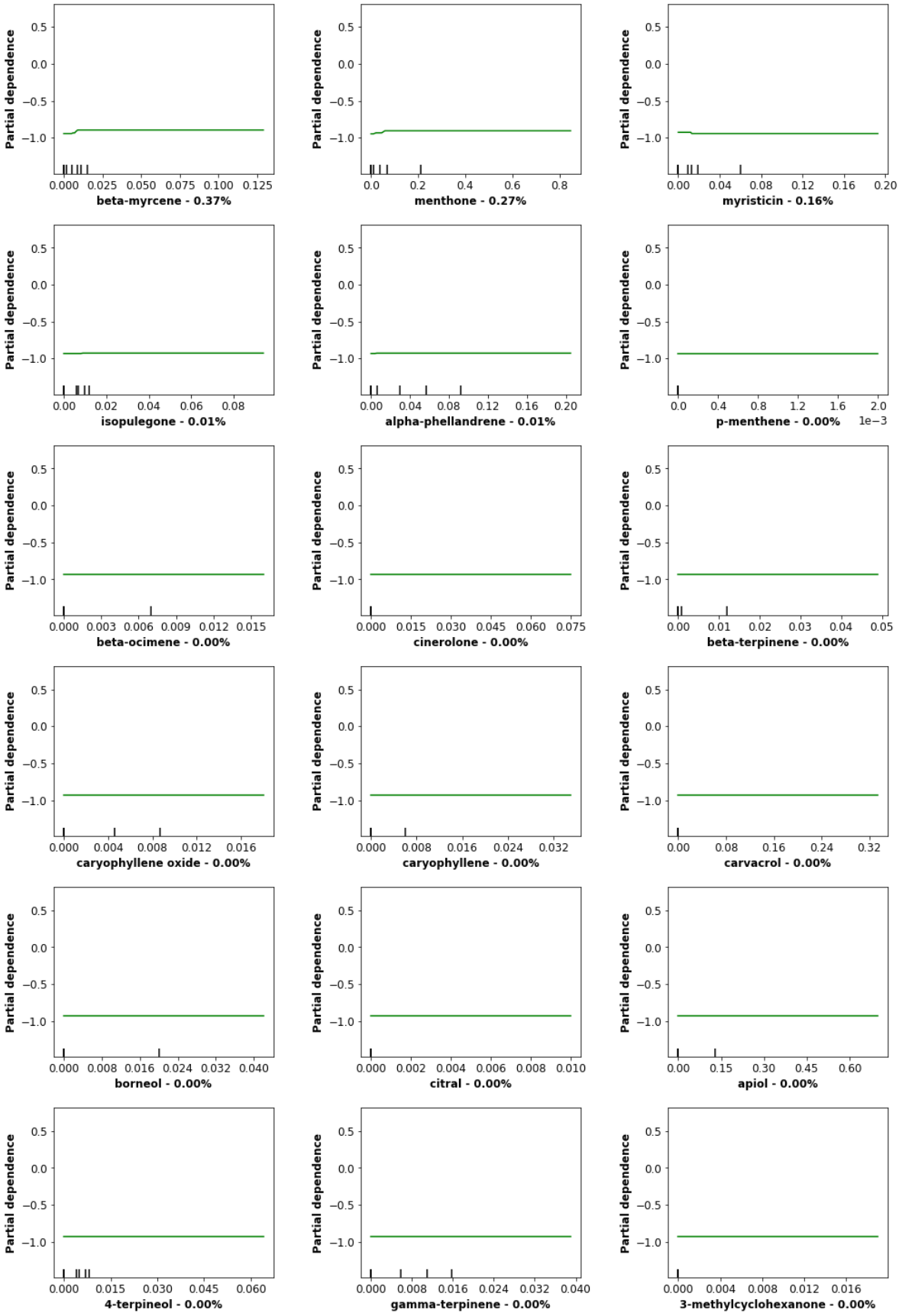
