Supplementary Information

Metabolomics Reveals How Seasonal Variation in the Essential Oils of *Ocotea odorifera* (Vell.) Rohwer Impacts Its Anti-Inflammatory Activity

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Additional supporting results are provided in the Supplementary Information, including environmental metadata (Table S1), VIP scores (Table S2), correlation coefficients (Table S3), the Q-Q plot (Figure S1), the full heatmap including safrole (Figure S2), the PC2-PC3 score plot highlighting seasonal separation (Figure S3), the VIP score plot (Figure S4), and the Python scripts provided as .txt files. In addition, the MS spectral data are publicly available in Zenodo at DOI: https://zenodo.org/records/19153319

Table S1. Environmental metadata including information on sample collection, such as date and time of collection, temperature and humidity recorded at the time of collection, plant part collected (sample), mass used in hydrodistillation, and essential oils yield expressed in grams and as a percentage relative to the initial mass

Date	time	Temperature / ºC	Humidity / %	Sample	Mass / g	Yield / g	Yield / %	Season
26/04/2021	17:00	22	57	leaf	130	1.674	1.288	fall
17/05/2021	17:00	19	60	leaf	130	2.857	2.198	fall
21/06/2021	17:20	19	52	leaf	130	2.961	2.278	winter
19/07/2021	17:05	13	21	leaf	130	2.134	1.642	winter
24/08/2021	17:30	24	33	leaf	130	2.415	1.858	winter
06/09/2022	17:00	28	17	leaf	130	1.484	1.142	spring
11/10/2021	17:00	18	43	leaf	130	0.559	0.430	spring
16/11/2021	17:00	26	32	leaf	130	0.774	0.595	spring
06/12/2021	17:00	25	68	leaf	130	1.279	0.984	summer
17/01/2021	17:00	26	35	leaf	130	2.363	1.818	summer
14/02/2022	17:00	29	25	leaf	130	0.628	0.483	summer
07/03/2022	17:00	30	12	leaf	130	2.373	1.825	fall

Table 2. Variable importance in projection (VIP) scores calculated to identify the variables most strongly associated with *ex vivo* anti-inflammatory activity

