**SUPPLEMENTARY MATERIAL**

**HPTLC Bioautography-Guided Isolation of Isogeranic Acid as the Main Antibacterial Constituent of *Artemisia santonicum* Essential Oil**

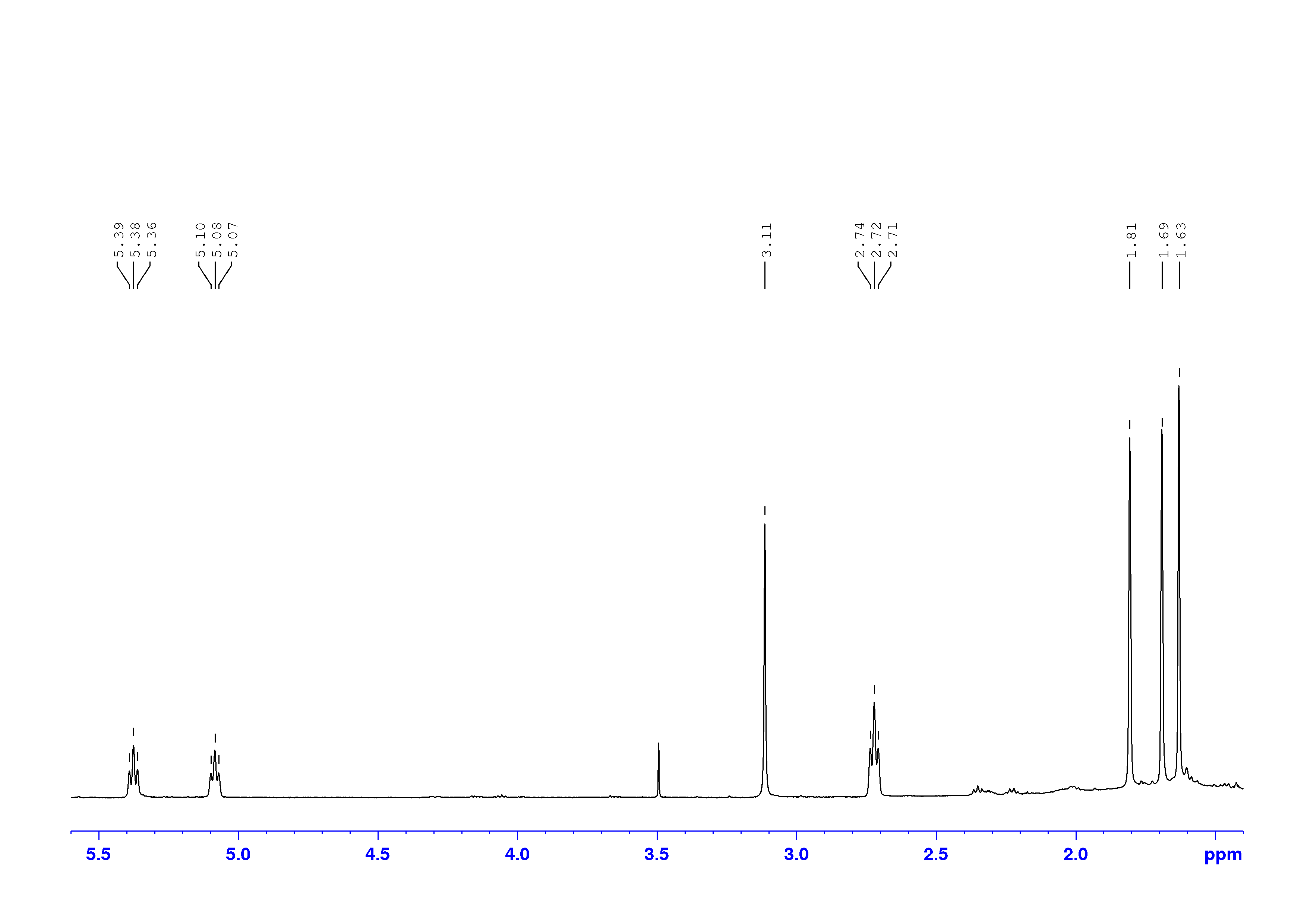
