Metabolic profiling and discrimination of *Cymbopogon* species using direct analysis real time mass spectrometry and principal component analysis

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Supplementary material

Experimental method

1. Chromatography

The analyses were performed on an Agilent 1200 HPLC system (Agilent Technologies, Waldbronn, Germany) consisting of a quaternary pump (G1311A), online vacuum degasser (G1322A), autosampler (G1329A), thermostatted column compartment (G1316C) and diode-array detector (G1315D). The outlet of the diode array detector was introduced into the electrospray (ESI) interface of the mass spectrometer through a flow splitter (1:1). The HPLC separation was carried out on an Agilent Betasil C8 column (4.6 × 250 mm, 2.7 μm; Thermo scientific) operated at 20°C. For positive mode, the mobile phase, which consisted of a 0.1% formic acid aqueous solution (A) and methanol (C), was delivered at a flow rate of 0.5 mL/min under a gradient program: 5–20% (C) from 0.1 to 2 min, 40–60% (C) from 5 to 12 min, 80–90% (C) from 20 to 25 min, 90–5% (C) from 30 to 35 min, and return to its initial condition over 5 min. The sample injection volume was 2 μL. For negative mode, the mobile phase, which consisted of a 0.1% formic acid aqueous solution (A) and methanol (C), was delivered at a flow rate of 0.5 mL/min under a gradient program: 5–20% (C) from 0.1 to 2 min, 40–60% (C) from 5 to 15 min, 70–80% (C) from 25 to 32 min, 90–90% (C) from 40 to 48 min, and return to its initial condition over 5 min. The sample injection volume was 2 μL.

2. Mass spectrometry

An Agilent 6520 Accurate Mass QTOF-MS/MS system was coupled with an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) via an ESI interface. In the ESI source, nitrogen was used as drying and collision gas. The heated capillary temperature was set

to 350°C and nebulizer pressure to 40 psi. The drying gas flow rate was 12 L/min. VCap, fragmentor, skimmer and octapole RF peak voltages were set to 3500 V, 150 V, 65 V and 750 V, respectively, in the ion source parameters. Detection was carried out within a mass range of m/z 50–1500 and resolving power above 15,000 (FWHM). The MS/MS analyses were acquired by auto fragmentation where the three most intense mass peaks where fragmented. Collision energy values for MS/MS experiments were fixed at 15 eV, 20 eV, and 25 eV for each selected mass. Accurate mass measurements were obtained by the auto mass calibration method which was performed using an external mass calibration solution (ESI-L Low Concentration Tuning Mix; Agilent calibration solution B). This solution includes a set of perfluorinated compounds that are distributed over the mass range (112.985587 to 2833.873139 Da in negative ionization mode) and highly volatile to keep the background in the system low. The chromatographic and mass spectrometric analyses, including the prediction of chemical formula and exact mass calculation, were performed by using Mass Hunter software version B.04.00 build 4.0.479.0 (Agilent Technology).

