Phytochemical, pharmacological and *in silico* studies on *Teucrium stocksianum* Bioss

Experimental data of Known Compounds 2-12

Ursolic Acid (2)

White crystalline powder. m.p 291˚C. UV λ_{max} ($CHCl_3$) nm (logε): 273 (3.8), 205 (3.0). IR ν_{max} (KBr) cm^{-1}: 3442 (OH), 1743 (carboxylic carbonyl), 1637 (olefinic). ^{1}H NMR (CD_3OD, 300 MHz) δ: 5.25 (1H, br s, H-12), 3.15 (1H, m, H-3), 2.61 (1H, d, *J* = 12.0 Hz, H-18), 2.05 (2H, m, H-11), 1.19 (3H, s, H-23), 1.11 (3H, s, H-27), 0.97 (3H, s, H-24), 0.95 (3H, s, H-25), 0.88 (3H, d, *J* = 6.9 Hz, H-30), 0.85 (3H, d, *J* = 6.9, H-29), 0.77 (3H, s, H-26), 0.73 (1H, br s, H-5). ^{13}C NMR (CD_3OD, 75 MHz) δ: 37.3 (C-1), 28.1 (C-2), 75.7 (C-3), 35.8 (C-4), 48.6 (C-5), 19.6 (C-6), 33.3 (C-7), 42.7 (C-8), 47.5 (C-9), 35.6 (C-10), 26.3 (C-11), 125.9 (C-12) ,138.9 (C-13), 43.7 (C-14), 24.1 (C-15), 23.4 (C-16), 43.2 (C-17), 53.2 (C 18), 35.5 (C-19), 32.5 (C-20), 27.4 (C-21), 32.3 (C-22), 27.1(C-23), 23.3 (C-24), 21.4 (C-25), 15.9 (C-26), 16.1 (C-27), 181.3 (C-28), 16.7 (C-29), 17.5 (C-30). EI-MS (70 e/v) (rel. Int %) *m/z*: 454 ($[M]^+$, 32), 438 (5), 408 (5), 353 (1.6), 248 (88.5), 231 (16.7), 203 (100), 187 (54), 175 (52), 159 (19), 146 (33), 119 (34), 105 (29), 81 (23), 67 (16), 55 (25). HR-EIMS *m/z*: 456.3613 $[M]^+$ (calcd for $C_{30}H_{48}O_3$ 456.3604) [1].

Apigenin (4', 5, 7-trihydroxyflavone) (3)

Yellow crystalline powder. m.p. 315 ˚C. UV λ_{max} (MeOH) nm (logε): 335 (4.1), 271 (2.3), 217 (3.2). IR ν_{max} (KBr) cm^{-1}: 3425 (OH), 1671 (α,β-conjugated carbonyl), 1605-1415 (aromatic moieties), 1385 (O-C). ^{1}H NMR (CD_3OD, 300 MHz) δ: 7.92 (2H, d, *J* = 9.0 Hz, H-2', -6'), 7.85 (2H, d, *J* = 9.0 Hz, H-3', -5'), 6.59 (1H, s, H-3), 6.45 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6). ^{13}C NMR (CD_3OD, 75 MHz) δ: 167.1 (C-2), 105.1 (C-3), 182.8 (C-4), 105.9 (C-4a), 163.0 (C-5), 101.0 (C-6), 160.9 (C-7), 95.9 (C-8), 167.1 (C-8a), 123.9 (C-1'), 129.1 (C-2', -6'), 117.0 (C-3', -5'), 163.9 (C-4'). EI-MS (70 e/v) (rel. Int %) *m/z*: 270 ($[M]^+$, 100), 242 (25), 213 (10), 153 (35), 152 (32), 124 (21), 69 (31), HR-EI-MS: *m/z* 270.0519 $[M]^+$ (calcd for $C_{15}H_{10}O_5$, 270.0528) [2].

3', 4', 7-trihydroxy-5, 6-dimethoxyflavone (4)

Yellow crystalline powder. m.p. 326 ˚C. UV λ_{max} (MeOH) nm (logε): 338 (3.5), 269 (4.1), 212 (3.2). IR ν_{max} (KBr) cm^{-1}: 3413 (OH), 1668 (C=O), 1601–1411 (aromatic moiety). ^{1}HNMR (CD_3OD, 300 MHz) δ: 7.44 (1H, d, *J* = 2.1 Hz, H-2'), 7.40 (1H, dd, *J* = 8.4, 2.1 Hz, H-6'), 6.90 (1H, d, *J* = 8.4 Hz, H-5'), 6.79 (1H, s, H-8), 6.60 (1H, s, H-3), 3.98 (3H, s, MeO (6)), 3.83 (3H, s,

MeO(5)). ^{13}C NMR(CD_3OD, 75 MHz) δ: 166.8 (C-2), 104.4 (C-3), 183.2 (C-4), 112.9 (C-4a), 155.4 (C-5), 140.1 (C-6), 157.2 (C-7), 99.4 (C-8), 158.6 (C-8a), 121.9 (C-1′), 113.7 (C-2′), 147.8 (C-3′), 152.2 (C-4′), 116.9 (C-5′), 122.5 (C-6′), 62.2 (5-OMe), 62.5 (6-OMe). EI-MS (70 e/v) (rel. Int %) *m/z*: 330 ($[M]^+$, 93), 315 (96), 287 (38), 255 (25), 181 (37), 153 (100), 105 (17), 77 (15), 69 (80). HR-EI-MS: *m/z* 330.0738 $[M]^+$ (calcd for $C_{17}H_{14}O_7$, 330.0742) [3].