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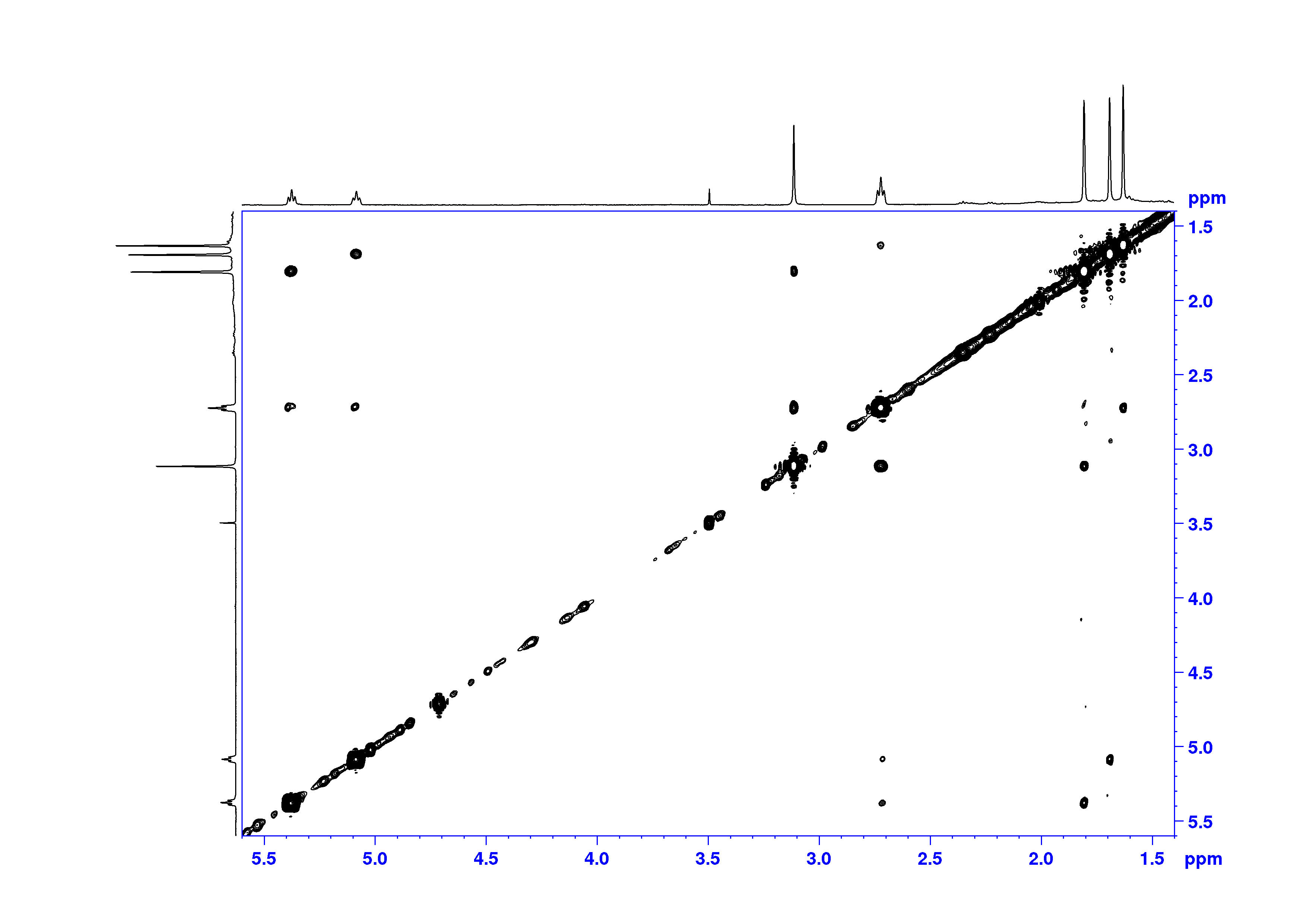
\*Corresponding author, e-mail:[dgodjev@chem.bg.ac.rs](mailto:dgodjev@chem.bg.ac.rs)