Results

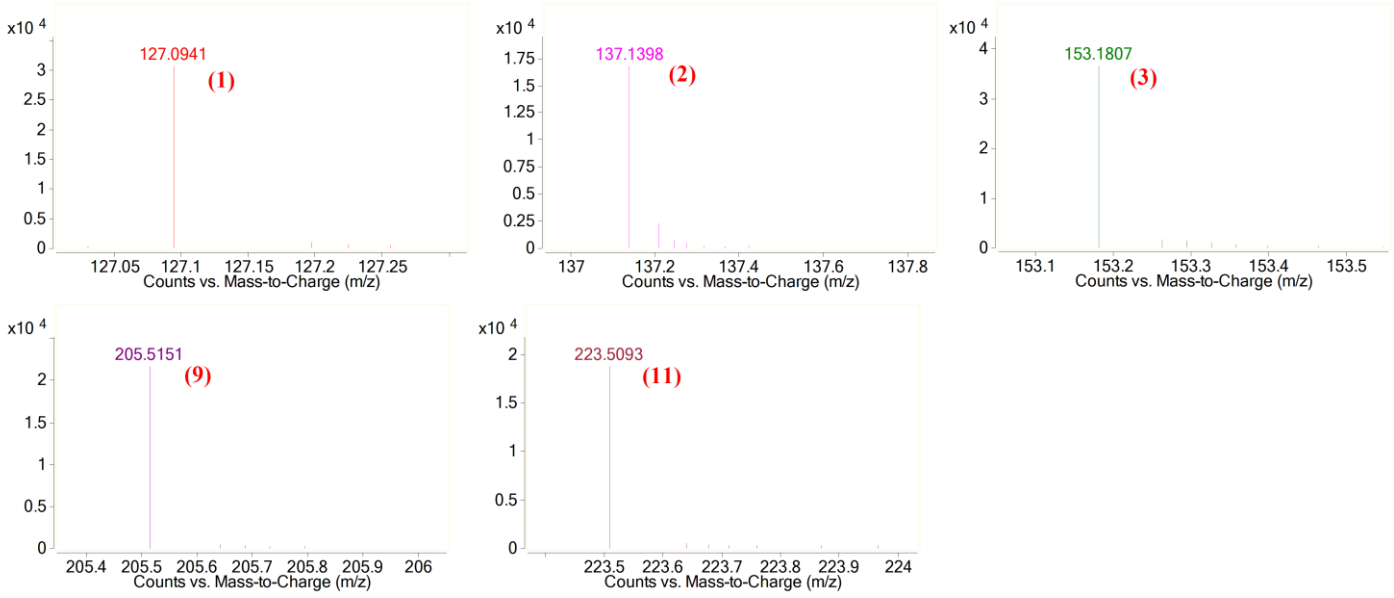


Fig. S1 (A): HPLC-ESI-QTOF-MS spectra of the compounds **(1)**, **(2)**, **(3)**, **(9)** and **(11)** of *Cymbopogon* species in positive mode.

Table S1: HPLC-ESI-QTOF-MS mass measurements of phytochemicals in leaf of *Cymbopogon* species in positive mode.

S. No.	R_t (min)	M. formula	Calculated mass $[M+H]^+$	Measured mass $[M+H]^+$	Compound	Error (ppm)
1	12.3	$C_8H_{14}O$	127.0935	127.0941	6-Methylhept-5-en-2-one	-4.72
2	11.5	$C_{10}H_{16}$	137.1390	137.1398	Myrcene	-3.90
3	10.5	$C_{10}H_{16}O$	153.1812	153.1807	Citral	3.21
9	31.9	$C_{15}H_{24}$	205.5160	205.5151	γ-1-cadinene	4.30
11	36.1	$C_{15}H_{26}O$	223.5090	223.5093	β-eudesmol	-1.32