Luteolin (3', 4', 5, 7-tetrahydroxyflavone) (5)

Yellow crystalline powder. m.p. 330 ˚C. UV λ_{max} (MeOH) nm (logε): 349 (1.8), 264 (3.0), 215 (3.8). IR ν_{max} (KBr) cm^{-1}: 3417 (OH), 1668 (α, β-conjugated carbonyl), 1611-1415 (aromatic moieties). ^{1}HNMR (CD_3OD, 300 MHz) δ: 7.38 (1H, d, *J* = 2.1 Hz, H-2'), 7.36 (1H, dd, *J* = 8.4, 2.1 Hz, H-6'), 6.89 (1H, d, *J* = 8.4 Hz, H-5'), 6.41 (1H, d, J = 1.8, H-8), 6.52 (1H, s, H-3), 6.19 (2H, d, J = 1.8, H-6). ^{13}C NMR (CD_3OD, 75 MHz) δ: 165.1 (C-2), 104.1 (C-3), 182.7 (C-4), 104.7 (C-4a), 158.9 (C-5), 99.8 (C-6), 165.1 (C-7), 94.7 (C-8), 163.8 (C-8a), 122.5 (C-1'), 111.9 (C-2'), 150.0 (C-3'), 148.9 (C-4'), 118.3 (C-5'), 127.1 (C-6'). EI-MS (70 e/v) (rel. int %) *m/z*: 286 ($[M]^+$, 100), 270 (10), 229 (15), 213 (11),153 (29), 152 (22), 134 (19), 111 (12), 78 (12). HR-EI-MS: *m/z* 286.0481 $[M]^+$ (calcd for $C_{15}H_{10}O_6$, 286.0471) [3].

Apigetrin (Apigenin 7-O-D-glucoside) (6)

Yellow needles powder. m.p. 229-232 ˚C. UV λ_{max} (MeOH) nm (logε): 335 (2.7), 267 (3.1), 217 (3.5). IR ν_{max} (KBr) cm^{-1}: 3425 (OH), 1669 (α, β-conjugated carbonyl), 1614-1415 (aromatic moieties). ^{1}H NMR (CD_3OD, 300 MHz) δ: 7.91 (2H, d, *J* = 8.7 Hz, H-2', -6'), 7.21 (2H, d, *J* = 8.7 Hz, H-3', -5'), 6.99 (1H, d, J = 1.8 Hz, H-6), 6.88 (1H, s, H-3), 6.81 (1H, d, *J* = 1.8 Hz, H-8), 4.57-4.33 (6H, m, H -2" – H-6"), 5.77 (1H, d, *J* = 7.5 Hz, H-1"). ^{13}C NMR (CD_3OD, 75 MHz) δ: 165.2 (C-2), 104.2 (C-3), 183.4 (C-4), 106.5 (C-4a), 162.2 (C-5), 100.6 (C-6), 164.3 (C-7), 95.3 (C-8), 167.8 (C-8a), 123.2 (C-1'), 129.2 (C-2', C-6'), 116.3 (C-3', C-5'), 162.4 (C-4'), 101.5 (C-1"), 79.4 –62.1 (C-2"–C-6"). EI-MS (70 e/v) (rel. int %) *m/z*: 270 ($[M\text{-glucose}]^+$, 100), 256 (10), 213 (11),153 (35), 152 (25), 124 (17), 69 (10). HR-FAB-MS: m/z 433.1125 $[M+H]^+$ (calcd for $C_{21}H_{21}O_{10}$, 433.1117) [4].

Cinnamic acid (7)

White crystalline Solid. m.p. 133 ˚C. UV ($CHCl_3$) λ_{max} nm (logε): 293(3.6), 255 (2.5), 233 (3.2). IR ν_{max} (KBr) cm^{-1}: 2650-2755 br (CO<u>OH</u>), 1747 (<u>CO</u>OH), 1640 (olefine), 1609-1408 (aromatic moiety). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.91 (2H, dd, *J* = 7.8, 1.5 Hz, H-2, -6), 7.44 (1H, dd, *J* = 7.2, 1.5, H-4), 6.88 (2H, dd, *J* = 7.8, 7.2 Hz, H-3, -5), 7.58 (1H, d, *J* = 15.4 Hz, H-3′), 6.26 (1H, d,

J = 15.4 Hz, H-2′). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 135.5 (C-1), 129.1 (C-2, -6), 130.3 (C-3, -5), 131.5 (C-4), 169.3 (C-1′), 120.4 (C-2′), 146.2 (C-3′). EI-MS (70 e/v) (rel. int %) *m/z*: 148 ($[M]^+$, 83), 147 (100), 131 (22), 120 (7), 103 (44), 91 (17), 77 (24), 63 (4), 51 (15). HR-EI-MS: *m/z* 148.0519 $[M]^+$ (calcd for $C_9H_8O_2$, 148.0522).

2H-Chromen-2-one (8)

White to light yellow. m.p. 71 ˚C, UV ($CHCl_3$) λ_{max} nm (logε): 321 (2.1), 283(2.5), 274 (3.4). IR ν_{max} (KBr) cm^{-1}: 1759 (OC=O, lactone), 1602-1415 (aromatic moiety), 1385 (O-C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.91 (1H, d, *J* = 9.6 Hz, H-4), 7.53-7.60 (2H, m, H-5, -6), 7.28-7.33 (2H, m, H-7, -8), 6.40 (1H, d, J = 9.6 Hz, H-3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 161.2 (C-2), 117.2 (C-3), 143.4 (C-4), 127.1 (C-5), 125.6 (C-6), 131.0 (C-7), 116.0 (C-8), 119.5 (C-9), 154.4 (C-10). EI-MS (70 e/v) (rel. int %) *m/z*: 146 ($[M]^+$, 30), 117 (100), 88 (31), 85 (37), 62 (23). HR-EI-MS: *m/z* 146.0377 $[M]^+$ (calcd for $C_9H_6O_2$, 146.0367) .

Benzoic Acid (9)

Colorless crystalline solid. m.p. 122.4 ˚C. UV ($CHCl_3$) λ_{max} nm (logε): 293 (2.8), 255 (3.2), 233 (4.0). IR ν_{max} (KBr) cm^{-1}: 2660-2770 br (CO<u>OH</u>), 1747 (<u>CO</u>OH), 1609-1478 (aromatic moiety). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.91 (2H, dd, *J* = 7.8, 1.5 Hz, H-2, -6), 7.44 (1H, dd, *J* = 7.2, 1.5, H-4), 6.88 (1H, dd, *J* = 7.8, 7.2 Hz, H-3, -5). ^{13}C-NMR (CD_3Cl, 75 MHz) δ: 169.0 (CO), 131.3 (C-1), 131.1 (C-2, -6), 130.6 (C-3, -5), 134.6 (C-4). EI-MS (70 e/v) (rel. int %) *m/z*: 122 ($[M]^+$, 7), 105 (100), 94 (5), 77 (59), 65 (6), 51 (22), 43 (7). HR-EI-MS: *m/z* 122.0368 $[M]^+$ (calcd for $C_7H_6O_2$, 122.0375).