slika gcms1.tif

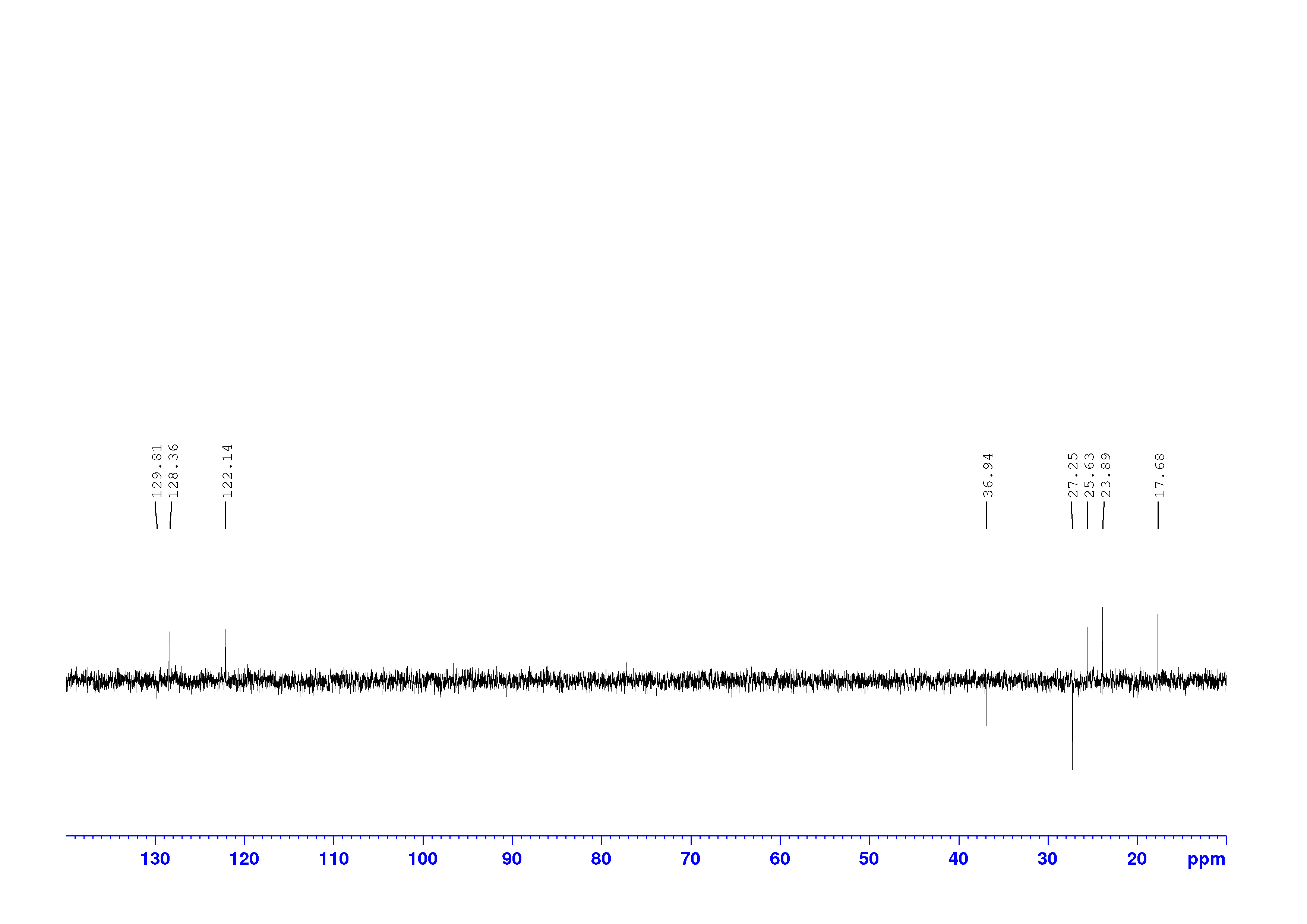
**Figure S1.** GC/FID chromatogram of the essential oil (up) and pure isogeranic acid (down)



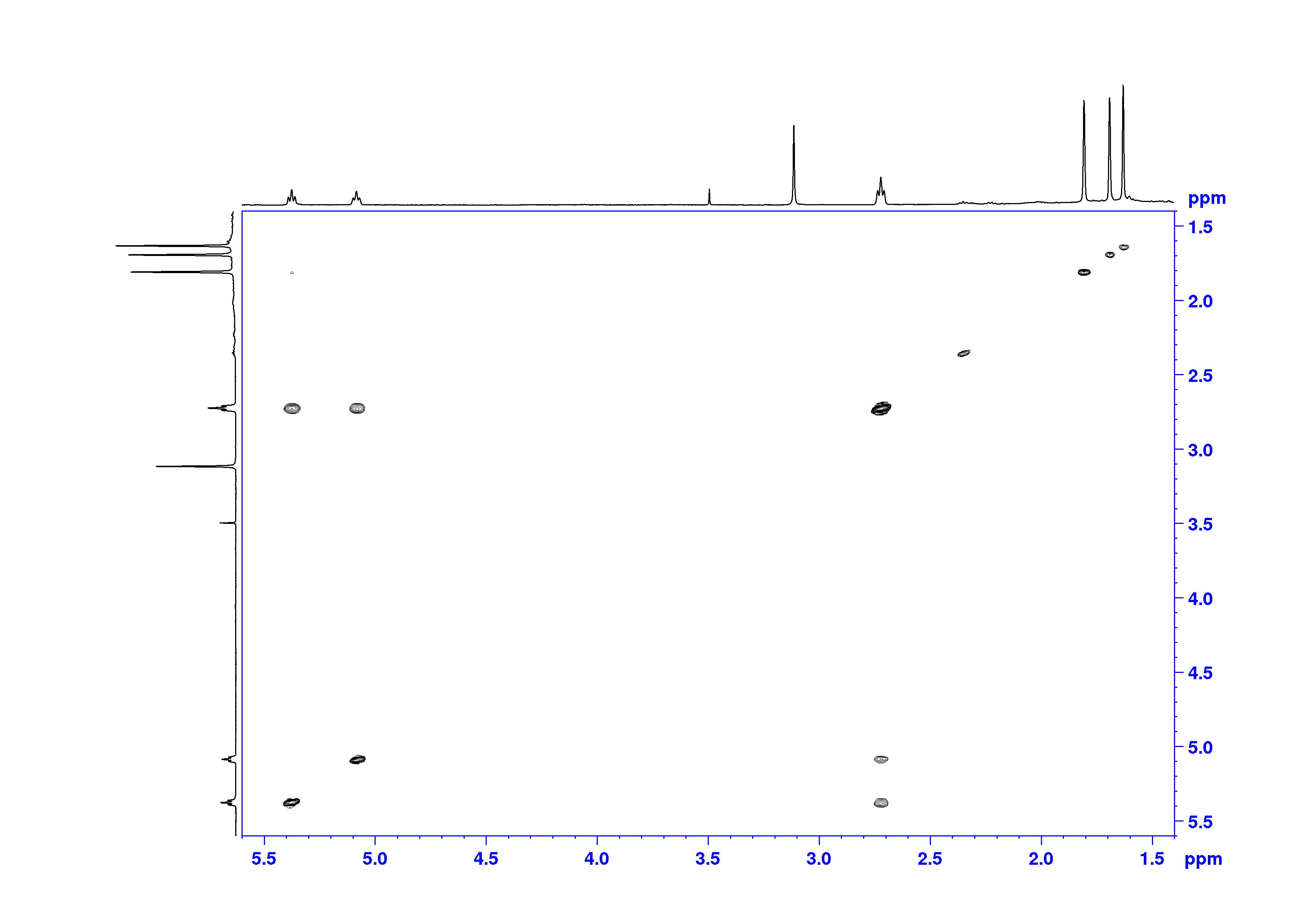
**Figure S2**. 1H NMR spectrum of isogeranic acid