	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6	Comp. 7	Comp. 8
bicyclogermacrene	3.2501	2.322	2.1862	2.1783	2.173	2.1659	2.1648	2.1638
cadina-1,4-diene	2.7594	1.9321	1.8466	1.8338	1.831	1.825	1.8246	1.8239
safrole	2.202	3.1786	2.9646	2.9462	2.9389	2.9302	2.9285	2.9274
spathulenol	1.7338	2.2313	2.0777	2.0629	2.0578	2.0587	2.0577	2.0574
4-terpineol	1.4685	1.2542	1.2261	1.225	1.2226	1.2215	1.2212	1.2207
beta-gurjunene	1.4129	1.0146	1.0135	1.0136	1.0149	1.0121	1.0115	1.0111
limonene	1.268	0.89508	0.85334	0.86166	0.85965	0.85701	0.85681	0.85649
camphor	1.2459	1.6451	1.7043	1.7216	1.7191	1.7363	1.7353	1.7354
(+)-sabinene	1.1225	0.82032	0.85745	0.88204	0.87982	0.89174	0.89237	0.89202
alpha-cadinol	0.98778	1.0911	1.0153	1.0083	1.0088	1.0058	1.0068	1.0069
alpha-copaene	0.84362	0.69543	0.6525	0.66138	0.66783	0.6661	0.66594	0.66574
selina-3,11-dien-6-?-ol	0.7997	0.90562	0.86975	0.86353	0.86404	0.86233	0.86391	0.86384
globulol	0.74416	0.92106	0.89518	0.89238	0.89495	0.89197	0.89219	0.89182
beta-myrcene	0.65076	0.58637	0.54635	0.54741	0.54859	0.56094	0.56347	0.56558
eucalyptol	0.63986	0.44576	0.65191	0.66291	0.73486	0.73253	0.73437	0.73422
beta-pinene	0.55557	0.48317	0.50992	0.51369	0.51852	0.51691	0.51661	0.51915
aromadendrene	0.38692	0.82655	0.92169	0.91535	0.91623	0.92648	0.92891	0.92911
camphene	0.37176	0.39888	0.41926	0.45165	0.45645	0.45578	0.45619	0.45671
carotol	0.34568	0.48853	0.64275	0.64684	0.64524	0.64309	0.64307	0.64292
muurola-4,10(14)-dien-1-?-ol	0.28684	0.40537	0.53333	0.53673	0.53541	0.53362	0.5336	0.53348
palustrol	0.2834	0.40051	0.52695	0.5303	0.52899	0.52723	0.52721	0.52709
alpha-phellandrene	0.26997	0.26513	0.34747	0.34552	0.34703	0.39679	0.39708	0.40058
alpha-cadinene	0.26556	0.3753	0.49377	0.49692	0.49569	0.49404	0.49402	0.49391
beta-elemene	0.26185	0.37005	0.48686	0.48997	0.48876	0.48713	0.48711	0.487
NI_24.776	0.25037	0.35383	0.46553	0.4685	0.46734	0.46578	0.46577	0.46566
viridiflorol	0.25037	0.35383	0.46553	0.4685	0.46734	0.46578	0.46577	0.46566
Eudesm-7(11)-en-4-ol	0.24242	0.3426	0.45075	0.45362	0.4525	0.45099	0.45098	0.45087
eudesma-3,7(11)-diene	0.2342	0.33098	0.43546	0.43824	0.43716	0.4357	0.43569	0.43559
cubenol	0.2213	0.31275	0.41148	0.4141	0.41307	0.4117	0.41168	0.41159
alpha-terpineol	0.21601	0.15157	0.19433	0.24855	0.2612	0.26533	0.27043	0.28498
eudesma-4(15),7-dien-1?-ol	0.21226	0.29998	0.39467	0.39719	0.39621	0.39489	0.39487	0.39478
alpha-bulnesene	0.20437	0.5968	0.87591	0.87676	0.87702	0.87408	0.87479	0.87441
gamma-terpinene	0.20283	0.28664	0.37712	0.37953	0.37859	0.37733	0.37732	0.37723
guaiol	0.20283	0.28664	0.37712	0.37953	0.37859	0.37733	0.37732	0.37723
linalool	0.17077	0.11983	0.15364	0.19649	0.2065	0.20977	0.2138	0.22529
alpha-amorphene	0.14834	0.59693	0.92736	0.93364	0.93197	0.92869	0.92863	0.92825
alpha-pinene	0.1432	0.56999	0.57496	0.59362	0.59297	0.60679	0.60884	0.60897
alpha-caryophyllene	0.10123	0.64117	0.75669	0.75401	0.75276	0.75176	0.75281	0.75253
ledol	0.066612	0.59895	0.5619	0.57015	0.5694	0.57219	0.5719	0.57201
caryophyllene	0.016067	0.68118	0.92921	0.93175	0.93063	0.92741	0.92776	0.92742

Table S3. Correlation coefficients of variables positively correlated with the spring-winter-fall (SWF) sample group (most active seasons)

	Overall
bicyclogermacrene	100
cadina-1,4-diene	91.593
spathulenol	89.009
camphor	70.874
4-terpineol	65.769
safrole	47.389
alpha-cadinol	43.539
beta-gurjunene	43.474
selina-3,11-dien-6-?-ol	34.361
globulol	34.332
limonene	33.397
aromadendrene	32.62
(+)-sabinene	31.266
alpha-bulnesene	25.627
eucalyptol	24.656
beta-myrcene	23.45
caryophyllene	21.347
carotol	20.125
alpha-amorphene	19.618
alpha-caryophyllene	18.993
alpha-copaene	15.652
muurola-4,10(14)-dien-1-?-ol	15.475
palustrol	15.204
alpha-pinene	13.898
alpha-cadinene	13.794
beta-elemene	13.501
NI_24.776	12.594
viridiflorol	12.594
Eudesm-7(11)-en-4-ol	11.966
eudesma-3,7(11)-diene	11.316
cubenol	10.296
beta-pinene	9.9289
eudesma-4(15),7-dien-1?-ol	9.5824
ledol	8.9956
gamma-terpinene	8.8365
guaiol	8.8365
camphene	3.0586
alpha-phellandrene	2.9249
alpha-terpineol	1.9051
linalool	0