Salicylic Acid (2-Hydroxybenzoic acid) (10)

Colorless amorphous powder. m.p. 156-158 ˚C. UV ($CHCl_3$) λ_{max} nm (logε): 231 (4.2), 251 (3.1), 295 (2.8), IR ν_{max} (KBr) cm^{-1}: 2580-2750 br (CO<u>OH</u>), 1762 (<u>CO</u>OH), 1609-1430 (aromatic). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.91 (1H, dd, *J* = 7.8, 1.5 Hz, H-6), 7.44 (1H, dd, J = 7.2, 7.2, 1.5, H-4), 6.84-6.91 (2H, m, H-3, -5). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 173.4 (C=O), 113.2 (C-1), 163.4 (C-2), 116.3(C-3), 135.2 (C-4), 120.6 (C-5), 130.8 (C-6). EI-MS (70 e/v) (rel. int %) *m/z*: 138 ($[M]^+$, 53), 120 (100), 92 (83), 81 (2), 64 (25), 53 (5), 46 (3). HR-EI-MS: *m/z* 138.0311 $[M]^+$ (calcd for $C_7H_6O_3$, 138.0316).

Stearic Acid (11)

White amorphous powder. m.p. 70 ˚C. UV ($CHCl_3$) λ_{max} nm (logε): 276, IR ν_{max} (KBr) cm^{-1}: 2730 br (OH), 1717 (carboxylic acid), 1350 (O-C). ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.34 (2H, t, *J* = 6.4 Hz, H-2), 1.71 (2H, qn, *J* = 7.0 Hz, H-3), 1.26 (28H, br, H-4 – H-17), 0.86 (3H, t, *J* = 6.8 Hz, H-18). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 181.7 (C-1), 33.1 (C-2), 27.2 (C-3), 30.1-33.2 (C-4– C-16), 23.1 (C-17), 13.0 (C-18). EI-MS (70 e/v) (rel. int %) *m/z*: 284 ($[M]^+$, 9), 256 (40), 241 (14), 239 (9), 213 (33), 185 (22), 171 (21), 157 (23), 143 (18), 129 (53), 115 (19), 111 (21), 101 (14), 97 (37), 87 (24), 85 (63), 83 (44), 73 (100), 71 (41), 69 (51), 60 (82), 57 (70), 43 (72). HR-EI-MS: *m/z* 284.2810 $[M]^+$ (calcd for $C_{18}H_{36}O_2$, 284.2815) [5].

1-Dotriaconatanol (12)

White amorphous powder. m.p. 89.3 ˚C. UV ($CDCl_3$) λmax nm (logε): 201 (4.1), 203 (3.5), 193 (4.2). IR ν_{max} (KBr) cm^{-1}: 3306 (OH), 3072 (CH_3), 2812 (CH_2), 1440 (O-C). ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.69 (2H, t, *J* = 7.2 Hz, H-1), 1.52 (2H, br, H-2), 1.23 (58H, br, H-3 – H-31), 0.86 (3H, t, *J* = 7.0 Hz, H-32). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 63.0 (C-1) , 35.7 – 22.4 (C-2 – C-31) , 14.1 (C-32). EI-MS (70 e/v) (rel. Int %) *m/z*: 448 ($[M-H_2O]^+$, 6), 420 (15), 392 (20), 378 (10), 364 (19), 350 (10), 336 (12), 307 (10), 279 (10), 251 (10), 236 (10), 208 (14), 194 (17), 182 (22), 168 (29), 154 (38), 139 (57), 125 (88), 111 (92), 97 (90), 83 (95), 71 (100), 57 (96). HR-FAB-MS: *m/z* 467.5189 $[M+H]^+$ (calcd for $C_{32}H_{67}O$, 467.5195) [6].

Pharmacological evaluation assays

Antibacterial bioassay

The antibacterial potential of ethanolic extract and its fractions and new isolate of *T. stocksianum* was determined against Gram-positive *Staphylococcus aureus*, *Streptococcus,* and Gram-negative *Escherichia coli* and *Klebsiella pneumonia* bacteria by agar well diffusion method using Mueller-Hinton agar medium [7]. Test samples were prepared by dissolving 3 mg extract in 1 mL DMSO. The strains were grown in Mueller-Hilton agar and broth. The strains were incubated at 37 ˚C for 24 hrs and diluted to approximately 10^6 CFU/mL. 100 µL of 10^6 CFU/mL containing bacterial suspension was spread over Mueller-Hilton agar and homogenized. After solidification, wells of 6 mm-diameters were made using sterile crock-borer. 100 µL test samples, ceftriaxone (positive control) and DMSO (negative control) were inoculated in respective wells and all wells were incubated at 37 ˚C for 24 hrs. Antibacterial activity was determined by measuring the diameter of zone of inhibition and results were expressed in mm. All reactions were performed in triplicate.

Antifungal bioassay

The *in vitro* antifungal activity of ethanolic extract and its fractions and new isolate of *T. stocksianum* were evaluated by agar tube dilution method [8] against *Candida albicans, Candida glabrata, Fusarium solani, Microsporum canis* and *Aspergillus flavus* fungi. Each extract (20 mg) was dissolved in 1 mL sterile DMSO. The strains were cultured in sabouraud dextrose agar (SDA), the acidic (pH 5.5-5.6) media was prepared by mixing 32.5 g/500 ml distilled water containing glucose or maltose 2%. The media (4 mL) was transferred to screw caps tube and autoclaved at 121 ˚C for 15 min. Cooled the tubes at 50 ˚C and 66.6 µL test samples were employed. Solidify the tubes and each tube was inoculated with 4 mm diameter fungus plates. The plates were incubated for 3-7 hours at 27-29 ˚C. For positive control, amphotericin B used for *A. flavus* and miconozale used for others. DMSO was used as negative control. The test solution diffused with the growth of inoculated microorganisms. The percentage inhibition of fungal growth was measured. The results were expressed in percentage inhibition as: 0-39 low active; 40-59 moderate active; 60-69 good active; >70 significant active. All reactions were performed in triplicate.