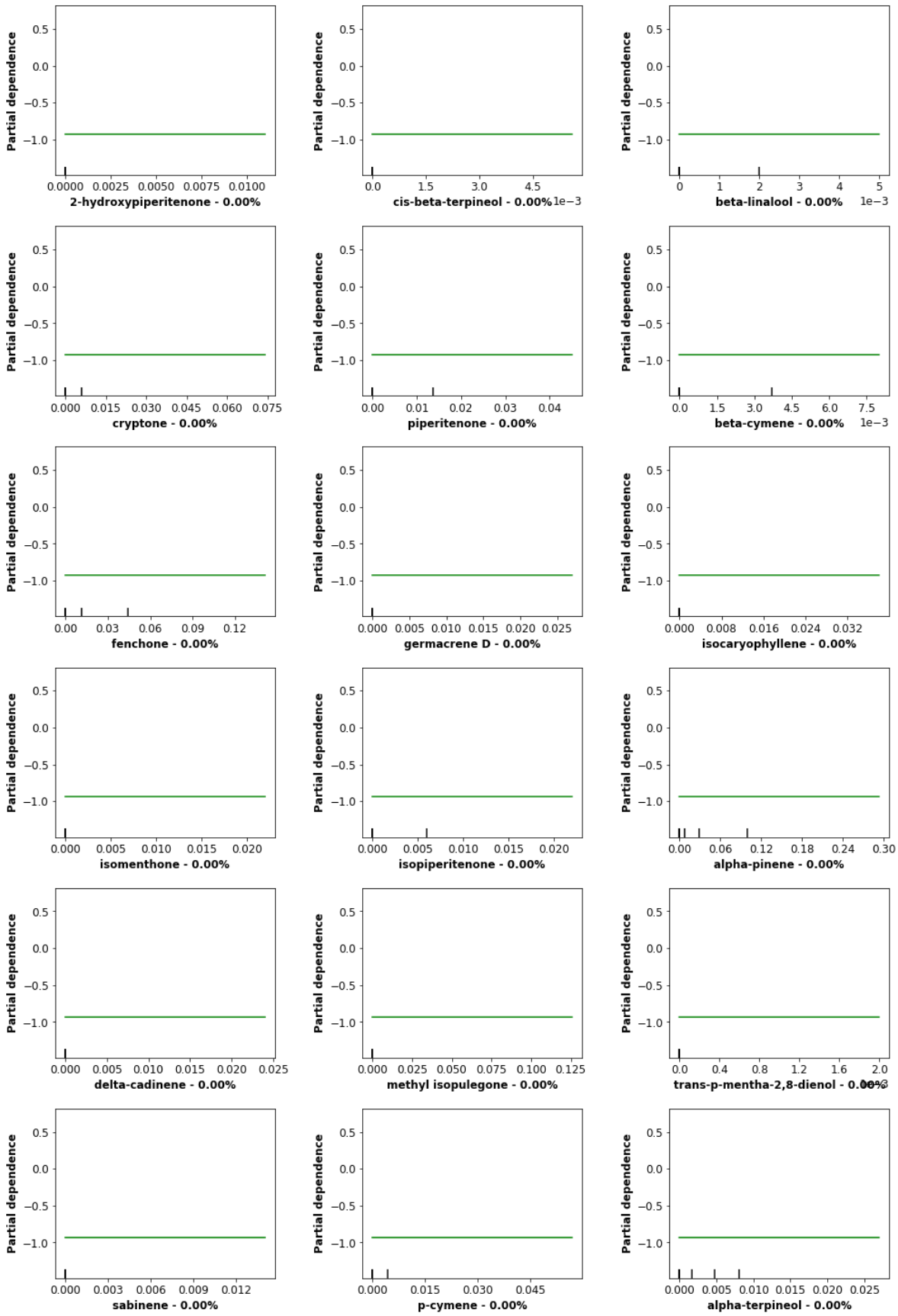