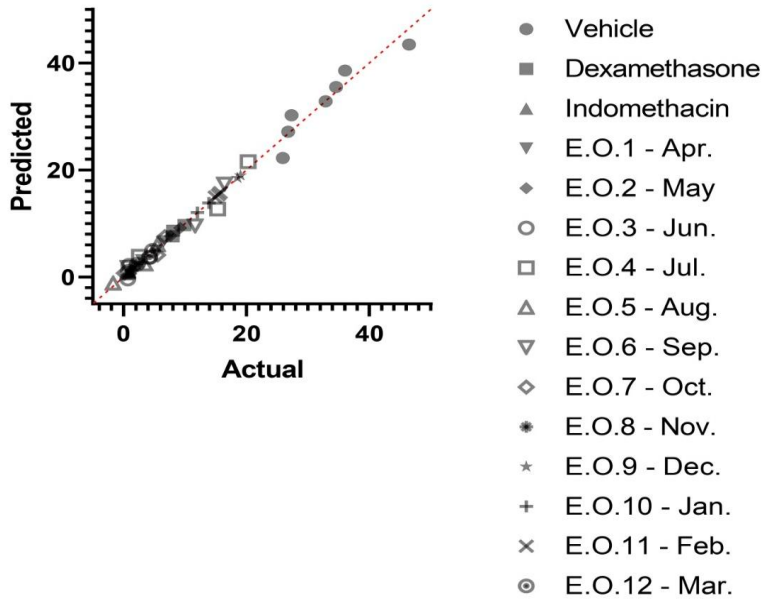

Figure S1. Q-Q plot used to assess the data distribution.