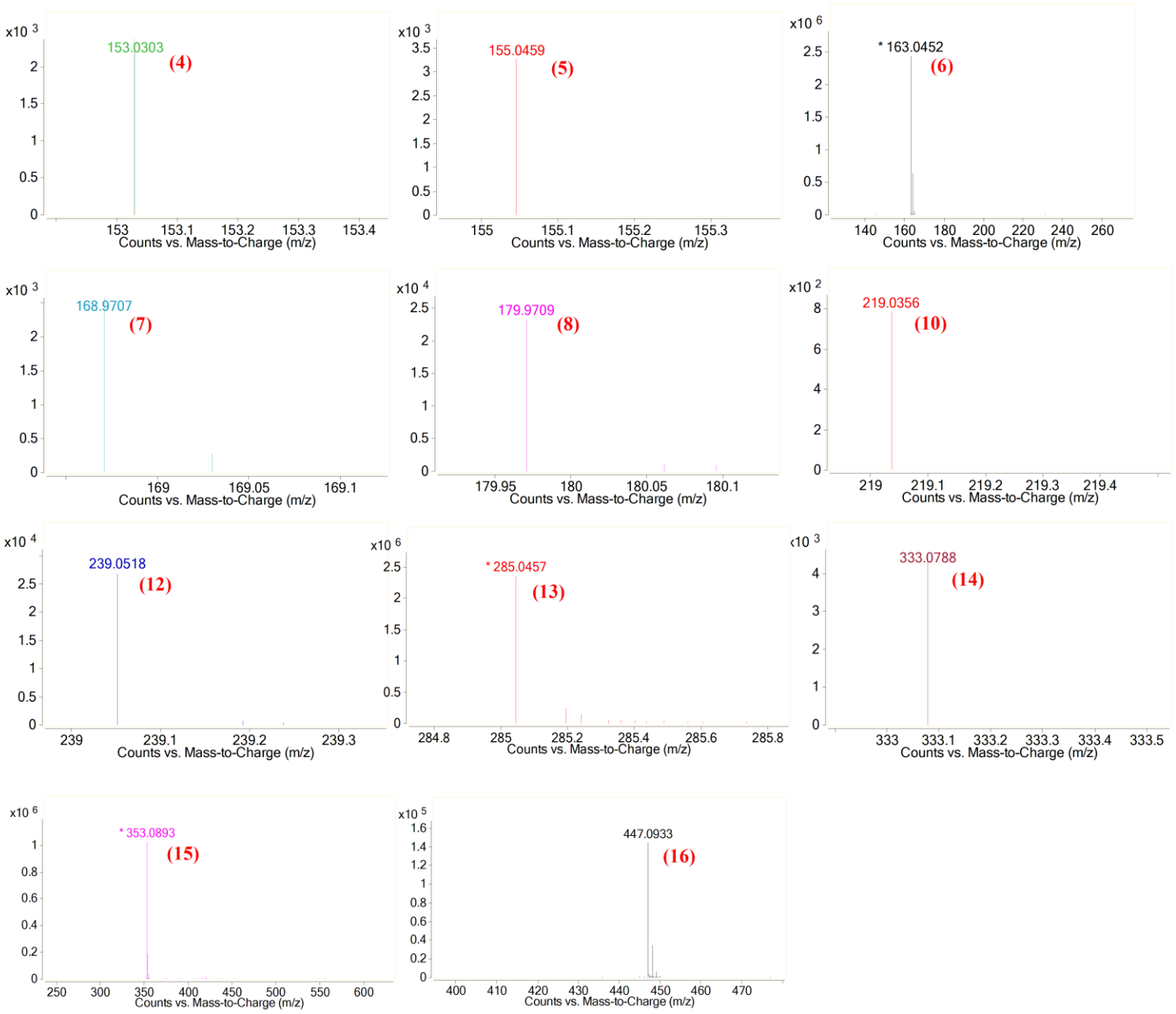
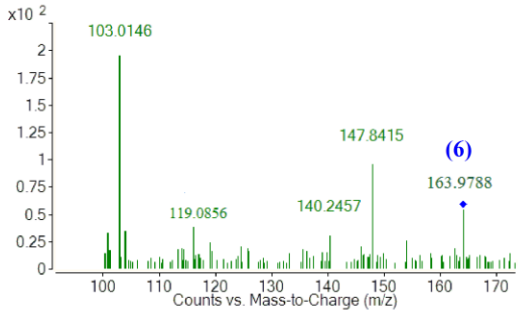
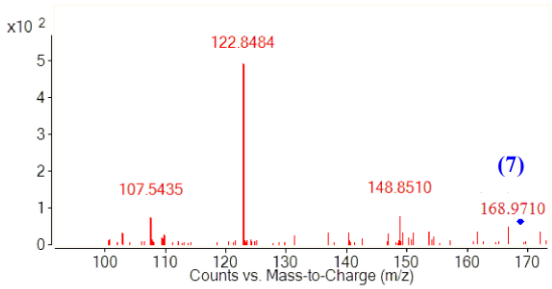
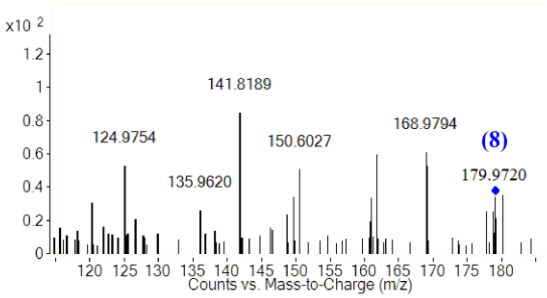
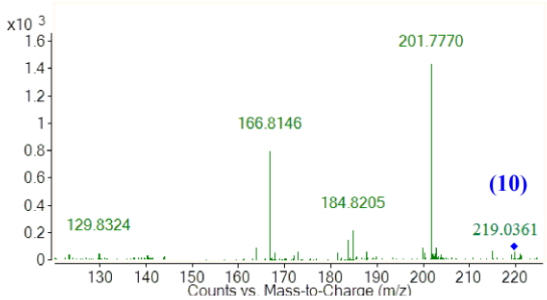