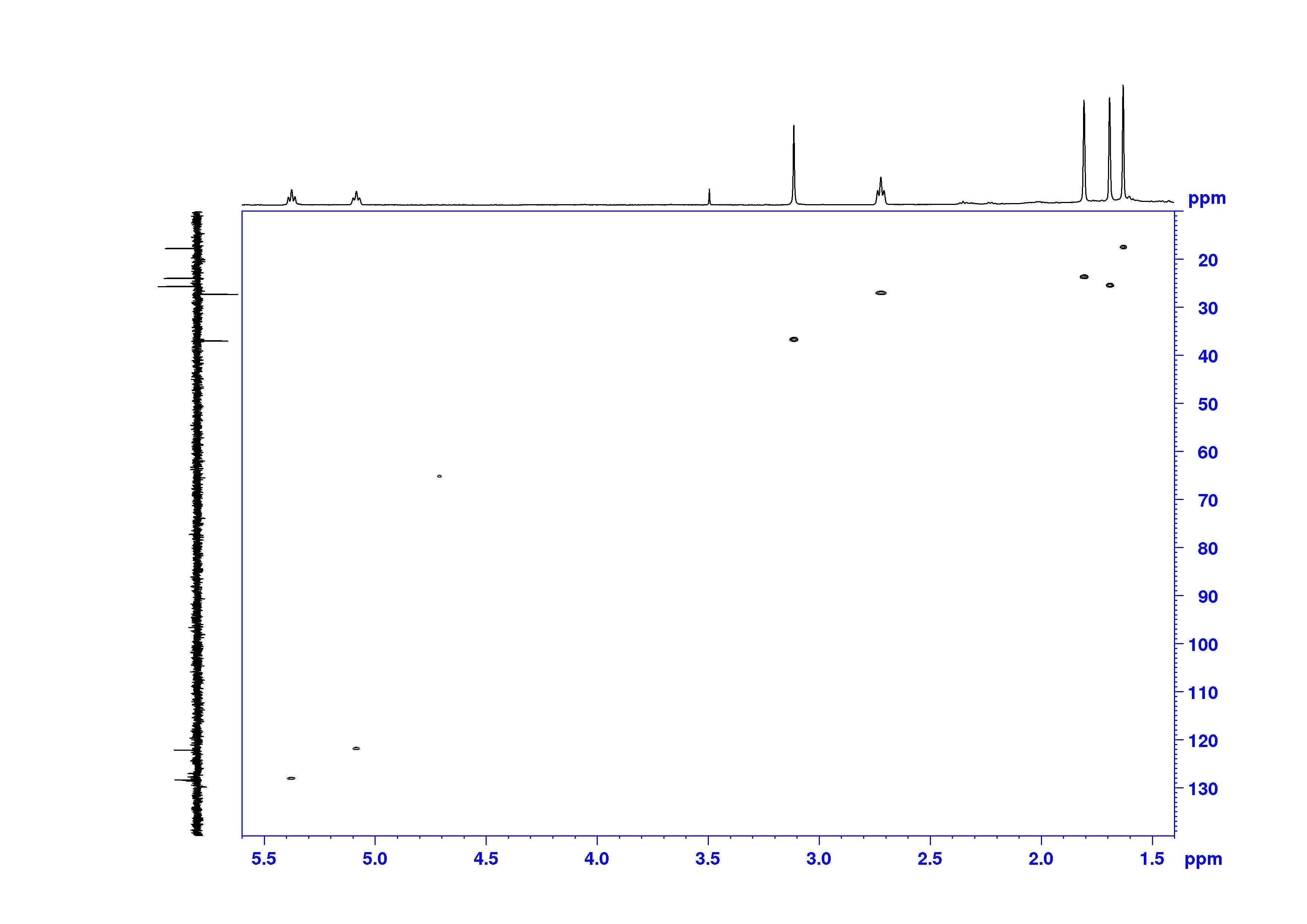
**Figure S3**. NOESY spectrum of isogeranic acid



