# Supplementary Information

## Metabolite Fingerprinting and Profiling of the Medicinal Grass *Eleusine indica* Based on HPLC-DAD, UPLC-DAD-MS/MS and NMR Analyses

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**Table S1.** Calibration curve of phenolic standards used for quantification in HPLC-DAD analysis of *Eleusine indica* extracts

| Compound | Linearity range[a] / ($\mu g.mL^{-1}$) | Calibration equation | Correlation factor ($r^2$) | LOD[b] / ($\mu g.mL^{-1}$) | LOQ[c] / ($\mu g.mL^{-1}$) |
|---|---|---|---|---|---|
| Gallic acid | 3.63-48.00 | $y = 59283.08x - 86870.61$ | 0.9959 | 1.20 | 3.64 |
| *p*-Coumaric acid | 2.43-78.40 | $y = 144446.89x - 5767.81$ | 0.9987 | 0.80 | 2.43 |
| Vitexin | 0.63-47.50 | $y = 55038x - 6027.4$ | 0.9995 | 0.21 | 0.63 |

[a]Six data points (n = 3); [b]LOD: limit of detection; [c]LQD: limit of quantification.

**Table S2.** Yields of the aqueous extraction from *Eleusine indica* aerial and underground parts from specimens collected in different Brazilian localities

| Sample | | Lyophilized aqueous extract | | |
|---|---|---|---|---|
| | | Mean ± SE / g | RSD / % | $Y_m$ (g *per* 100 g) / % |
| BH | aerial parts | 0.68 ± 0.02 | 1.83 | 13.69 |
| | underground parts | 0.23 ± 0.01 | 1.31 | 4.60 |
| BP | aerial parts | 0.74 ± 0.03 | 3.23 | 14.91 |
| | underground parts | 0.28 ± 0.01 | 3.26 | 5.71 |
| IF | aerial parts | 0.76 ± 0.05 | 4.65 | 15.24 |
| | underground parts | 0.43 ± 0.03 | 4.97 | 8.70 |
| PA | aerial parts | 0.54 ± 0.02 | 3.12 | 10.83 |
| | underground parts | 0.22 ± 0.01 | 2.17 | 4.41 |

SE: standard error; RSD: relative standard deviation; $Y_m$: yield mean of the aqueous extract lyophilized inter-day replicates extraction (n = 3 days for aerial parts and n = 2 days for underground parts) for each 100 g of plant material; BH: Belo Horizonte, Minas Gerais State; BP: Barra do Piraí, Rio de Janeiro State; IF: Ilha do Fundão, Rio de Janeiro State; PA: Porto Alegre, Rio Grande do Sul State.

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**Table S3.** Phenolic profile by HPLC-DAD analyses (270, 300 and 330 nm) of lyophilized extracts from *Eleusine indica* underground parts (Ei-UGP) collected in different Brazilian localities