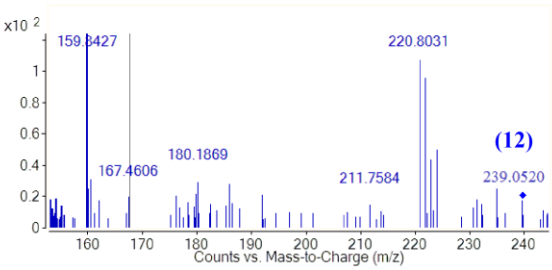
Fig. S1 (B): HPLC-ESI-QTOF-MS spectra of the compounds of *Cymbopogon* species in negative mode.

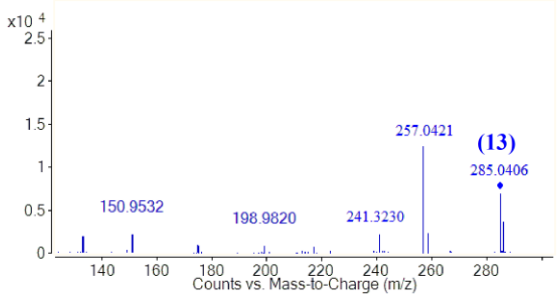

x10 2
103.0146
147.8415
(6)
163.9788
119.0856
140.2457
Counts vs. Mass-to-Charge (m/z)

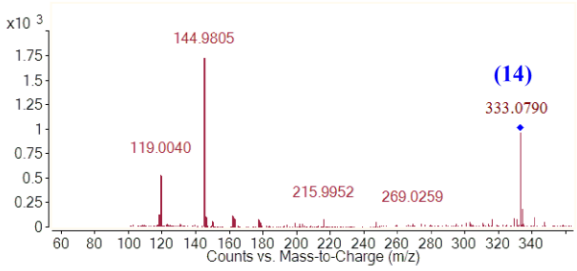

x10 2
122.8484
(7)
107.5435
148.8510
168.9710
Counts vs. Mass-to-Charge (m/z)

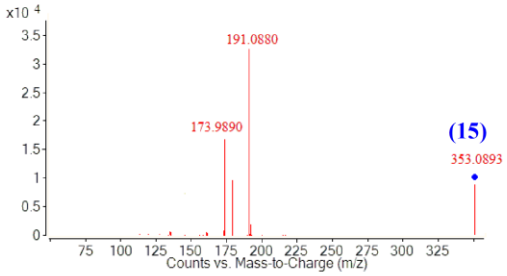

x10 2
141.8189
124.9754
150.6027
168.9794
(8)
135.9620
179.9720
Counts vs. Mass-to-Charge (m/z)


x10 3
201.7770
166.8146
(10)
184.8205
129.8324
219.0361
Counts vs. Mass-to-Charge (m/z)