Insecticidal activity

Crude ethanolic extract and all its fractions and new isolate of *T. stocksianum* were evaluated against insects viz *Tribolium castaneum* and *Rhyzopertha dominica* by impregnated filter paper test [9]. Each extract sample was prepared by dissolving 20 mg in 2 mL volatile solvent (methanol/acetone). A filter paper placed in the petri plate (5 cm or 50 mm) and the whole sample of different concentrations was loaded over the filter paper and plates left for 24 hours to evaporate the solvent. After 24 hours, 10 healthy and active insects of same size and age of each species were placed in each petri plate and the plates were incubated at 27 ˚C for 24 hours with 50% relative humidity in growth chamber. Permethrin was used as positive control and volatile solvent was used as negative control. Total number of survivors per petri plate were counted and recorded on the third day. The % mortality was calculated by the following formula:

$$\% \text{ Mortality} = 100 - \frac{\text{No. of insects alive in test sample}}{\text{No. of insects alive in control}} \times 100$$

Cytotoxicity/Brine shrimp lethality bioassay

To check *in vitro* cytoatoxicity/brine shrimp activity, reported protocol was employed [Alves et al. 2000]. The *Artemia salina* (shrimps) eggs were stored at 4 ˚C. Artificial seawater was prepared

by dissolving 38 g sea salt in 1 liter double distilled water (pH 7.4), filtered it and was placed in small partition of hatching tray. 1 mg of shrimp eggs (*A. salina*) was added to the large partition of the tray, which was darkened with aluminum foil and incubated at 37 ˚C. The shrimp's larvae were attracted by illuminated partition through perforation of the wall. It was permitted to stand for 48 hours at 25 ℃ to hatch and mature the shrimps. Each extract (20 mg) was dissolved in 2 mL respective solvent. From these solution, 5, 50 and 500 µL were transferred to vials in triplicate and adjusted at 10, 100, 1000 µg/mL concentrations and the solvents were evaporated. After 2 days, the shrimp larvae were matured. In each veil, added 10 shrimp's larvae using Pasteur pipette and 5 mL seawater, and then incubated at 25-27 ˚C 24 hours under illumination. After 24 hours, the surviving larvae were counted and determine the LD_{50} using Probit method by Finney computer program.

Phytotoxic bioassay

Phytotoxic activity of ethanolic extract and its fractions of *T. stocksianum* were evaluated against the *Lemna minor* using standard procedure [10]. The growth medium for *Lemna* was prepared by dissolving various constituents in distilled water (1L) and its pH was adjusted at 5.5-5.6 by adding KOH pellets. The medium was then autoclaved at 121 ˚C for 15 min. The extracts (30 mg) were dissolved in methanol/ethanol (10 ml) to prepare a stock solution. Three concentrations were prepared of 10, 100 and 1000 µg/ml by taking 10, 100 and 1000 µl of the stock in petri dish as three petri plates of each concentration. The solvent was allowed to evaporate overnight under sterile conditions. Each plate was inoculated with growth medium (20 mL) and *Lemna minor* plants (10), each containing a rosette of three fronds. Three other plates each were supplemented with growth media and reference growth inhibitors, served as negative control. All plates were kept in the growth cabinet for seven days. Total number of fronds per petri plate was counted and recorded on seventh day. Percent growth inhibition was calculated as % regulation as follows.

$$\% \text{ Regulation} = 100 - \frac{\text{No. of fronds in test sample}}{\text{No. of frond in –ve control}} \times 100$$

Antioxidant: DPPH radical scavenging activity bioassay

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH). The solution of DPPH was prepared by dissolving 8 mg in methanol (250 mL, 80%). 0.2 Microliters of methanolic solution of each test sample of different concentration (5-500 µg) was mixed with 3

mL of DPPH solution. The mixture was then dispersed in 96 well plates. The mixture was shaken and kept for an hour at room temperature. The absorbance was measured at 515 nm by microtitre plate reader (Spectramax plus 384 Molecular Device, U.S.A.). Acrobic acid was used as positive control and DPPH as negative control. The percentage inhibition was determined by the following formula. All reactions were carried in triplicate.

$$\% \text{ Inhibition} = [1 - (A_{sample} / A_{control})] \times 100$$

Computational studies

Presently, First-principles methods are interesting tools to explore various properties of interests [11-14]. Density functional theory (DFT) was comprehensively applied for prediction of electronic properties which also effectively replicated the experimental data. The DFT is reliable tool for the ground state (S_0) geometries and electronic properties. The DFT was found an effective and reliable approach for the S_0 geometries optimizations of different compounds [15-16]. The B3LYP is rational for the S_0 geometries of various organic compounds [17-18]. Previously, it was concluded that after optimizing the geometries of organic compounds by B3LYP functional with a number of basis sets has no noteworthy effect on the geometrical parameters. In the present study, the optimizations were performed by B3LYP functional and triple zeta with 2 polarization function (TZ2P) basis set [19-20]. All the calculations were performed by ADF.

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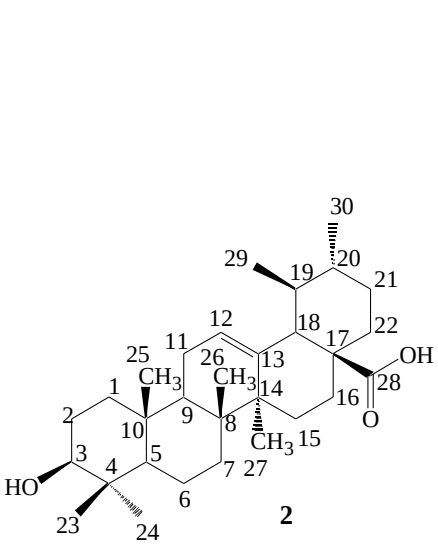

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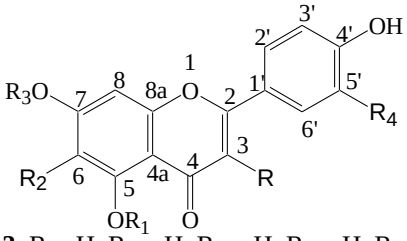
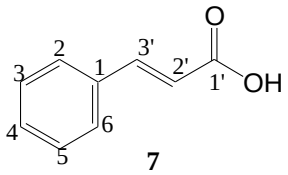


