

Chemical Composition and Evaluation of Antinociceptive Activity of the Essential Oil of *Stevia serrata* Cav. from Guatemala

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Abstract

The composition and the antinociceptive activity of the essential oil of *Stevia serrata* Cav. from a population located in the west highlands of Guatemala were evaluated. A yield of 0.2% (w/w) of essential oil was obtained by hydrodistillation of the dried aerial parts of the plant. The essential oil analyzed by GC-FID and GC-MS showed a high content of sesquiterpenoids, with chamazulene (60.1%) as the major component and 91.5% of the essential oil composition was identified. To evaluate antinociceptive activity in mice, the essential oil of *S. serrata* Cav. was administered as gavage, using three different doses. In the formalin test, the animals were pre-treated with oral doses of the essential oil before the administration of formalin. Oral administration of *S. serrata* Cav. essential oil produced a marked antinociceptive activity. Therefore, the plant could be domesticated as a source of essential oil rich in chamazulene for developing medicinal products.

Experimental

1 Plant material:

Aerial parts of *S. serrata* were collected in September 2014 from a population found near San José Chacayá, a village in Sololá at 2341 m (91°13' 56.41" W/14°46' 40.66" N). The plant was identified using the dichotomous key according to Pruski & Robinson (2015). A voucher specimen is kept at the Herbarium of the School of Biology of the Faculty of Chemistry and Pharmacy of the University of San Carlos, Guatemala (BIGU 72832). The plant material was dried in a solar dryer at a temperature between 30 and 35 °C, and immediately extracted.

2 Extraction of essential oil:

The oil from 40.0 g of aerial parts of *S. serrata* was extracted by hydrodistillation using a Clevenger-type apparatus for 2 h. The essential oil was collected in pentane which was later removed in a rotatory evaporator at 40 °C. It was then weighed with an analytical scale. A yield of 0.2% (w/w) was obtained. This oil was used for chromatographic analysis and administered to animals.

3 GC-MS:

GC–MS analyses were performed using a chromatograph Shimadzu 2010 Plus system coupled with a SHIMADZU QP-2010 Plus selective detector (MSD) and equipped with a DB5-MS capillary fused silica column (60 m, 0.25 mm I.D., 0.25 µm film thickness). The oven temperature program initiated at 60 °C, then rise by 3 °C/min to 246 °C, then held for 20 min. Other operating conditions were as follows: carrier gas, He (99.999%), with a flow rate of 1.03 mL/min; injector temperature, 220 °C; split ratio of 1:50; Injection volume of 1 µL. Mass spectra were taken at 70 eV. The m/z values were recorded in the range of m/z 40–700 Da.

4 Quantification by GC-FID:

GC analyses were carried out using a Shimadzu 2010 GC apparatus (Tokyo, Japan) equipped with dual flame ionization detector (FID) system and DB5 fused-silica capillary columns (60 m, 0.22 mm i.d., film thickness 0.25 μm , Restek, France). The oven temperature was programmed from 60 °C to 246 °C at 3 °C/min and then held isothermally at 246 °C for 20 min. Injector and detector temperatures were maintained at 220 °C and 290 °C respectively. Samples were injected in the split mode with split ratio of 1:50, using H₂ as carrier gas (1.44 mL/min). Retention indices (RI) of compounds were determined relative to the retention times of series of n-alkanes (C₇–C₃₀) with linear interpolation, using Van den Dool and Kratz (1963) equation. Relative amounts of components were calculated based on GC peak areas without using correction factors.

5 Animals:

All experiments were performed with female Swiss Webster mice (20-25g) donated by Instituto Vital Brazil (Niterói, Brazil). Animals were kept in a room with controlled temperature 22±2°C for 12h light/dark cycle with free access to food and water. Twelve hours before each experiment animals received only water, in order to avoid food interference with substances absorption. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA), approved by the Biomedical Science Institute/UFRJ, Ethical Committee for Animal Research, and received the number DFBCICB015–04/16.

6 Essential oil of *S. serrata* administration:

Essential oil was dissolved in oil (Sigma-Aldrich, USA) to prepare a stock solution at 100 mg/mL. Groups composed by six animals were administered by oral gavage, with essential oil of *S. serrata* Cav. at doses of 10, 30 or 100 mg/kg (final volume 0.1mL) and vehicle (PBS with the same amount of oil used in the highest dose) 60 min prior to experiments. The vehicle and essential oil of *S. serrata* Cav. were administered by oral gavage.

7 Formalin test:

This procedure was similar to the method described by Hunskaar and Hole (1987) and Gomes et al. (2007). Mice received an injection of 20 μ L of formalin (2.5% v/v) in to the dorsal surface of the left hind paw. The time that the animal spent licking the injected paw was immediately recorded. The nociceptive and inflammatory response consists of the following two phases: the first phase lasts until 5 min after the formalin injection (neurogenic pain response), and the second phase occurs 15–30 min after the formalin injection (inflammatory pain response). The animals were pre-treated with oral doses of essential oil of *S. serrata Cav.*, 60 min before the administration of formalin.