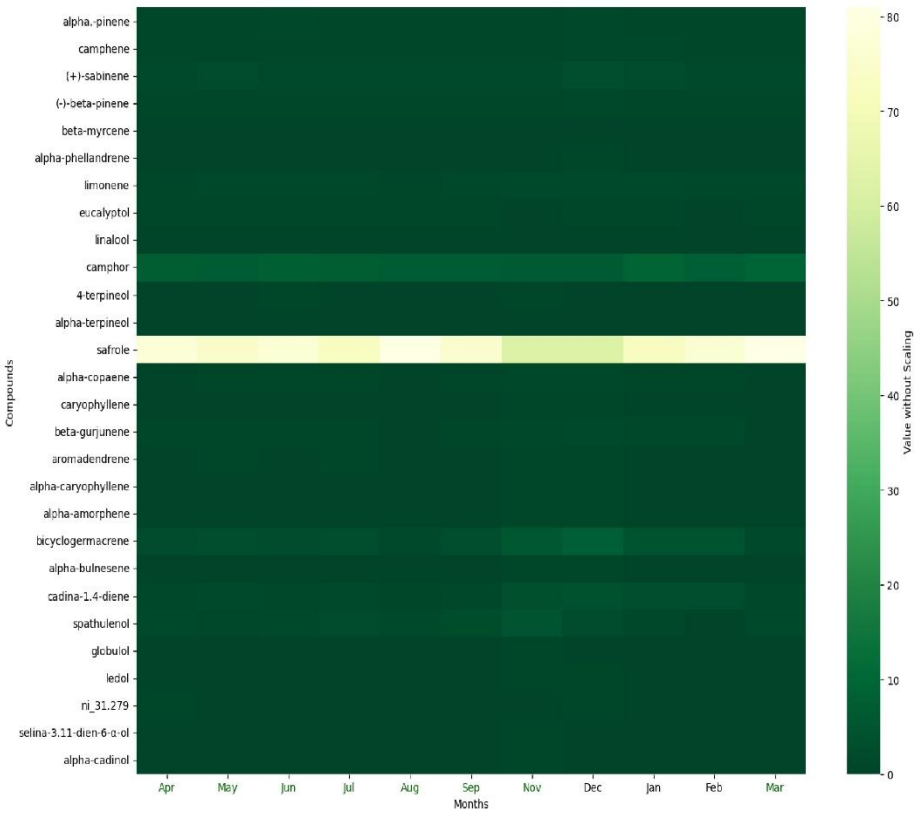

Figure S2. Full heatmap used to visually represent seasonal variation in the metabolite profile of *O. odorifera* essential oils, including safrole.

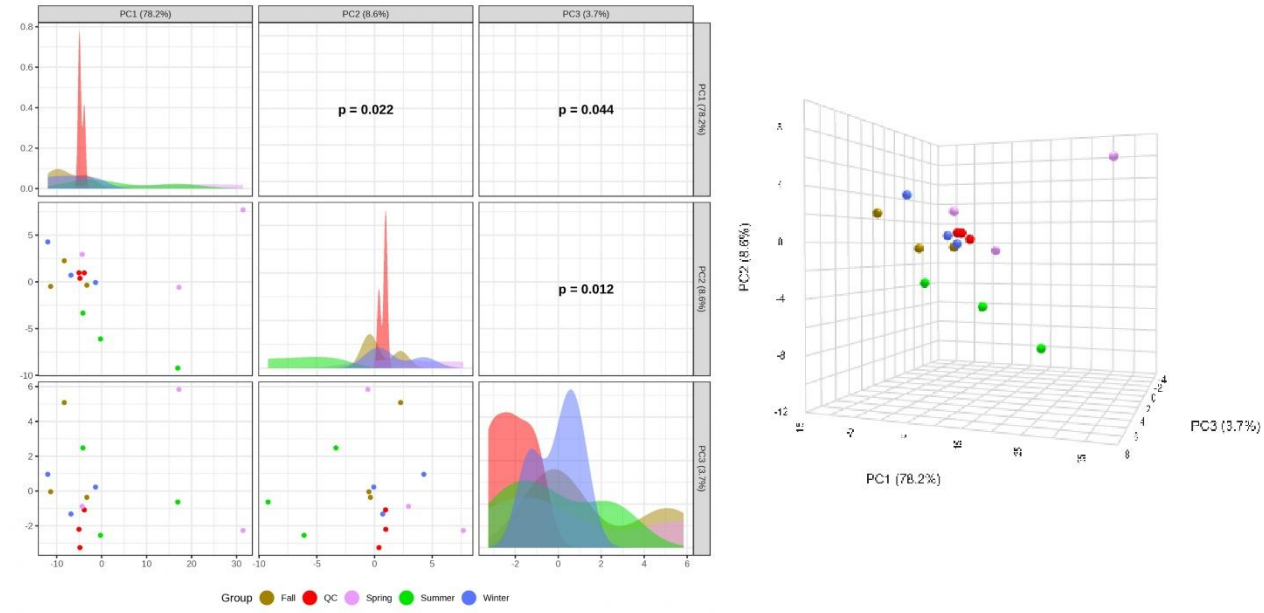

Figure S3. PC2-PC3 of PCA score scatterplots showing summer samples at negative PC2 and winter at positive PC2, indicating a strong seasonal contrast.