Figure S4. Partial Dependence plot obtained for the GB classification models at 48.8 μg/ml for the first 18 EOs' chemical components



continue **Figure S4.** Partial Dependence plot obtained for the GB classification models at 48.8 μg/ml for the second 18 EOs' chemical components



continue **Figure S4.** Partial Dependence plot obtained for the GB classification models at 48.8 μg/ml for the last 18 EOs' chemical components

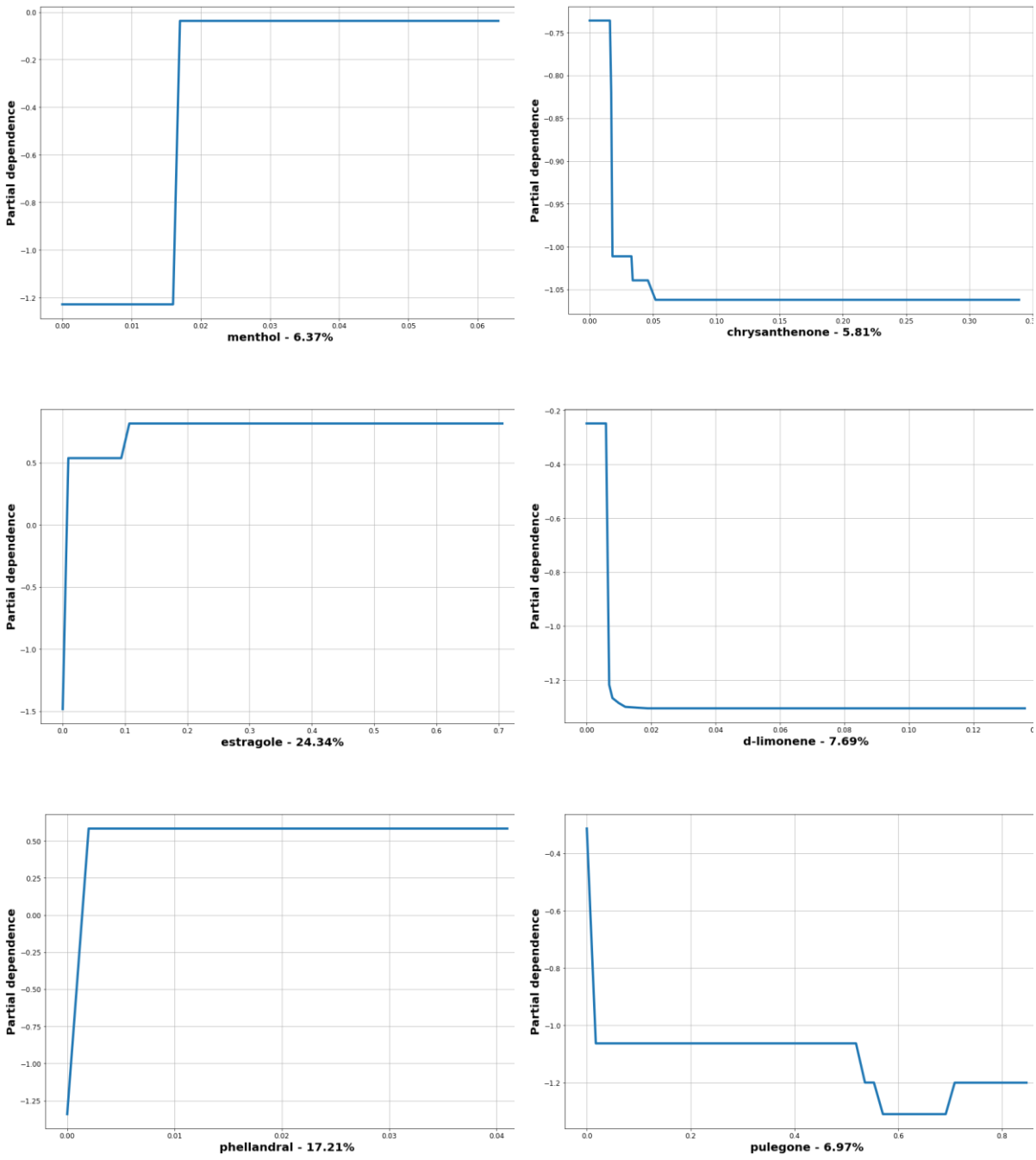


Figure S5. Partial Dependence plot obtained for the GB classification models at 48.8 μg/ml for the most important chemical components.