References:

- Gomes NM, Rezende CM, Fontes SP, Matheus ME, Fernandes PD. 2007. Antinociceptive activity of Amazonian Copaiba oils. *J Ethnopharmacol.* 109:486–492.
- Hunskaar S, Hole K. 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain.* 30:103-114.
- Pruski JF, Robinson H. 2015. Asteraceae Bercht. & J. Presl, nom. cons. (Compositae Giseke, nom. alt.). *Flora Mesoamericana.* 5(2): 554-571. [cited 14 Jan 2017]. Available from:<http://www.tropicos.org/docs/meso/asteraceae.pdf>
- Van den Dool H, Kratz PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatogr A.* 11:463-471.

Table S1. Percentage composition of the essential oil of aerial parts of *Stevia serrata* Cav. analyzed by GC-FID

Compound	RI	%
linalool	1104	0.8
eugenol	1352	0.3
β -elemene	1389	0.4
caryophyllene	1423	2.9
α -humulene	1459	0.4
germacrene D	1484	5.4
bicyclogermacrene	1497	1.0
selinene	1519	0.4
(<i>E</i>)-nerolidol	1560	7.3
(-)-spathulenol	1581	4.3
caryophyllene oxide	1587	6.3
humulene epoxide II	1614	0.4
caryophyllene<14-hydroxy-9-epi-(<i>E</i>)->	1660	1.5
chamazulene	1737	60.1
N.I.	1783	1.3
Total		92.8

N.I. Not identified

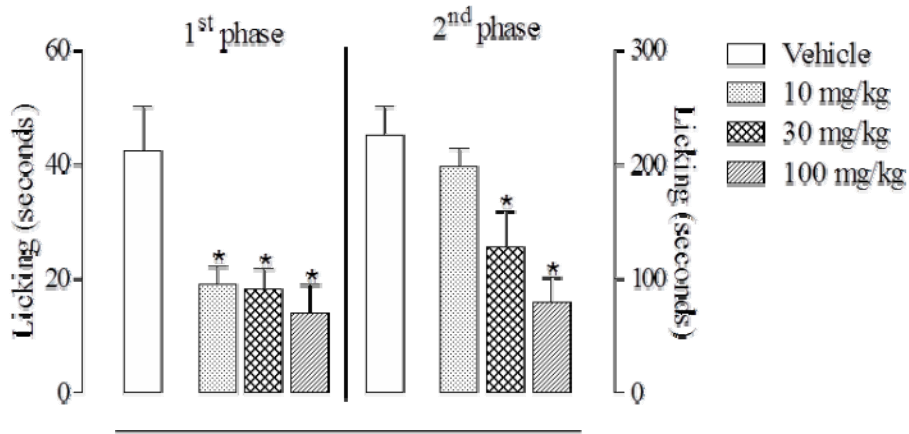


Figure S1. Effect of essential oil of *Stevia serratea* Cav. on formalin-induced licking response in mice. Animals were pre-treated with different doses (10, 30 or 100 mg/kg, p.o.) of essential oil or vehicle. The results are presented as mean±S.D. ($n=6$) of the time that the animal spent licking the formalin-injected paw. Statistical significance was calculated by ANOVA followed by Bonferroni's test. * $P<0.05$ when compared to vehicle-treated mice.