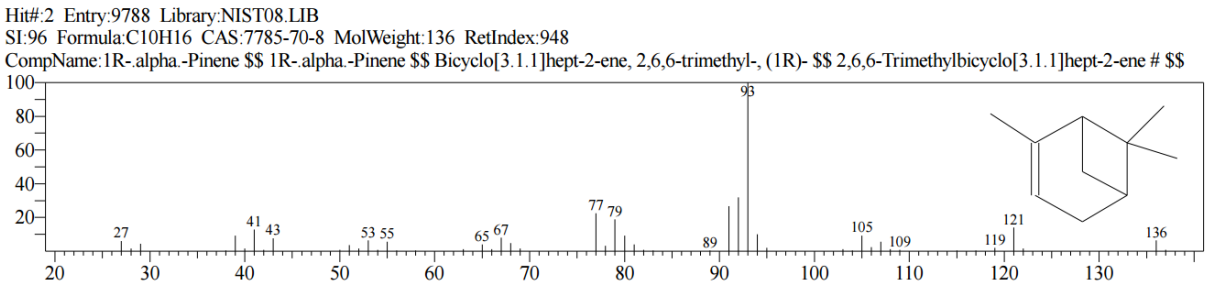

Figure S4. EI-MS of α-pinene.

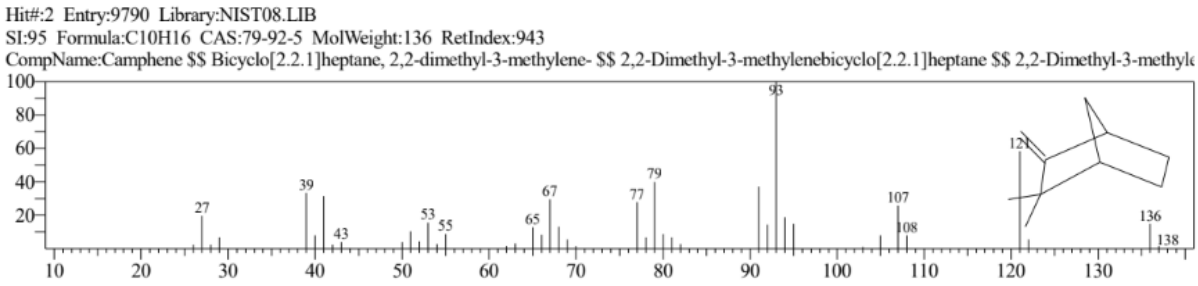

Figure S5. EI-MS of camphene.

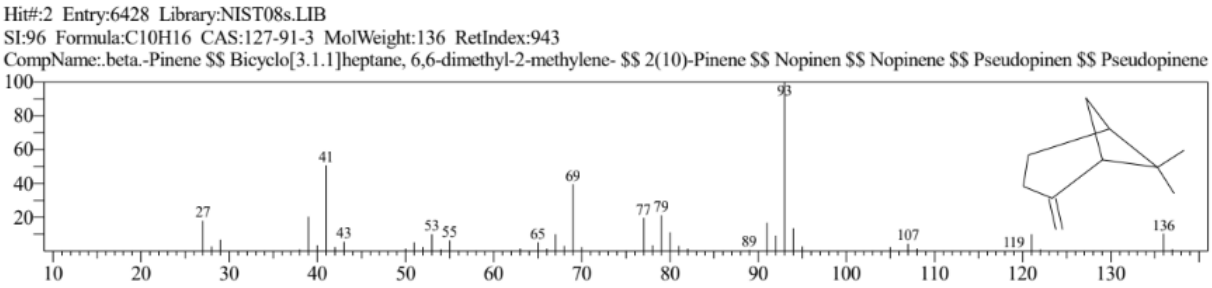

Figura S6. EI-MS of (–)-β-pinene.

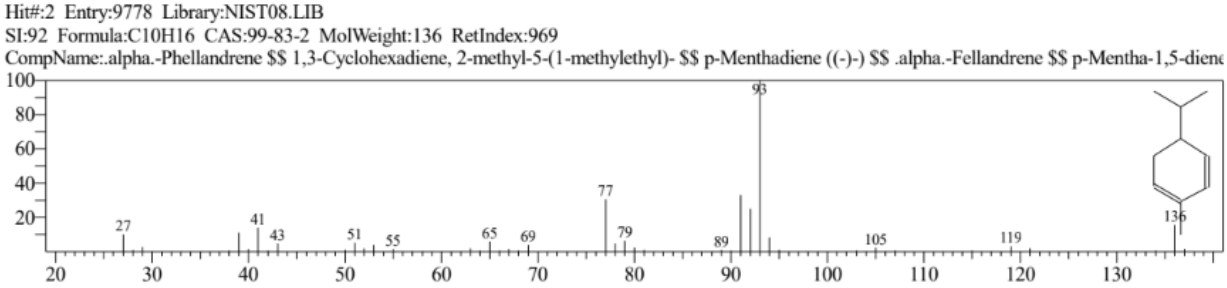

Figure S7. EI-MS of α-phellandrene.