3, R = H, R_1 = H, R_2 = H, R_3 = H, R_4 = H
4, R = H, R_1 = Me, R_2 = OMe, R_3 = H, R_4 = OH
5, R = H, R_1 = H, R_2 = H, R_3 = H, R_4 = OH
6, R = H, R_1 = H, R_2 = H, R_3 = β-D-O-glu, R_4 = H



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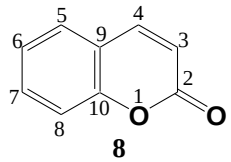

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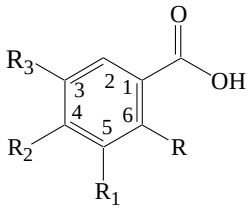
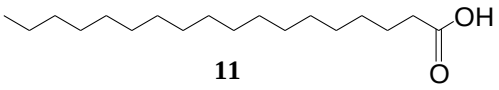


9, R = H, R_1 = H, R_2 = H, R_3 = H
10, R = OH, R_1 = H, R_2 = H, R_3 = H



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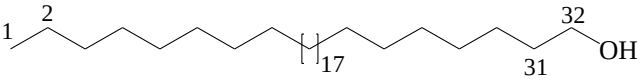
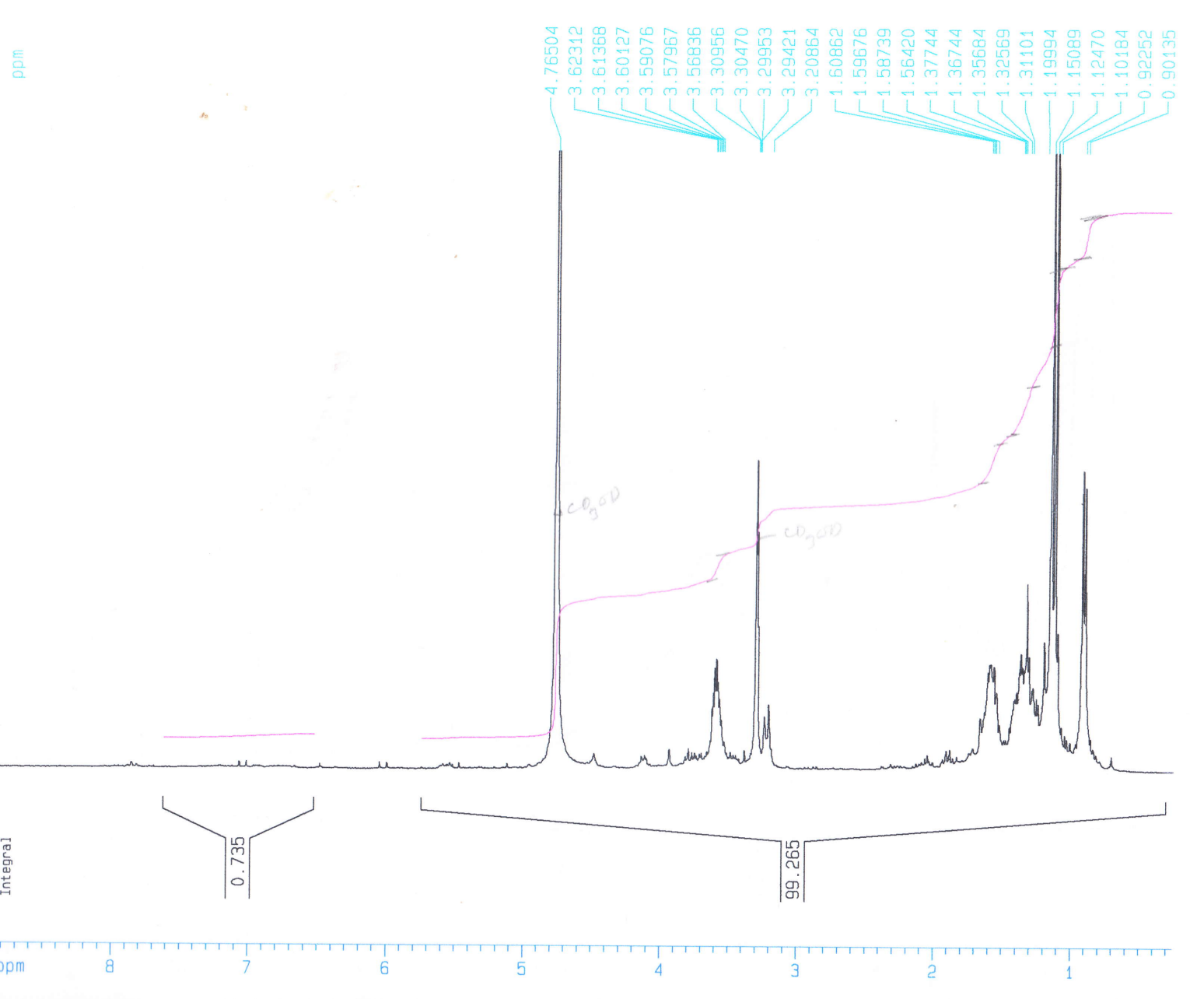


12

Figure S1: Structures of the compounds **2-12**.