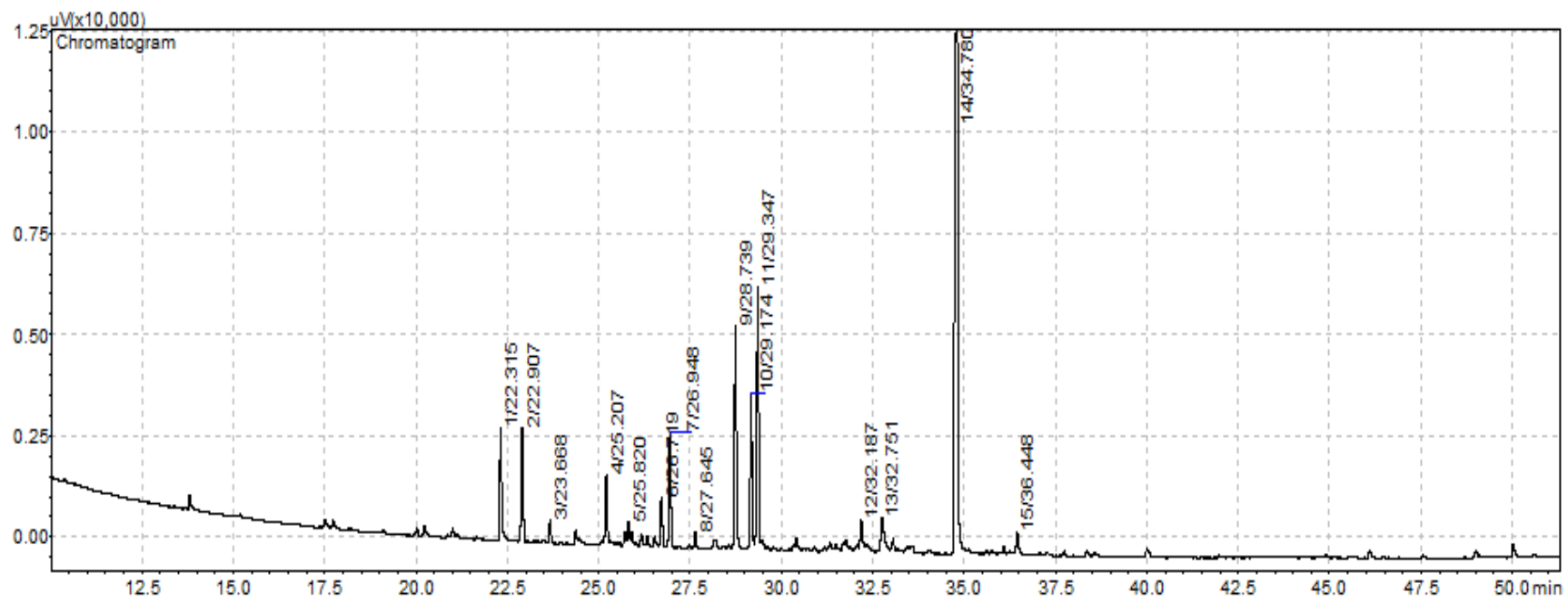


Figure S2. GC-FID Chromatogram.

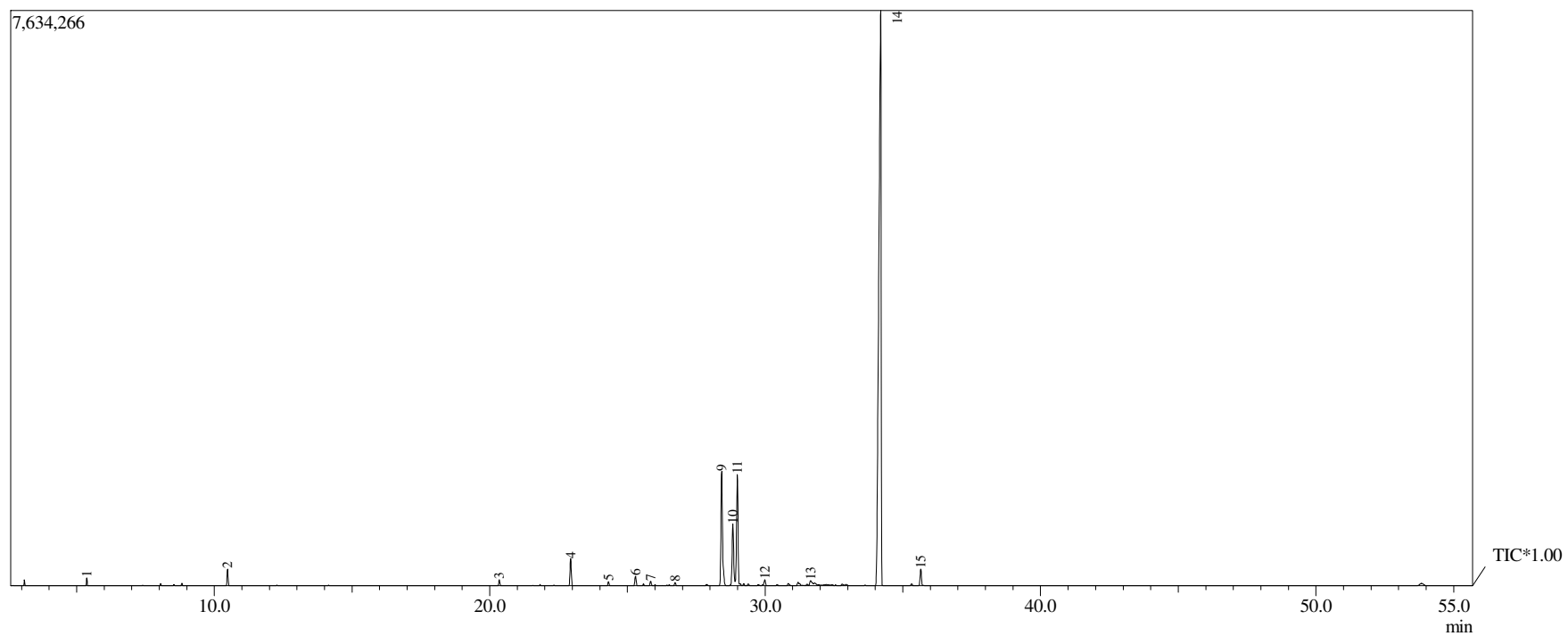


Figure S3. GC-MS Chromatogram.