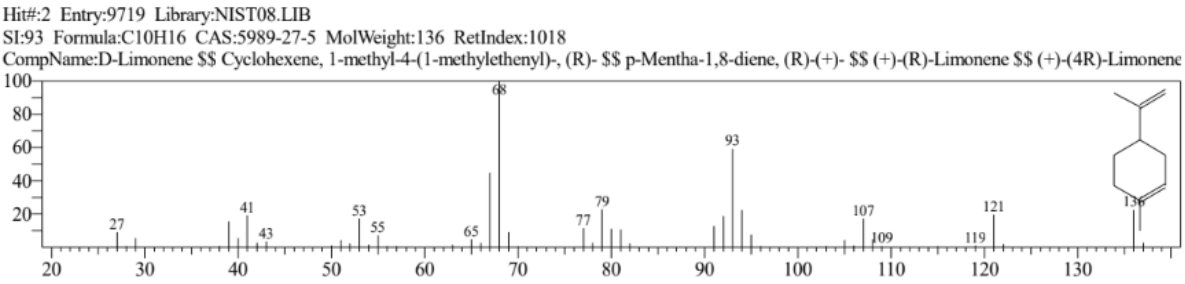

Figure S8. EI-MS of limonene.

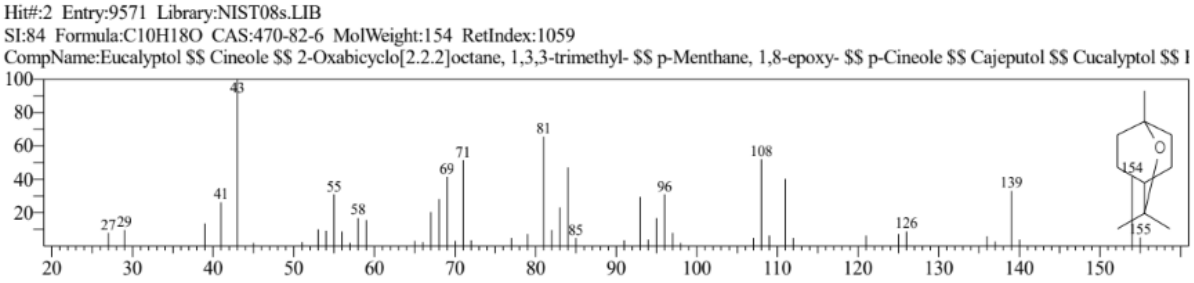

Figure S9. EI-MS of eucalyptol.

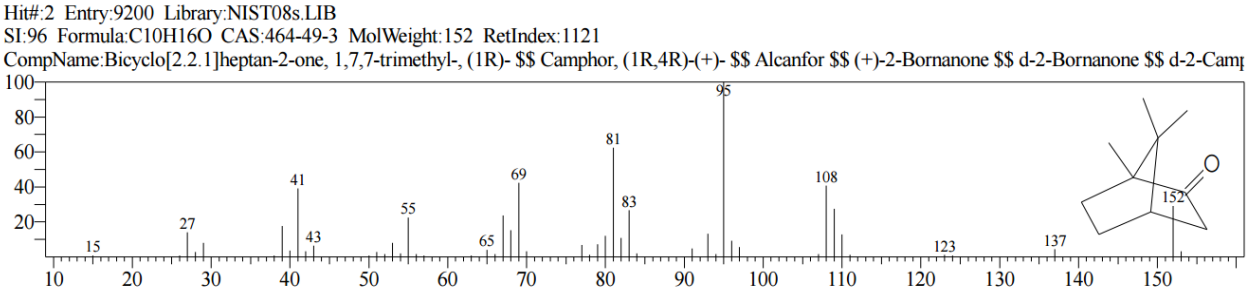

Figure S10. EI-MS of camphor.