| Peak | $t_R$[a] / min | UV $\lambda_{max}$ / nm | Mean ± SE (RSD / %) / (mg *per* 100 g) | | | |
|---|---|---|---|---|---|---|
| | | | Belo Horizonte | Barra do Pirai | Ilha do Fundão | Porto Alegre |
| 1[b] | 3.28 | 268 | n.q. | 42.2 ± 1.1 (7.2) | 42.2 ± 0.9 (5.9) | 53.1 ± 3.1 (16.5) |
| **2**[b] | 3.80 | 273 | 58.5 ± 6.0 (30.9) | 79.8 ± 1.1 (3.9) | 48.8 ± 0.8 (4.8) | 52.5 ± 0.9 (4.7) |
| 3[b] | 6.24 | 260 | 53.3 ± 4.1 (23.4) | 85.9 ± 5.1 (16.9) | n.q. | n.q. |
| 4[b] | 7.61 | 290 | n.q. | n.q. | 46.7 ± 6.9 (42.1) | n.q. |
| 5[b] | 8.17 | 266 | n.q. | 50.8 ± 0.5 (2.8) | 41.8 ± 2.2 (14.6) | 44.9 ± 1.3 (8.2) |
| 6[b] | 8.64 | 252, 272sh | 103.3 ± 5.7 (16.6) | 172.1 ± 4.7 (7.7) | n.q. | 80.0 ± 4.7 (16.5) |
| **7**[b] | 9.03 | 260 | 36.5 ± 1.5 (12.5) | 120.9 ± 3.2 (7.5) | 147.6 ± 5.4 (10.3) | 59.6 ± 10.9 (52.1) |
| 8[b] | 9.36 | 257, 320sh | 86.9 ± 9.3 (32.3) | 193.7 ± 7.2 (10.5) | n.q. | 62.8 ± 5.7 (25.9) |
| **9**[b] | 9.78 | 254, 273sh, 312sh | 52.1 ± 4.2 (23.9) | 55.3 ± 12.3 (62.9) | 46.3 ± 0.8 (4.7) | 54.3 ± 1.1 (5.8) |
| **10**[b] | 10.05 | 258, slope | 36.3 ± 4.6 (38.1) | 67.1 ± 8.3 (35.1) | 49.8 ± 0.3 (1.7) | 66.9 ± 1.7 (7.4) |
| **11**[b] | 10.61 | 259 | 47.9 ± 2.3 (14.2) | 184.8 ± 6.4 (9.8) | 101.6 ± 0.7 (1.8) | 78.2 ± 8.8 (31.9) |
| **12**[b] | 10.81 | 253, 270sh | 144.0 ± 7.7 (16.0) | 336.3 ± 7.7 (6.5) | 102.5 ± 0.9 (2.8) | 102.5 ± 4.3 (11.9) |
| **13**[b] | 11.27 | 257 | 65.0 ± 10.9 (50.6) | 224.5 ± 0.7 (0.9) | 94.2 ± 3.7 (11.0) | 83.5 ± 6.5 (21.9) |
| 14[b] | 11.69 | 257 | n.q. | 42.9 ± 1.2 (7.8) | 38.9 ± 0.3 (2.3) | n.q. |
| 15[b] | 12.42 | 265 | 36.1 ± 3.5 (28.9) | 49.8 ± 2.3 (13.3) | 48.8 ± 0.1 (0.8) | n.q. |
| 16[b] | 14.77 | 271 | n.q. | n.q. | 40.9 ± 0.7 (4.8) | n.q. |
| **17**[b] | 17.39 | 254, 273sh, 312sh | 48.7 ± 2.3 (13.9) | 41.1 ± 0.3 (2.0) | 57.2 ± 1.9 (9.4) | 64.4 ± 2.1 (9.4) |
| 18[b] | 18.42 | 280 | n.q. | n.q. | n.q. | 38.1 ± 1.1 (8.3) |
| **19**[b] | 19.11 | 260, 290 | 48.7 ± 2.0 (12.5) | 38.8 ± 0.7 (4.9) | 44.1 ± 1.7 (10.9) | 47.2 ± 0.4 (2.4) |
| 20[b] | 21.25 | 283 | 51.4 ± 5.2 (30.4) | 40.2 ± 2.4 (16.8) | n.q. | n.q. |
| **21**[c] | 23.69 | 309 | 147.1 ± 6.4 (13.0) | 128.1 ± 2.8 (6.1) | 112.8 ± 0.6 (1.6) | 104.9 ± 13.8 (37.4) |
| 22[d] | 26.16 | 291, 322 | 83.6 ± 2.8 (9.9) | n.q. | 10.1 ± 0.1 (2.6) | 23.8 ± 6.3 (74.6) |

[a]Mean of retention time ± 0.05 (min); [b]compounds of group I: benzoic acid derivatives, amino acids, and nucleosides ($\lambda_{max}$ between 250-280 nm) were expressed as 1 mg of gallic acid *per* 100 g of lyophilized aqueous extract; [c]peaks of group II: cinnamic acid derivatives ($\lambda_{max}$ between 295-330 nm) were expressed as 1 mg of *p*-coumaric acid *per* 100 g of lyophilized aqueous extract; [d]peaks of group III: flavone derivatives ($\lambda_{max}$ between 310-330 nm for band I and 260-280 nm for band II) were expressed as 1 mg of vitexin *per* 100 g of lyophilized aqueous extract. All 22 peaks were detected by high-performance liquid chromatography coupled to diode array detector (HPLC-DAD), but only 10 (in bold) were quantified for the four localities. RSD: percentage of relative standard deviation are in parentheses; n.q.: not quantified; sh: shoulder. Each value content in the table is the mean of inter- and intra-day replicate analysis (n = 2 days, 3 replicates *per* day) ± standard error (SE).