Line#:1 R.Time:5.367(Scan#:333) MassPeaks:20
RawMode:Averaged 5.358-5.375(332-334) BasePeak:93.05(26045)
BG Mode:None Group 1 - Event 1 Scan

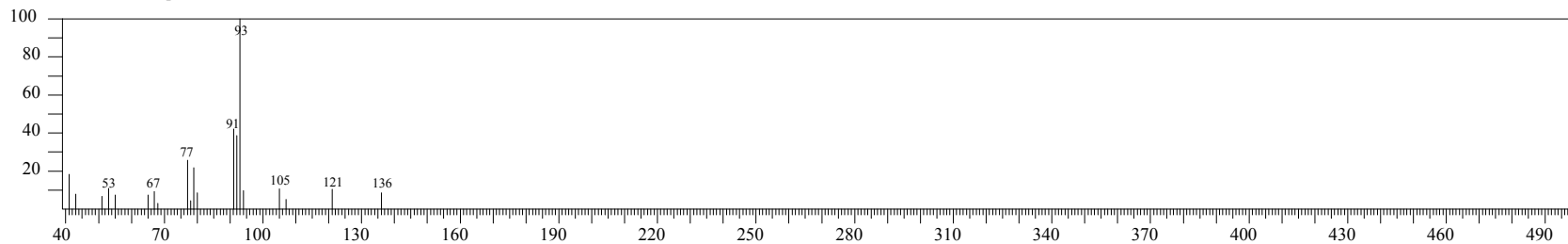


Figure S4. Linalool mass spectrum.

Line#:2 R.Time:10.483(Scan#:947) MassPeaks:39
RawMode:Averaged 10.475-10.492(946-948) BasePeak:71.00(27739)
BG Mode:None Group 1 - Event 1 Scan

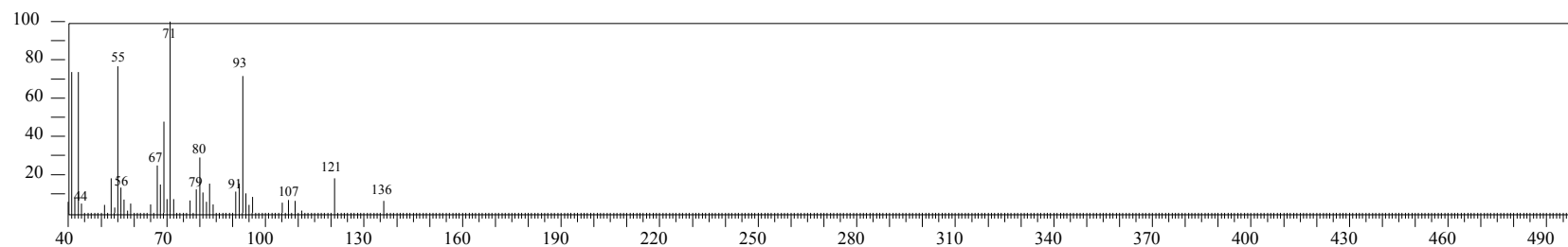


Figure S5. Eugenol mass spectrum.

Line#:3 R.Time:20.350(Scan#:2131) MassPeaks:26
RawMode:Averaged 20.342-20.358(2130-2132) BasePeak:164.00(11556)
BG Mode:None Group 1 - Event 1 Scan

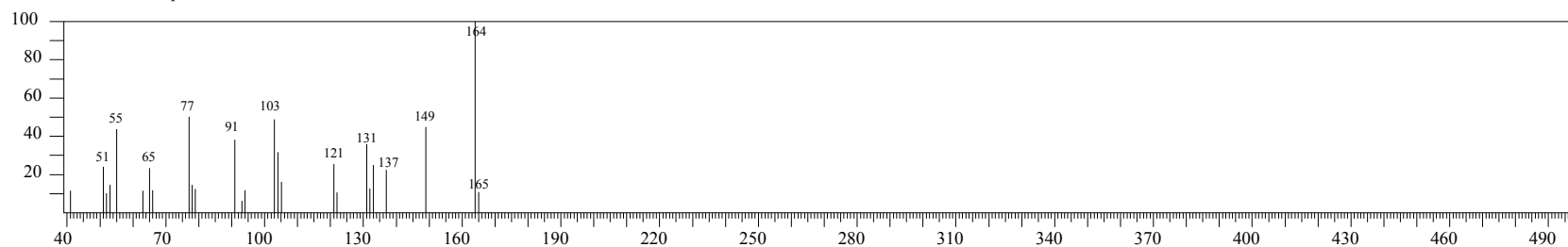


Figure S6. β -elemene mass spectrum.