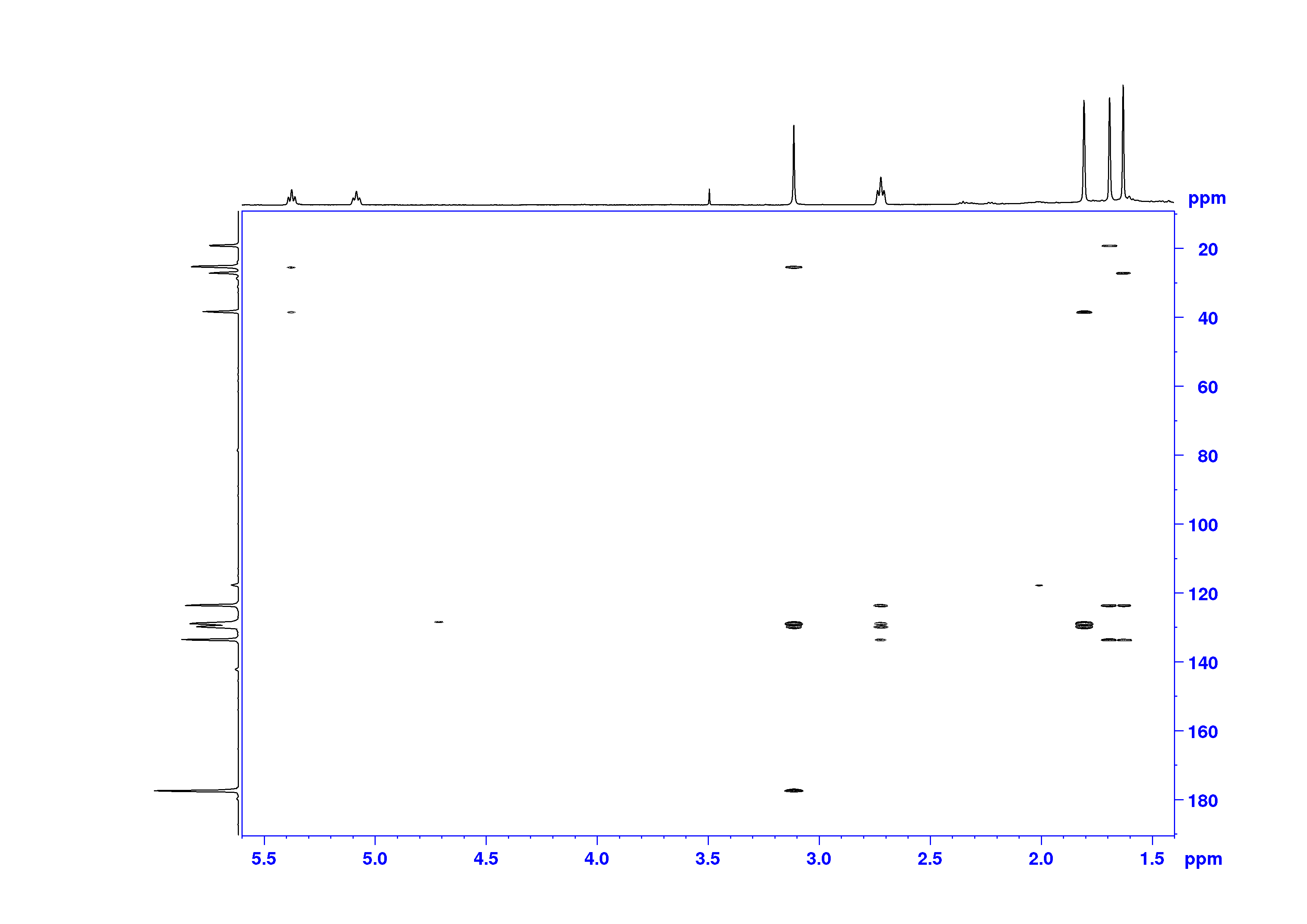
**Figure S4.** DEPT spectrum of isogeranic acid