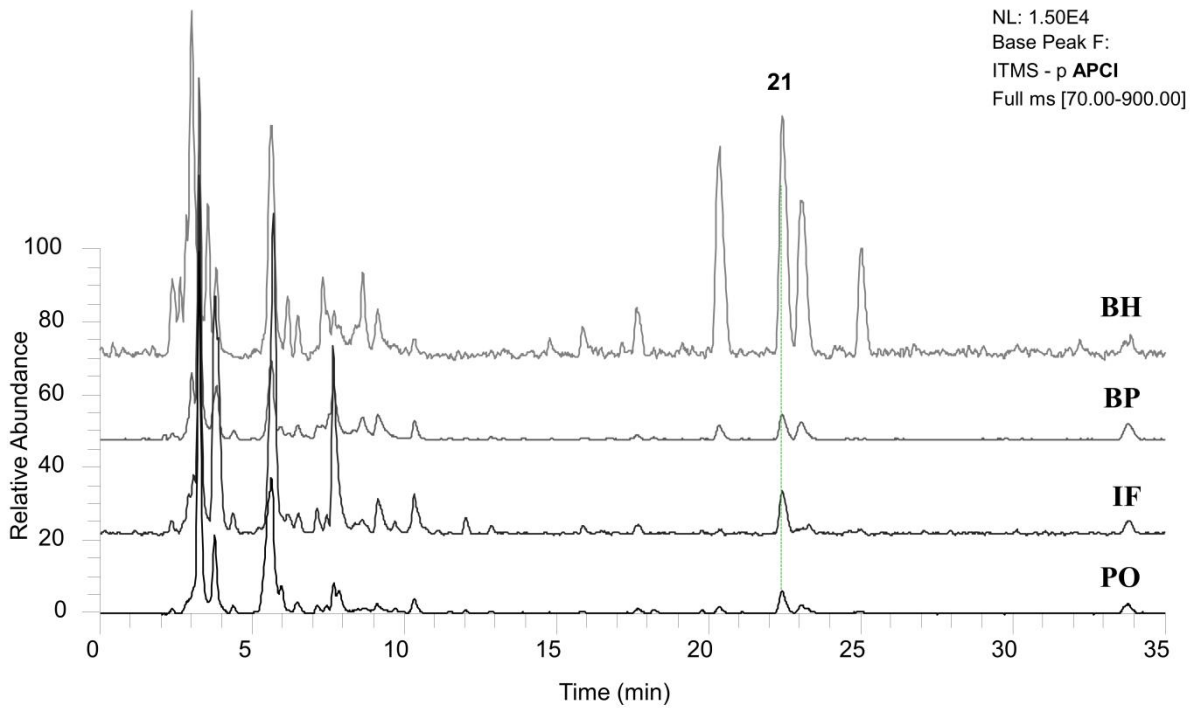


**Figure S1.** UPLC-DAD-MS/MS chromatograms of lyophilized extracts from *Eleusine indica* underground parts. Samples from four localities: BH = Belo Horizonte, Minas Gerais State; BP = Barra do Piraí and IF = Ilha do Fundão, Rio de Janeiro State; and PO = Porto Alegre, Rio Grande do Sul State, Brazil. The only peak common to four locality specimens (peak **21** = *p*-coumaric acid) was ionized and identified by (–)-APCI.