Spectra of the teucrol can be found on next pages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3.20864
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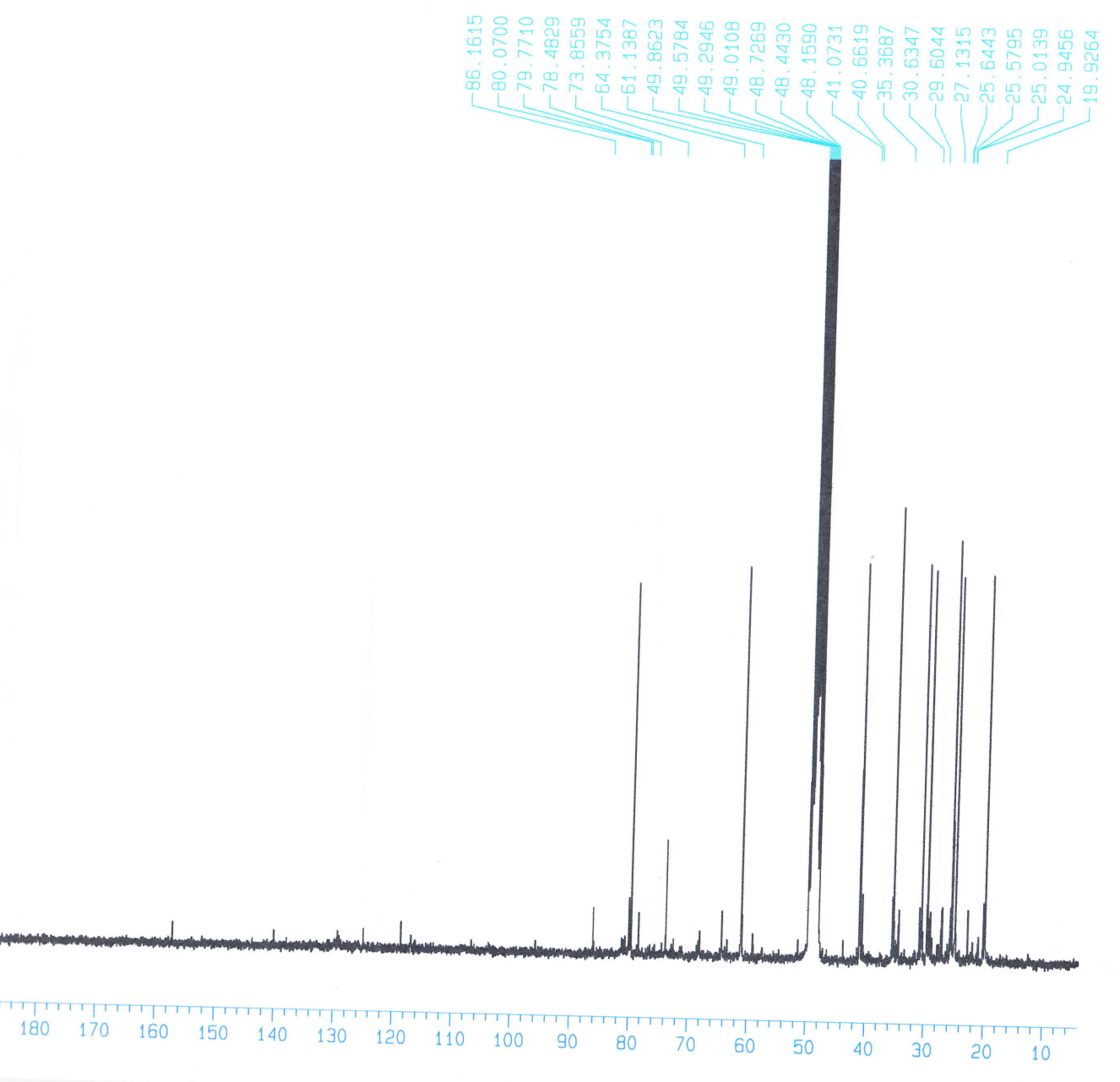
ppm
8
7
6
5
4
3
2
1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30.6347
29.6044
27.1315
25.6443
25.5795