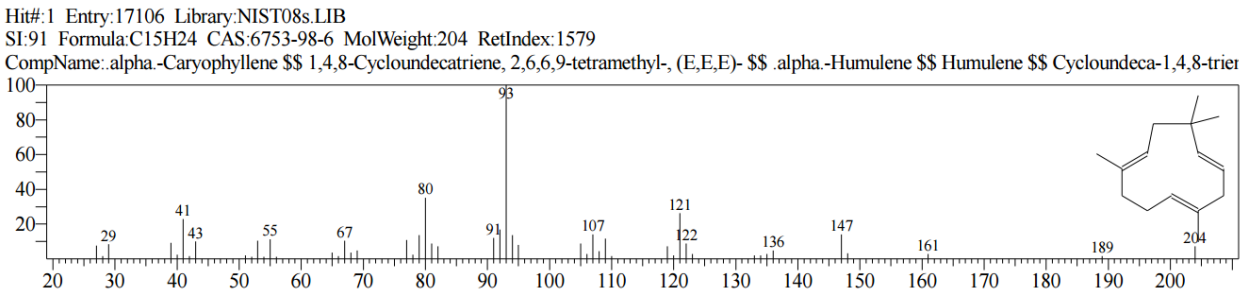

Figure S11. EI-MS of α-caryophyllene.

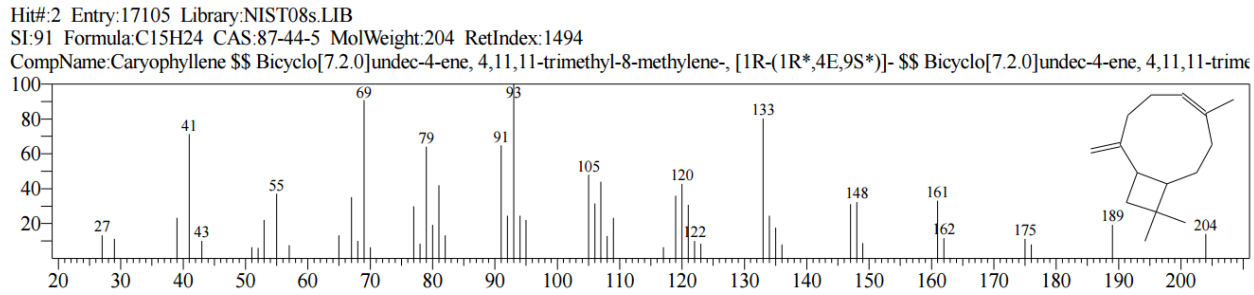

Figure S12. EI-MS of caryophyllene.

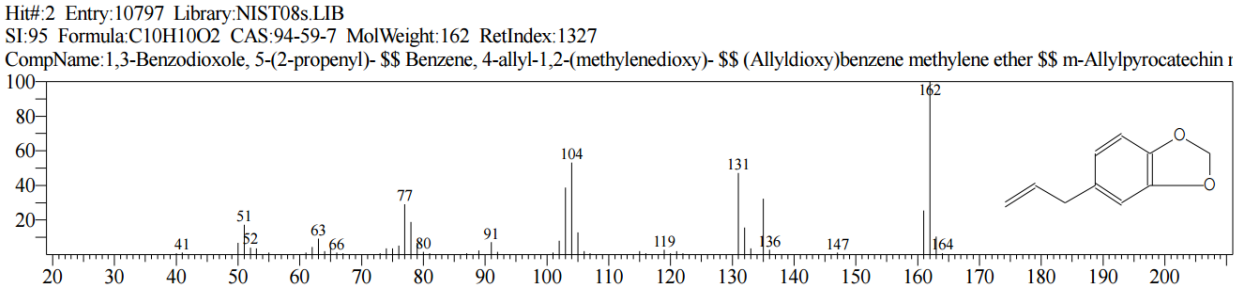

Figure S13. EI-MS of safrole.