Line#:4 R.Time:22.942(Scan#:2442) MassPeaks:53
RawMode:Averaged 22.933-22.950(2441-2443) BasePeak:41.00(26423)
BG Mode:None Group 1 - Event 1 Scan

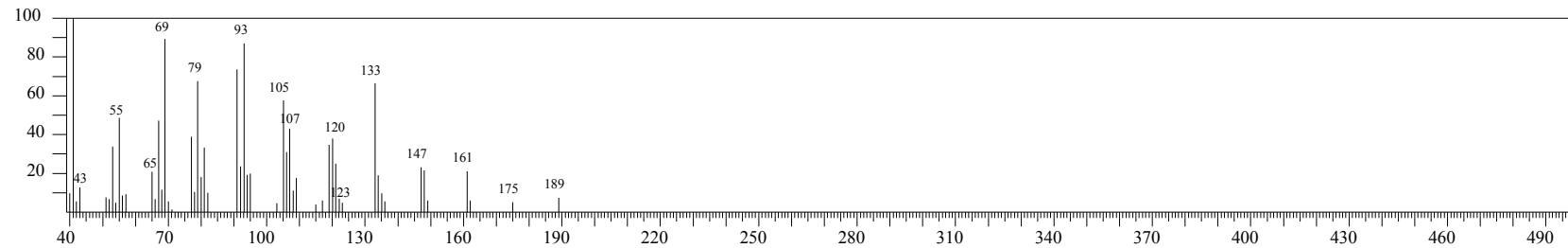


Figure S7. Caryophyllene mass spectrum.

Line#:5 R.Time:24.317(Scan#:2607) MassPeaks:21
RawMode:Averaged 24.308-24.325(2606-2608) BasePeak:93.00(11619)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

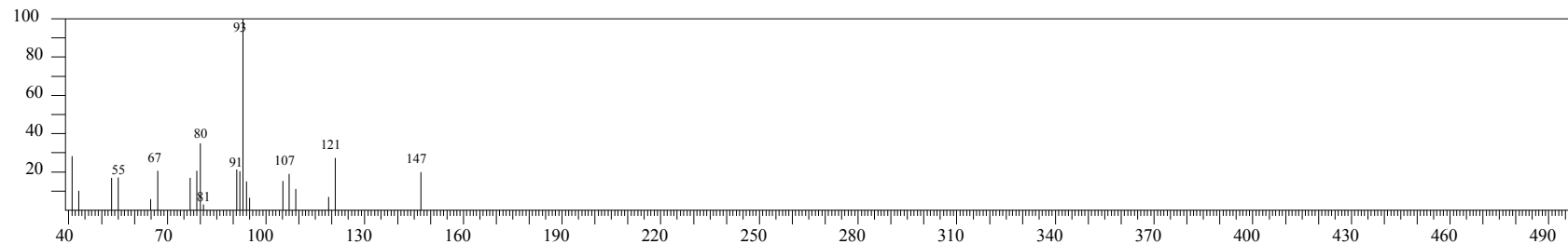


Figure S8. α -humulene mass spectrum.

Line#:6 R.Time:25.300(Scan#:2725) MassPeaks:29
RawMode:Averaged 25.292-25.308(2724-2726) BasePeak:161.10(13412)
BG Mode:None Group 1 - Event 1 Scan

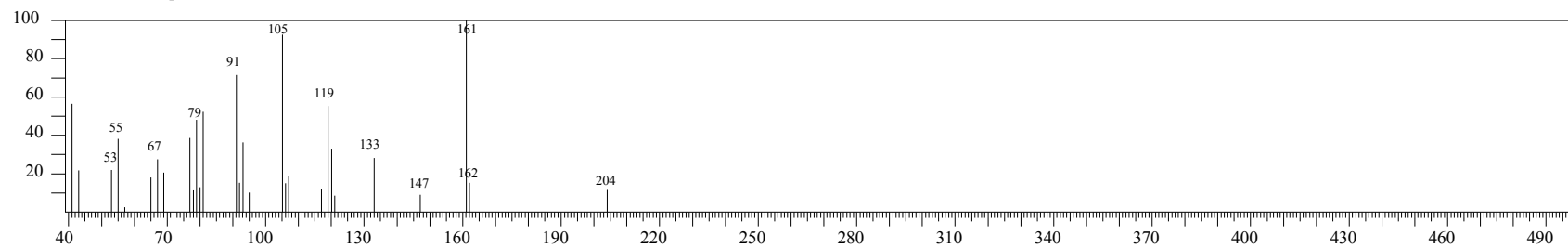


Figure S9. Germacrene D mass spectrum.