25.0139
24.9456
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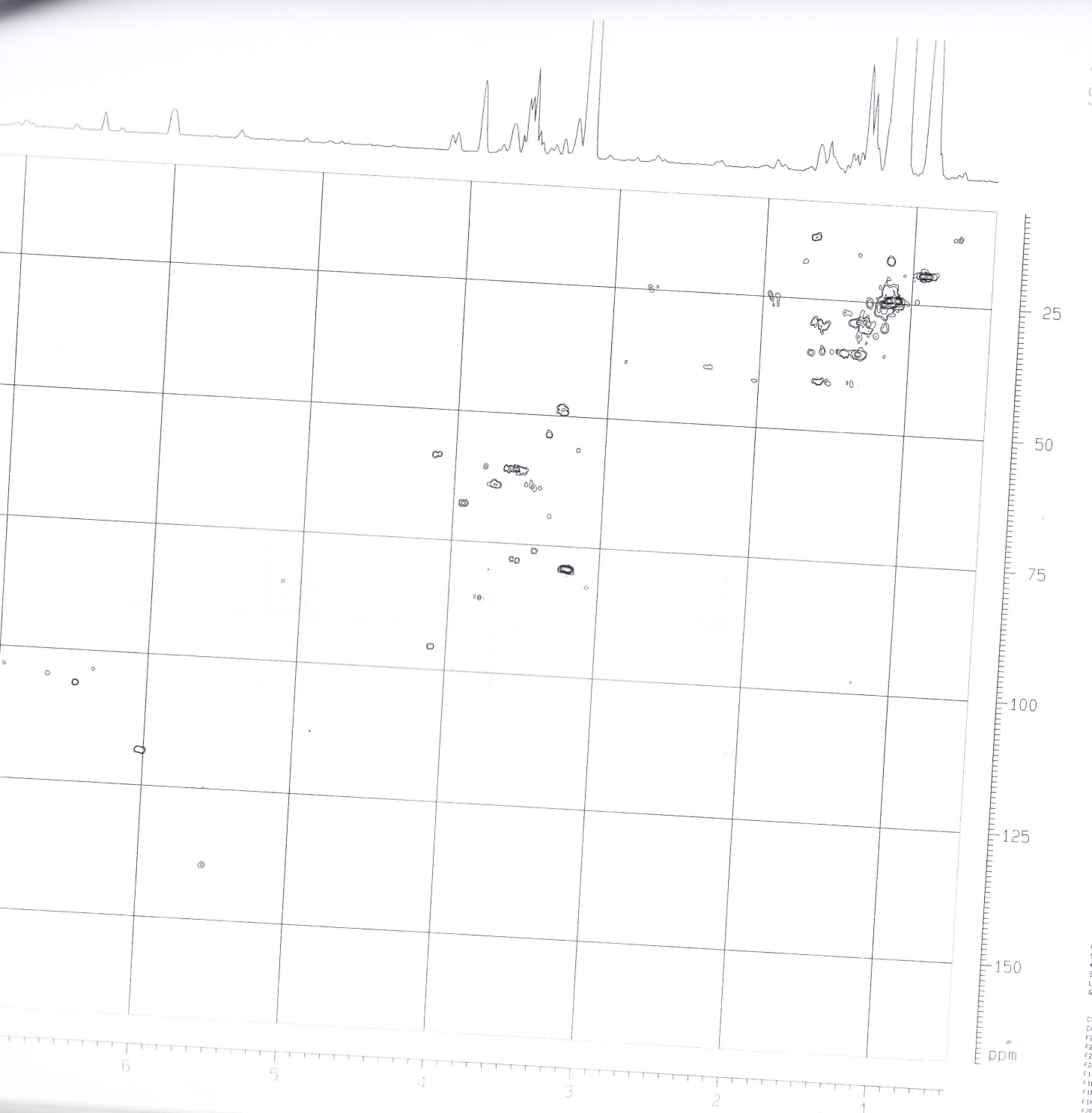
20
10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2
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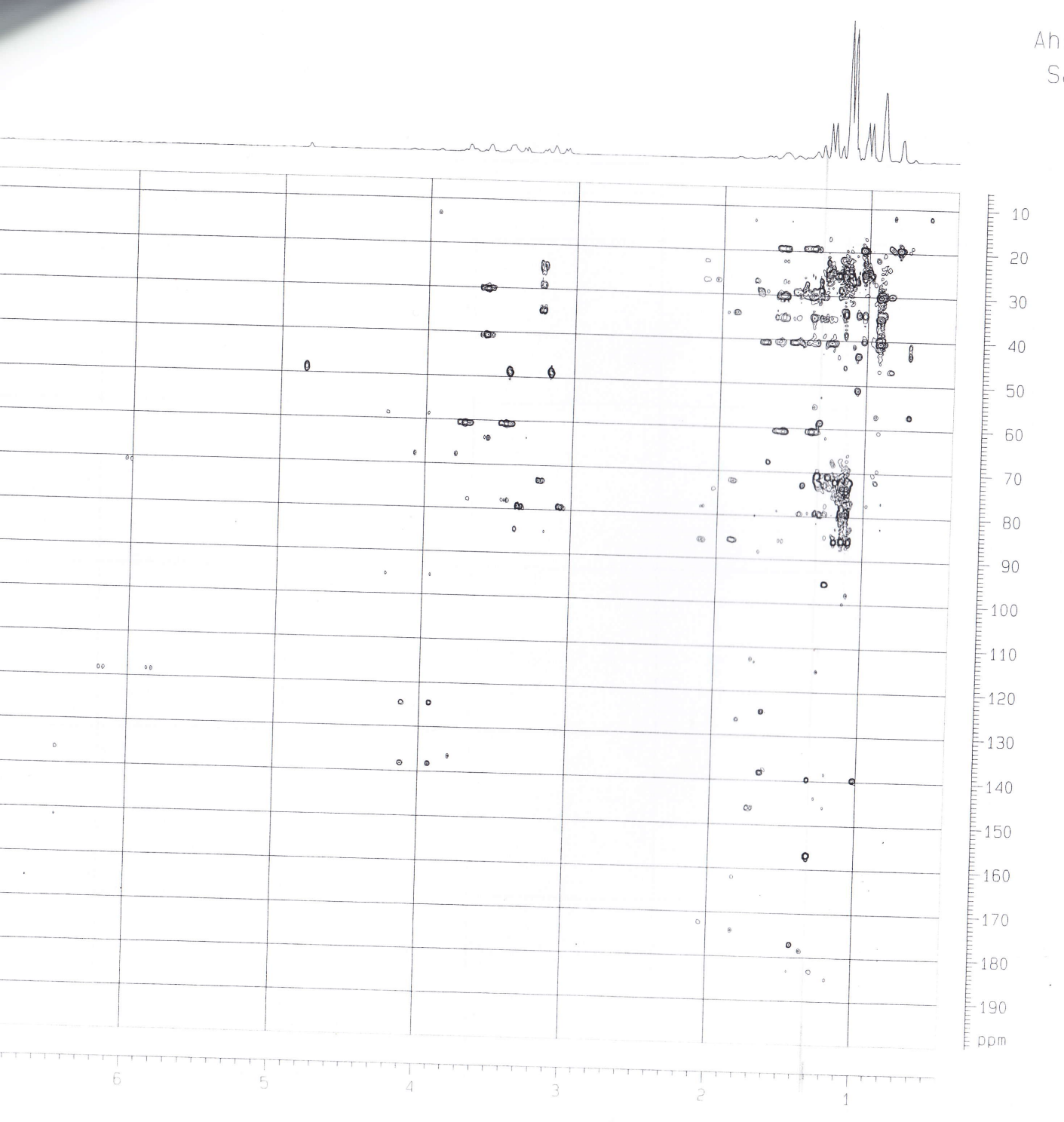
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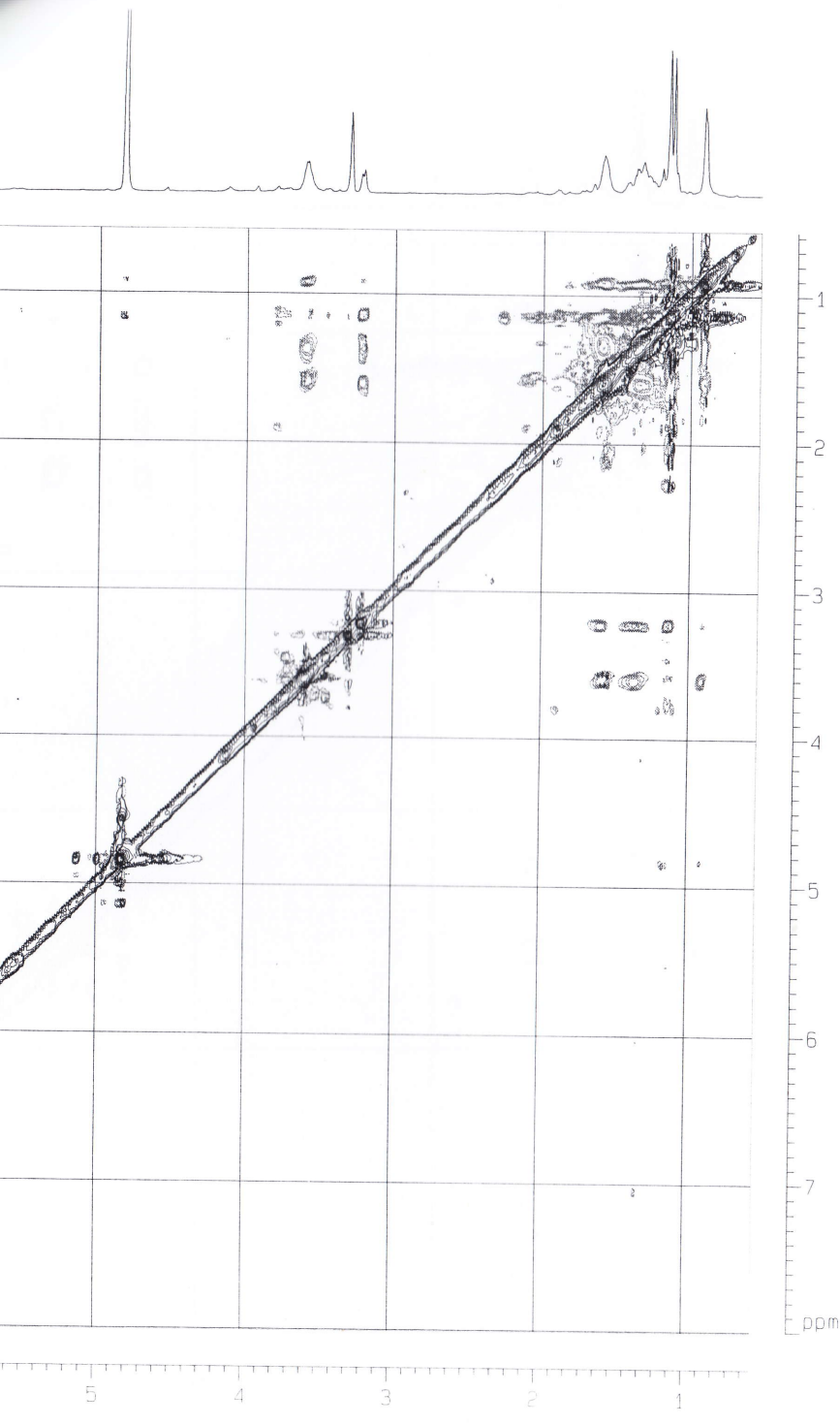