x10 2
159.8427
220.8031
(12)
180.1869
167.4606
211.7584
239.0520
Counts vs. Mass-to-Charge (m/z)


x10 4
257.0421
(13)
285.0406
150.9532
198.9820
241.3230
Counts vs. Mass-to-Charge (m/z)


x10 3
144.9805
(14)
333.0790
119.0040
215.9952
269.0259
Counts vs. Mass-to-Charge (m/z)


x10 4
191.0880
173.9890
(15)
353.0893
Counts vs. Mass-to-Charge (m/z)

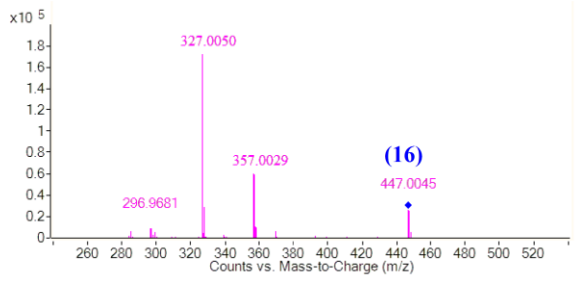

x10 5
327.0050
357.0029
(16)
447.0045
296.9681
Counts vs. Mass-to-Charge (m/z)

Fig. S2: HPLC-ESI-QTOF-MS/MS spectra of the compounds of *Cymbopogon* species in negative mode.

Table S2: HPLC-ESI-QTOF-MS mass measurements and fragmentation of phytochemicals in leaf of *Cymbopogon* species in negative mode.

S. No.	t_R (min)	M. formula	Calculated mass $[M-H]^-$	Measured mass $[M-H]^-$	Compound	Error (ppm)	Fragments (intensity)
4	25.0	$C_{10}H_{18}O$	153.0309	153.0303	Citronellal	3.90	-
5	27.0	$C_{10}H_{20}O$	155.0453	155.0459	Citronellol	-3.80	-
6	20.0	$C_9H_8O_3$	163.0401	163.0452 (10)	*p*-coumaric acid*	1.21	147.8415 (49), 140.2457 (16), 119.0805 (100), 103.0146
7	5.90	$C_{10}H_{18}O_2$	168.9711	168.9707 (28)	*Cis*-Linalool oxide	2.36	148.8510 (16), 122.8484 (100), 107.5435 (15)
8	17.2	$C_9H_8O_4$	179.9713	179.9709 (28)	Caffeic acid	2.22	168.9794 (31), 150.6027 (26), 141.8189 (43), 135.9620 (100), 124.9754 (27)
10	13.8	$C_{15}H_{24}O$	219.0360	219.0356 (15)	Caryophyllene oxide	1.82	201.7770 (100), 184.8205 (12), 166.8146 (32), 129.8324 (40)
12	25.1	$C_{15}H_{28}O_2$	239.0515	239.0518 (16)	Cryptomeridiol	-1.25	220.8031 (29), 211.7584 (10), 180.1869 (30), 167.4606 (12), 159.8427 (100)
13	30.0	$C_{15}H_{10}O_6$	285.0405	285.0457 (20)	Kaempferol*	-0.69	257.0421 (100), 241.3230 (10),198.9820 (4), 150.9532 (8)
14	31.8	$C_{20}H_{30}O_4$	333.0782	333.0788 (55)	Cymbodiacetal	-0.60	269.0259 (10), 215.9952 (15), 144.9805 (100), 119.4040 (31)
15	15.0	$C_{16}H_{18}O_9$	353.0878	353.0893	Cholorogenic	3.58	191.0889 (100), 179.3890 (30), 173.9890 (49)

				(25)	acid*		
16	17.9	$C_{21}H_{20}O_{11}$	447.0933	447.0937 (13)	Orientin*	-0.89	356.9829 (34), 326.9763 (100), 296.9681 (6)

*Matched with authentic standards