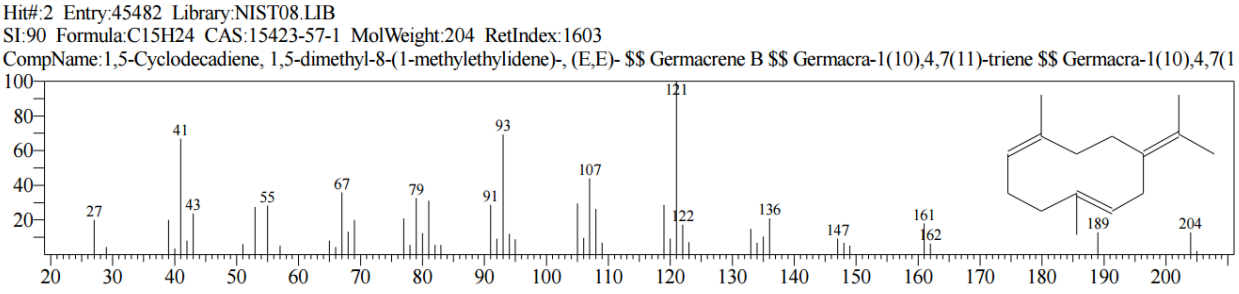

Figure S14. EI-MS of bicyclogermacrene.

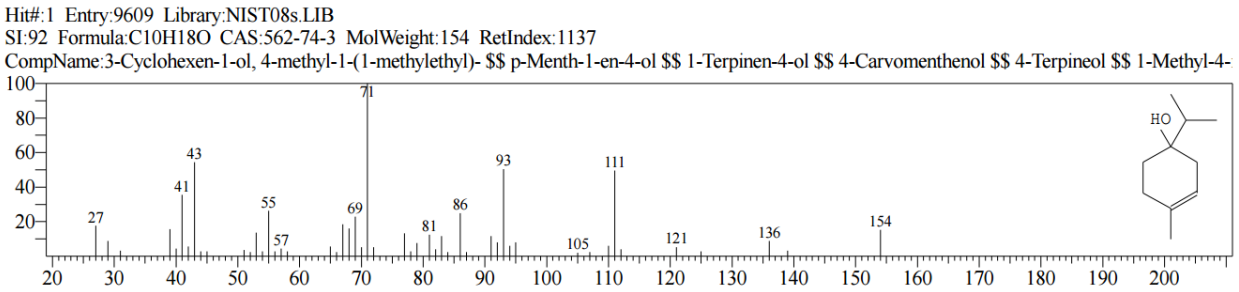

Figure S15. EI-MS of 4-terpineol.

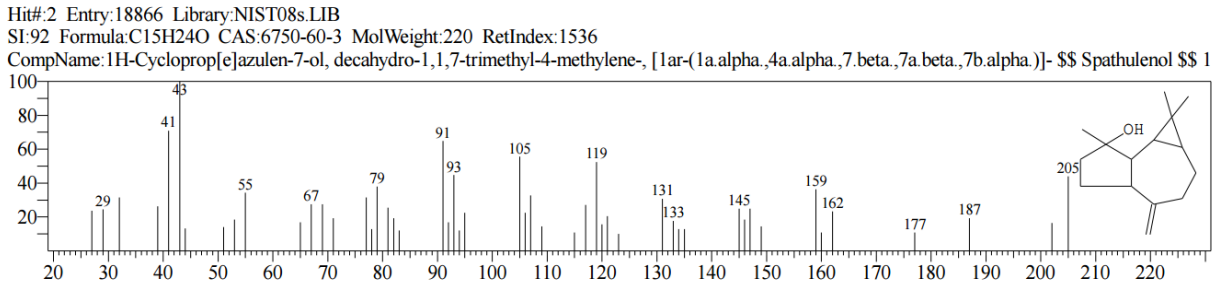

Figure S16. EI-MS of spathulenol.