Line#:7 R.Time:25.842(Scan#:2790) MassPeaks:25
RawMode:Averaged 25.833-25.850(2789-2791) BasePeak:121.05(6529)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

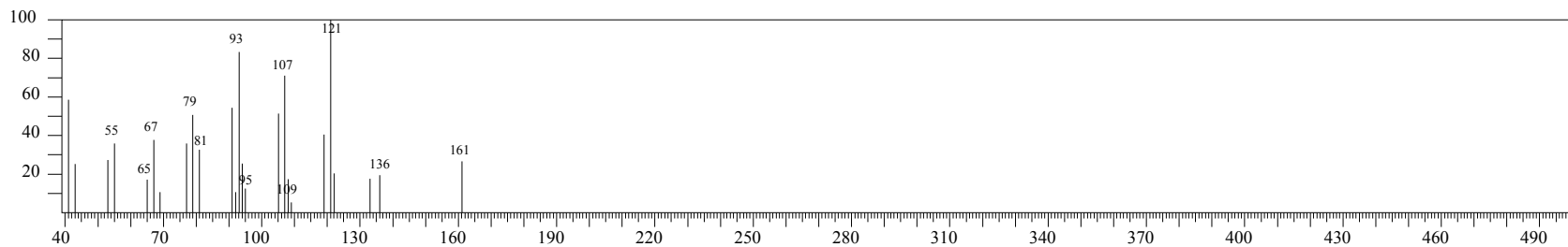


Figure S10. Bicyclogermacrene mass spectrum.

Line#:8 R.Time:26.733(Scan#:2897) MassPeaks:17
RawMode:Averaged 26.725-26.742(2896-2898) BasePeak:161.10(5814)
BG Mode:None Group 1 - Event 1 Scan

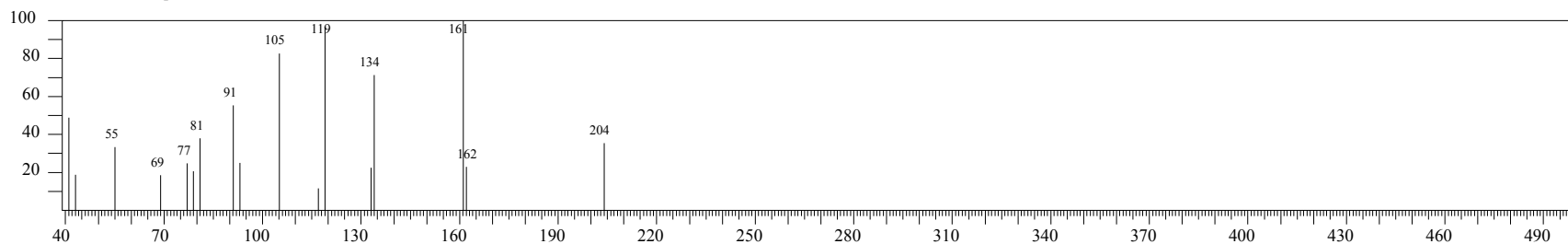


Figure S11. Selinene mass spectrum.

Line#:9 R.Time:28.425(Scan#:3100) MassPeaks:62
RawMode:Averaged 28.417-28.433(3099-3101) BasePeak:69.00(201297)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

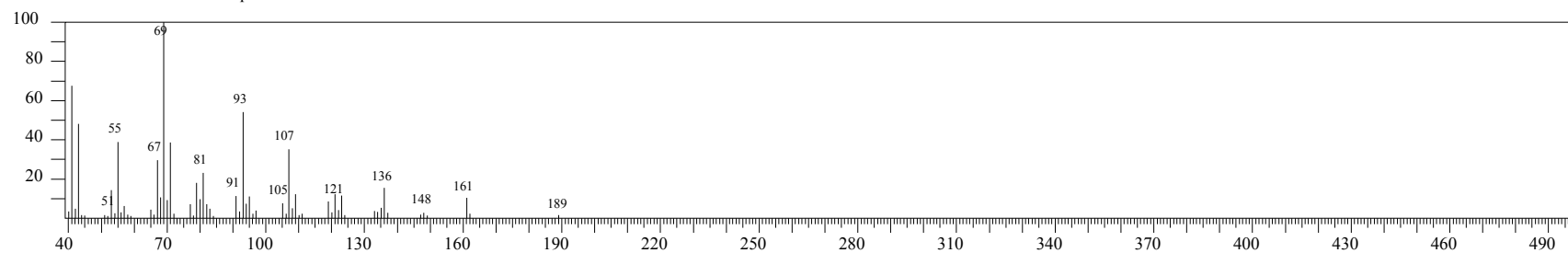


Figure S12. (*E*)-nerolidol mass spectrum.