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ppm
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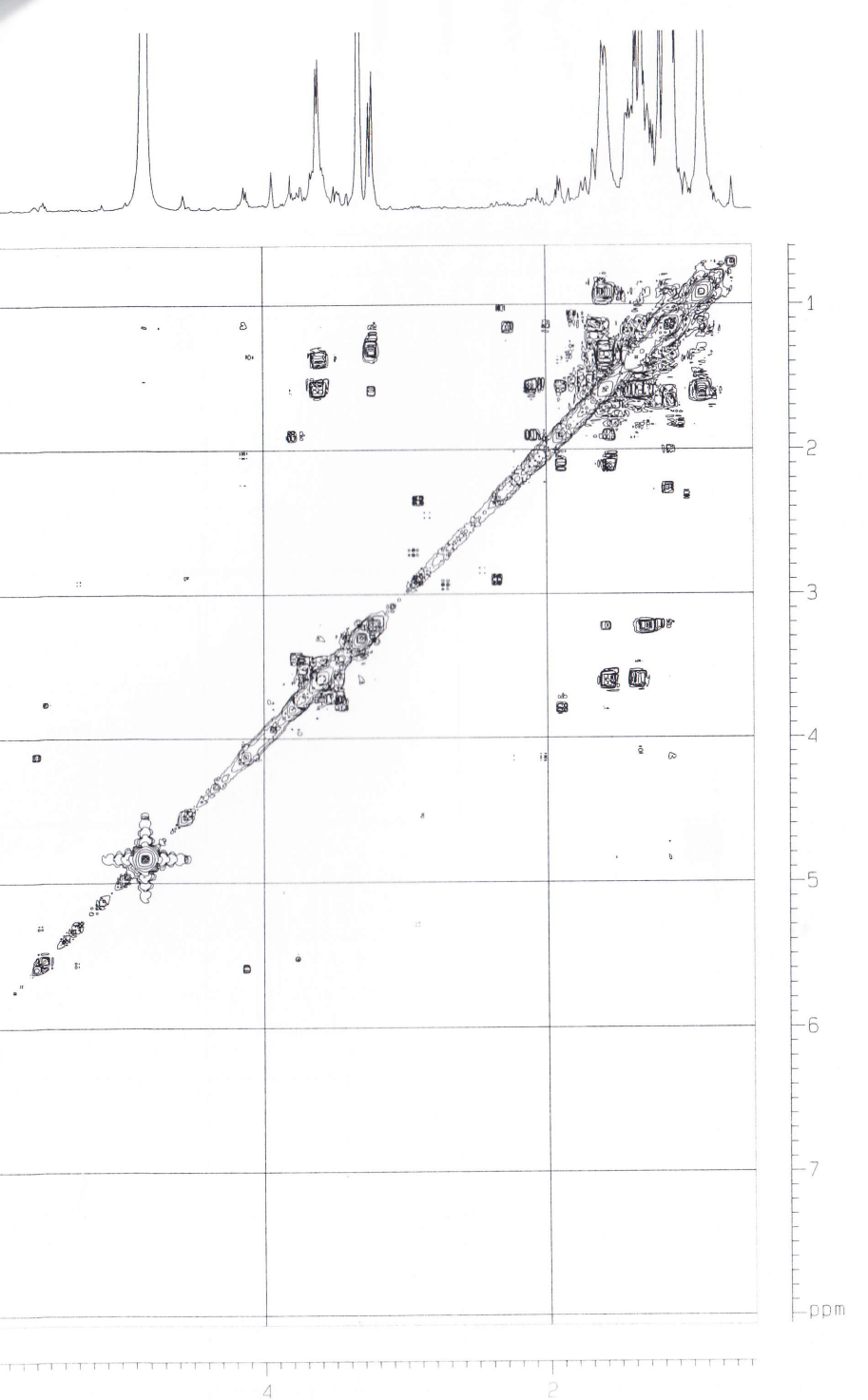
2
1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R.T.: 1.12

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