**Figure S5.** COSY spectrum of isogeranic acid



**Figure S6.** HSQC spectrum of isogeranic acid



**Figure S7.** HMBC spectrum of isogeranic acid

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table S1.** Compositiona of the Essential Oil from *A. santonicum* | | | | |
| No. | Compound | RIb | Rtc(min) | %a |
| 1 | 1,2,5,5-Tetramethyl-1,3-cyclopentadiene | 817 | 3.741 | 0.4 |
| 2 | NI | 868 | 4.599 | 0.1 |
| 3 | Tricyclene | 906 | 5.489 | 0.2 |
| 4 | *α*-Thujene | 914 | 5.579 | 0.2 |
| 5 | *α*-Pinene | 921 | 5.771 | 1.1 |
| 6 | Camphene | 936 | 6.171 | 0.8 |
| 7 | Thuja-2,4(10)-diene | 942 | 6.315 | 0.4 |
| 8 | Sabinene | 966 | 6.852 | 0.8 |
| 9 | *β*-Pinene | 971 | 6.972 | 0.3 |
| 10 | 1,8-Dehydro cineole | 985 | 7.379 | 0.3 |
| 11 | Mesitylene | 991 | 7.457 | 1.3 |
| 12 | Yomogi alcohol | 996 | 7.58 | 0.4 |
| 13 | *α*-Phellandrene | 999 | 7.808 | 0.2 |
| 14 | NI | 1006 | 8.081 | 0.1 |
| 15 | *α*-Terpinene | 1010 | 8.206 | 0.5 |
| 16 | 1,2,4-Trimethylbenzene | 1015 | 8.425 | 0.6 |
| 17 | *p*-Cymene | 1016 | 8.473 | 2.8 |
| 18 | 1,8-Cineole | 1022 | 8.739 | 18.8 |
| 19 | Santolina alcohol | 1026 | 8.848 | 0.3 |
| 21 | NI | 1036 | 9.300 | 0.1 |
| 22 | *γ-*Terpinene | 1046 | 9.698 | 0.9 |
| 23 | *cis*-Sabinene hydrate | 1056 | 10.006 | 0.3 |
| 24 | Artemisia alcohol | 1070 | 10.623 | 0.4 |
| 25 | NI | 1074 | 10.821 | 0.9 |
| 26 | NI | 1082 | 11.137 | 0.1 |
| 27 | Linalool | 1091 | 11.298 | 1.6 |
| 28 | Filifolone | 1094 | 11.455 | 2.6 |
| 29 | *cis*-Thujone | 1097 | 11.559 | 8.4 |
| 30 | *trans-*Thujone | 1110 | 11.969 | 1.6 |
| 31 | Isophorone | 1111 | 12.123 | 2.0 |
| 32 | Chrysanthenone | 1117 | 12.367 | 13.3 |
| 33 | *trans*-Sabinol | 1131 | 12.938 | 2.9 |
| 34 | Camphor | 1136 | 13.124 | 3.0 |
| 35 | *p*-Mentha-1,7-dien-8-ol | 1140 | 13.29 | 0.4 |
| 36 | NI | 1147 | 13.583 | 0.1 |
| 37 | Sabina ketone | 1150 | 13.669 | 0.2 |
| 38 | Pinocarvone | 1155 | 13.881 | 0.6 |
| 39 | Borneol | 1160 | 14.014 | 0.2 |
| 40 | *p*-Mentha-1,5-dien-8-ol | 1155 | 14.063 | 0.5 |
| 41 | Santolinyl acetate | 1159 | 14.253 | 0.2 |
| 42 | Terpinen-4-ol | 1171 | 14.522 | 1.5 |
| 43 | Thuj-3-en-10-al | 1178 | 14.848 | 0.4 |
| 44 | *α*-Terpineol | 1185 | 15.098 | 0.7 |
| 45 | Myrtenal | 1191 | 15.338 | 0.4 |
| 46 | Verbenone | 2004 | 15.898 | 0.8 |
| 47 | Cumin aldehyde | 1237 | 17.278 | 0.4 |
| 48 | Carvone | 1240 | 17.441 | 0.2 |
| 49 | Piperitone | 1250 | 17.891 | 0.3 |
| 50 | *cis*-Chrysanthenyl acetate | 1258 | 18.215 | 0.7 |
| 51 | *p*-Mentha-1,8-dien-3-one | 1268 | 18.673 | 0.5 |
| 52 | NI | 1276 | 19.013 | 0.1 |
| 53 | Bornyl acetate | 1277 | 19.303 | 0.3 |
| 54 | *trans*-Sabinyl acetate | 1285 | 19.636 | 3.3 |