Line#:10 R.Time:28.825(Scan#:3148) MassPeaks:93
RawMode:Averaged 28.817-28.833(3147-3149) BasePeak:43.00(57427)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

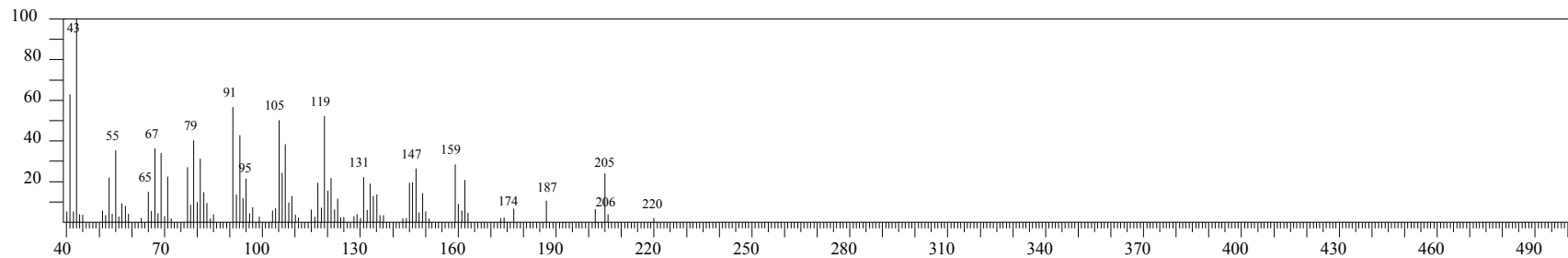


Figure S13. (-)-spathulenol mass spectrum.

Line#:11 R.Time:28.992(Scan#:3168) MassPeaks:90
RawMode:Averaged 28.983-29.000(3167-3169) BasePeak:41.00(94291)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

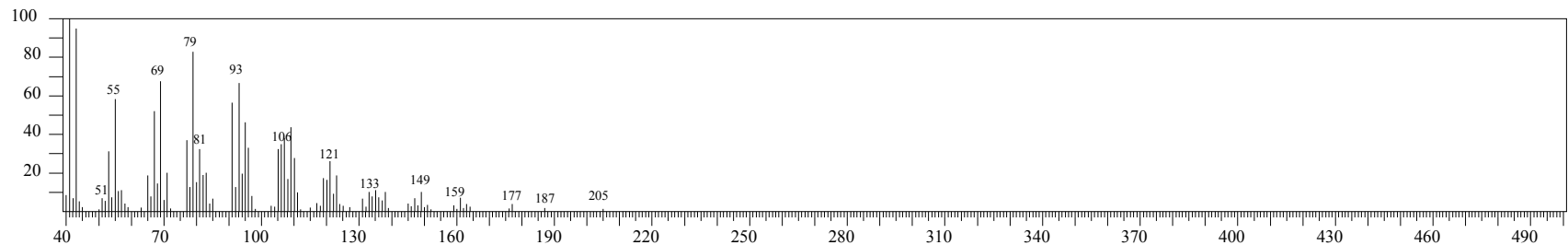


Figure S14. Cariophyllene oxide mass spectrum.

Line#:12 R.Time:29.992(Scan#:3288) MassPeaks:29
RawMode:Averaged 29.983-30.000(3287-3289) BasePeak:67.00(6249)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

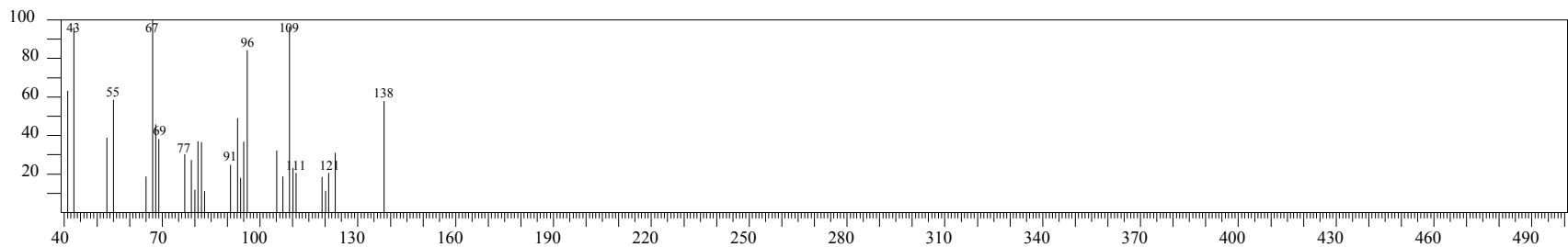


Figure S15. Humulene epoxide II mass spectrum.