| 55 | Terpinen-4-ol acetate | 1296 | 19.940 | 0.2 |
| 56 | Carvacrol | 1300 | 20.076 | 0.2 |
| 57 | NI | 1305 | 20.307 | 0.1 |
| 58 | NI | 1310 | 20.505 | 0.4 |
| 59 | Filifolide A | 1316 | 20.783 | 1.4 |
| 60 | NI | 1319 | 20.935 | 0.1 |
| 61 | NI | 1334 | 21.607 | 0.1 |
| 62 | Piperitenone | 1338 | 21.759 | 0.1 |
| 63 | Isogeranic acid | 1343 | 21.993 | 0.2 |
| 64 | *α*-Terpinyl acetate | 1345 | 22.127 | 0.1 |
| 65 | NI | 1348 | 22.205 | 0.1 |
| 66 | NI | 1352 | 22.371 | 0.2 |
| 67 | Eugenol | 1359 | 22.504 | Tr |
| 68 | *α*-Copaene | 1368 | 23.296 | 0.3 |
| 69 | NI | 1373 | 23.51 | 0.1 |
| 70 | *β*-Bourbonene | 1381 | 23.689 | 0.1 |
| 71 | NI | 1391 | 24.172 | 3.3 |
| 72 | (*Z*)-Jasmone | 1394 | 24.293 | 1.0 |
| 73 | NI | 1401 | 24.587 | 0.1 |
| 74 | NI | 1411 | 25.028 | 0.2 |
| 75 | (*E*)-Caryophyllene | 1415 | 25.184 | 1.2 |
| 76 | *α*-Humulene | 1449 | 26.641 | 0.1 |
| 77 | (*E*)-*β*-Farnesene | 1453 | 26.803 | Tr |
| 78 | Dehydro sesquicineole | 1464 | 27.303 | 0.3 |
| 79 | NI | 1471 | 27.586 | 0.6 |
| 80 | Germacrene D | 1479 | 27.837 | 1.8 |
| 81 | *β*-Selinene | 1482 | 28.033 | 1.6 |
| 82 | Chrysanthenyl pentanoate | 1486 | 28.174 | 0.8 |
| 83 | Bicyclogermacrene | 1494 | 28.48 | 0.4 |
| 84 | NI | 1501 | 28.742 | 0.3 |
| 85 | NI | 1512 | 29.247 | 0.3 |
| 86 | *δ*-Cadinene | 1516 | 29.597 | 0.1 |
| 87 | NI | 1534 | 30.304 | 0.1 |
| 88 | NI | 1550 | 30.818 | 0.1 |
| 90 | NI | 1562 | 31.359 | 0.1 |
| 91 | NI | 1571 | 31.691 | 0.2 |
| 92 | Spathulenol | 1574 | 31.81 | 1.1 |
| 93 | Caryophyllene oxide | 1580 | 32.023 | 0.5 |
| 94 | NI | 1587 | 32.382 | 0.6 |
| 95 | Ledol | 1599 | 32.842 | 0.1 |
| 96 | NI | 1604 | 33.073 | 0.1 |
| 97 | NI | 1607 | 33.172 | 0.1 |
| 98 | NI | 1609 | 33.282 | 0.1 |
| 99 | NI | 1619 | 33.636 | 0.1 |
| 100 | NI | 1624 | 33.855 | 0.2 |
| 101 | *γ*-Eudesmol | 1627 | 33.952 | 0.1 |
| 102 | NI | 1634 | 34.183 | 0.1 |
| 103 | *epi-α*-Murrolol | 1638 | 34.359 | 0.1 |
| 104 | *α*-Cadinol andNI | 1651 | 34.824 | 0.1 |
| 105 | NI | 1655 | 34.962 | 0.1 |
| 106 | NI | 1662 | 35.251 | 0.1 |
| 107 | 2,4-Dimethylether-phloroacetophenone | 1671 | 35.552 | 0.1 |
| 108 | NI | 1672 | 35.633 | 0.1 |
| 109 | Mustakone | 1675 | 35.748 | 0.1 |
| 110 | NI | 1677 | 35.883 | 0.1 |
| 111 | NI | 1764 | 39.008 | 0.1 |
| 112 | NI | 1782 | 39.684 | 0.1 |
| 113 | NI | 1798 | 40.263 | 0.1 |
| Monoterpene hydrocarbons | | | | 8.2 |
| Oxygenated monoterpenes | | | | 67.5 |
| Sesquiterpene hydrocarbons | | | | 5.6 |
| Oxygenated sesquiterpenes | | | | 3.3 |
| Normonoterpenes | | | | 2.6 |
| Homomonoterpenes | | | | 1.0 |
| Aromatics | | | | 1.9 |
| Total | | | | 90.1 |