Line#:13 R.Time:31.650(Scan#:3487) MassPeaks:27
RawMode:Averaged 31.642-31.658(3486-3488) BasePeak:95.05(5079)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

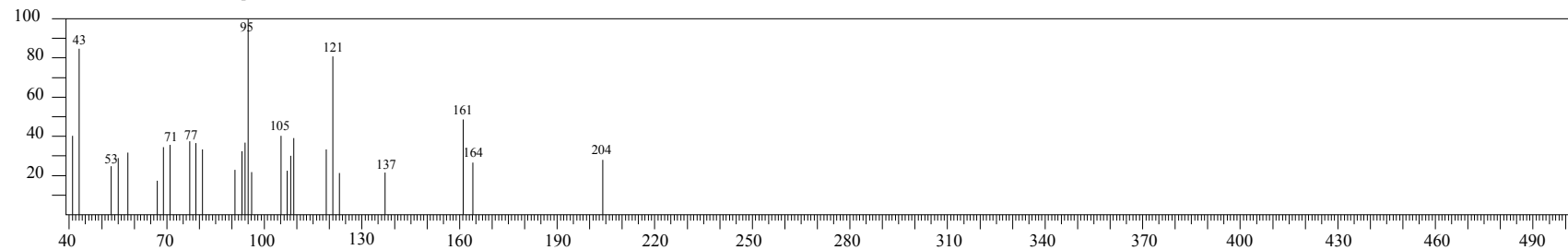


Figure S16. Caryophyllene<14-hydroxy-9-epi-(E)-> mass spectrum.

Line#:14 R.Time:34.200(Scan#:3793) MassPeaks:60
RawMode:Averaged 34.192-34.208(3792-3794) BasePeak:169.00(1111403)
BG Mode:None Group 1 - Event 1 Scan

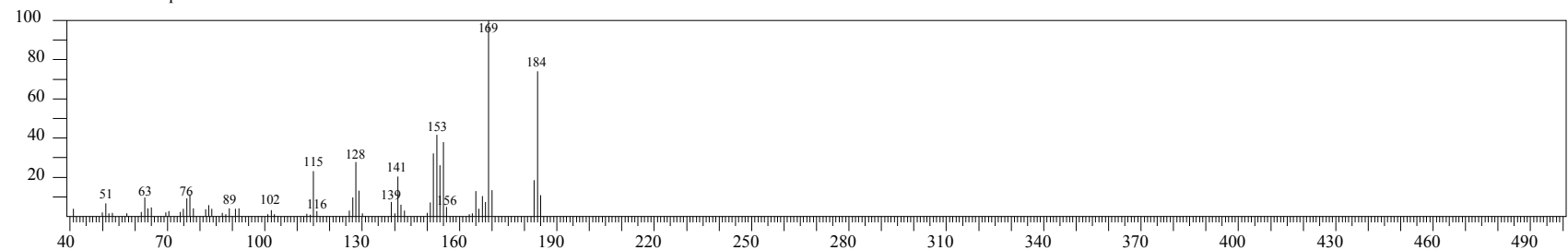


Figure S17. Chamazulene mass spectrum.

Line#:15 R.Time:35.650(Scan#:3967) MassPeaks:53
RawMode:Averaged 35.642-35.658(3966-3968) BasePeak:181.95(281105)
BG Mode:Calc. from Peak Group 1 - Event 1 Scan

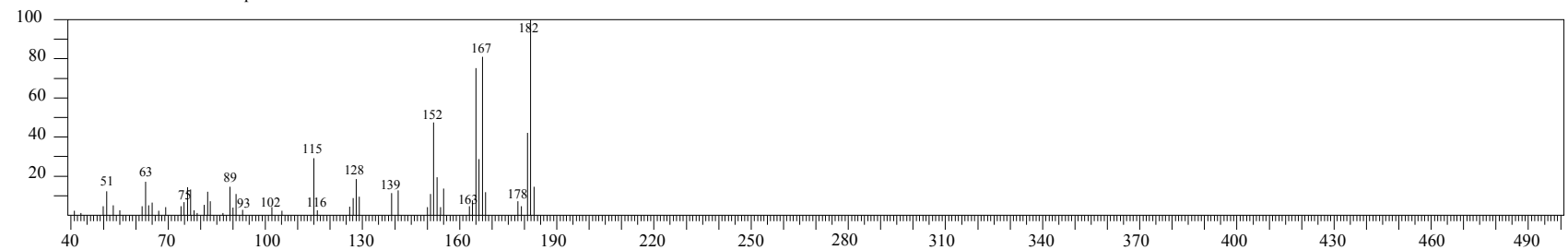


Figure S18. Not identified compound mass spectrum.