aRelative percentages obtained by FID peak area normalization;bRI: Non-ishothermal Kovats retention indices on HP-5 MS (from temperature programming, using definition of Van den Dool and Kratz);cRt: Retention time; tr: Trace (compound present in an amount less than 0.1 %);NI: Not identified.

**Table S2.** NMR Spectroscopic Data of Isogeranic Acid in CDCl3

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Atom No | 1H,*δ* (mult, *J*in Hz), integral | 13C, *δ* | NOESY | HMBC |
| 1 | 1.69 (s), 3H | 25.6 | H-3 | C-10 |
| 2 | / | 133.7 | / | H-10, H-1, H-4 |
| 3 | 5.08 (t, 7.1), 1H | 122.1 | H-1, H-4 | C-10, C-4, C-1 |
| 4 | 2.72 (t, 7.1), 2H | 27.2 | H-10,H-7, H-5,H-3 | C-3,C-5, C-6, C-2 |
| 5 | 5.37 (t, 7.2), 1H | 128.3 | H-9, H-4 | C-9, C-4, C-7 |
| 6 | / | 129.8 | / | H-9, H-4 |
| 7 | 3.11 (s), 2H | 36.9 | H-9, H-4 | C-5,C-6 |
| 8 | / | 177.6 | / | H-7 |
| 9 | 1.80 (s), 3H | 23.9 | H-7, H-5 | C-7 |
| 10 | 1.63 (s), 3H | 17.7 | H-4 | C-4 |

**Table S3**. Minimum Inhibitory (MIC) and Bactericidal Concentration (MBC) of *A. santonicum* Essential Oil and Isogeranic Acid

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Bacteria** | **Essential oil** MIC  MBC  mg/mL | **Isogeranic acid** MIC  MBC  mg/mL | **Streptomycin**  MIC  MBC  mg/mL | **Ampicillin**  MIC  MBC  mg/mL |
| *Staphylococcus aureus* | 15±2  50±3 | / | 0.04±0.003  0.10±0.006 | 0.25±0.008  0.40±0.009 |
| *Bacillus*  *cereus* | 2±0.1  4±0.2 | / | 0.10±0.005  0.20±0.006 | 0.25±0.006  0.40±0.008 |
| *Micrococcus*  *flavus* | 25±4  50±5 | 0.05  0.10 | 0.20±0.006  0.30±0.008 | 0.25±0.005  0.40±0.005 |
| [*Listeria*](http://www.springerlink.com/index/70274UJ0T5TYCB0P.pdf)  [*monocytogenes*](http://www.springerlink.com/index/70274UJ0T5TYCB0P.pdf) | 15±6  50±3 | 0.05  0.10 | 0.20±0.006  0.30±0.005 | 0.40±0.005  0.50±0.006 |
| *Pseudomonas aeruginosa* | 4±0.3  6±0.3 | 0.075  0.10 | 0.20±0.003  0.30±0.002 | 0.75±0.008  1.25±0.040 |
| *Salmonella typhimurium* | 25±4  50±6 | / | 0.15±0.004  0.30±0.006 | 0.40±0.003  0.75±0.003 |
| *Escherichia*  *coli* | 6±0.5  12±0.4 | 0.05  0.10 | 0.20±0.004  0.30±0.006 | 0.25±0.002  0.50±0.030 |
| *Enterobacter cloacae* | 6±0.4  12±0.6 | / | 0.25±0.006  0.50±0.008 | 0.40±0.003  0.75±0.020 |

/ - not tested

**Table S4.** Effects of Essential Oil and Isogeranic Acid on Inhibition of Biofilm Formation of *P.aeruginosa* (PAO1)

|  |  |  |  |
| --- | --- | --- | --- |
| **Samples** | **Inhibition of biofilm formation\*** | | |
| **0.5MIC**  **(%±SE)** | **0.25MIC**  **(%±SE)** | **0.125MIC**  **(%±SE)** |
| Essential oil | - | 1.52±0.62 | 42.54±2.77 |
| Isogeranic acid | 46.77±0.82 | 34.82±0.32 | 18.41±0.19 |
| Ampicillin | 65.28±0.65 | 58.94±0.46 | 85.24±0.37 |
| Streptomyicin | 52.46±0.46 | 74.57±0.36 | 89.31±0.43 |

\*Inhibition of biofilm formation values were calculated as: (mean A620 control well -mean A620 treated well)/(mean A620 control well) ×100. Values are expressed as means ± SE.

- not activity

**Table S5.** Effects of *Artemisia santonicum*Essential Oil and Isogeranic Acid on Twitching and Protrusions Motility of *Pseudomonas aeruginosa* PAO1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Agents** | **Colony diameter**  (mm ± SD) | **Protrusions diameter**  (μm) | **Colony**  **Colour** | **Colony edge on microscope** |
| Essential oil | 11.00±2.65 | - | white | reduced protrusion |
| Ampicillin | 8.33±1.53 | - | white | reduced protrusion |
| Streptomycin | 6.00±1.73 | - | white | reduced protrusion |
| Control (PAO1) | 29.34±3.05 | 96-160 | green | regular protrusion |
| Isogeranic acid | 10.67±1.15 | - | white | reduced protrusion |

**Table S6.** Reduction of the Pyocyanin Production

|  |  |  |
| --- | --- | --- |
| Isogeranic acid | 62.8 | 0.5 |
| *A. santonicum essential oil* | 60.6 | 1.9 |
| Streptomycin | 23.8 | 3.3 |
| Ampicillin | 32.5 | 1.5 |
| Control (PAO1) | 79.3 | 2.3 |