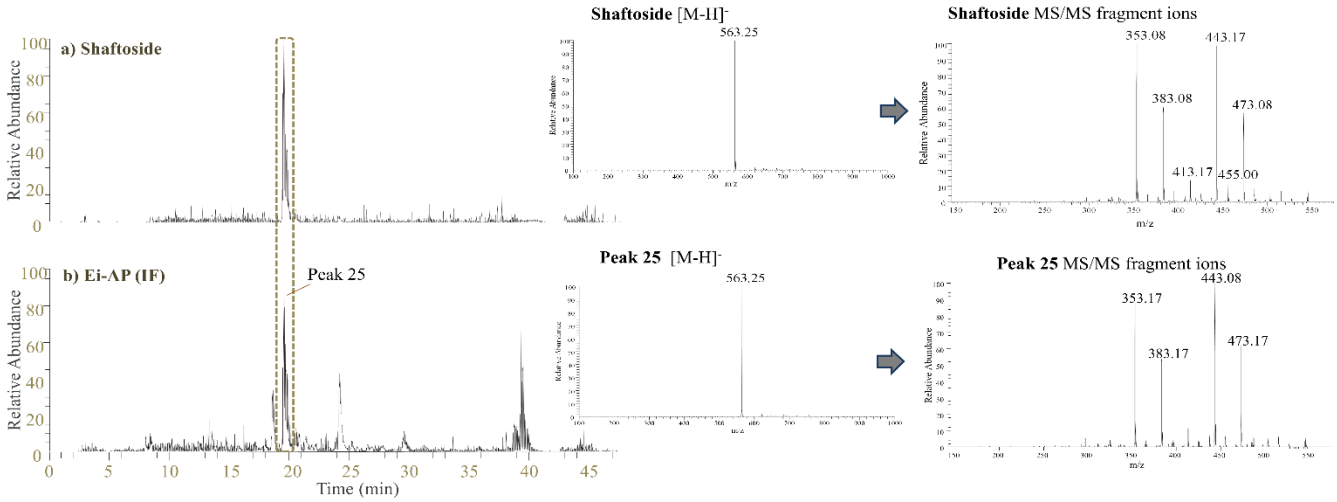


**Figure S2.** UPLC-DAD-MS/MS chromatograms and spectra: (a) reference standard of schaftoside and (b) peak 25 in the lyophilized extract of *Eleusine indica* aerial parts (Ei-AP). Locality: Ilha do Fundão (IF).

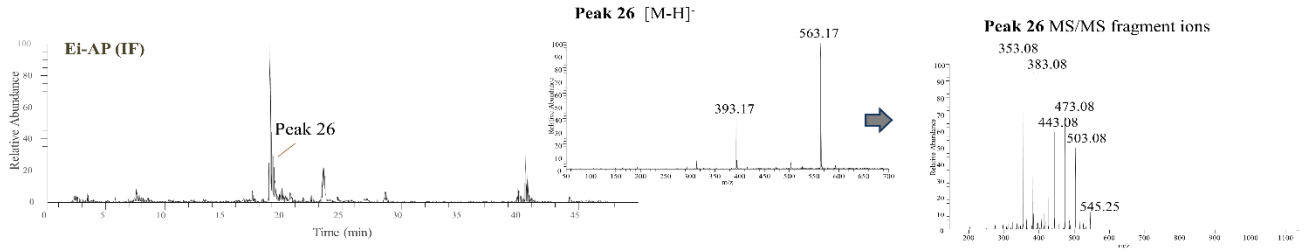


**Figure S3.** UPLC-DAD-MS/MS chromatograms and spectra: peak 26 in the lyophilized extract of *Eleusine indica* aerial parts (Ei-AP). Locality: Ilha do Fundão (IF).

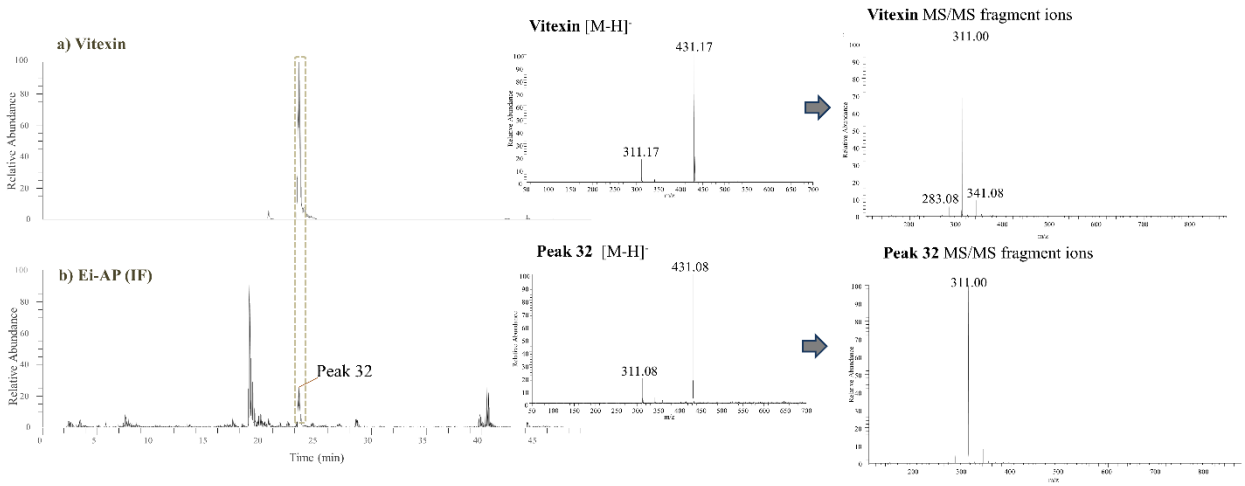


**Figure S4.** UPLC-DAD-MS/MS chromatograms and spectra: (a) reference standard of vitexin and (b) peak 32 in the lyophilized extract of *Eleusine indica* aerial parts (Ei-AP). Locality: Ilha do Fundão (IF).

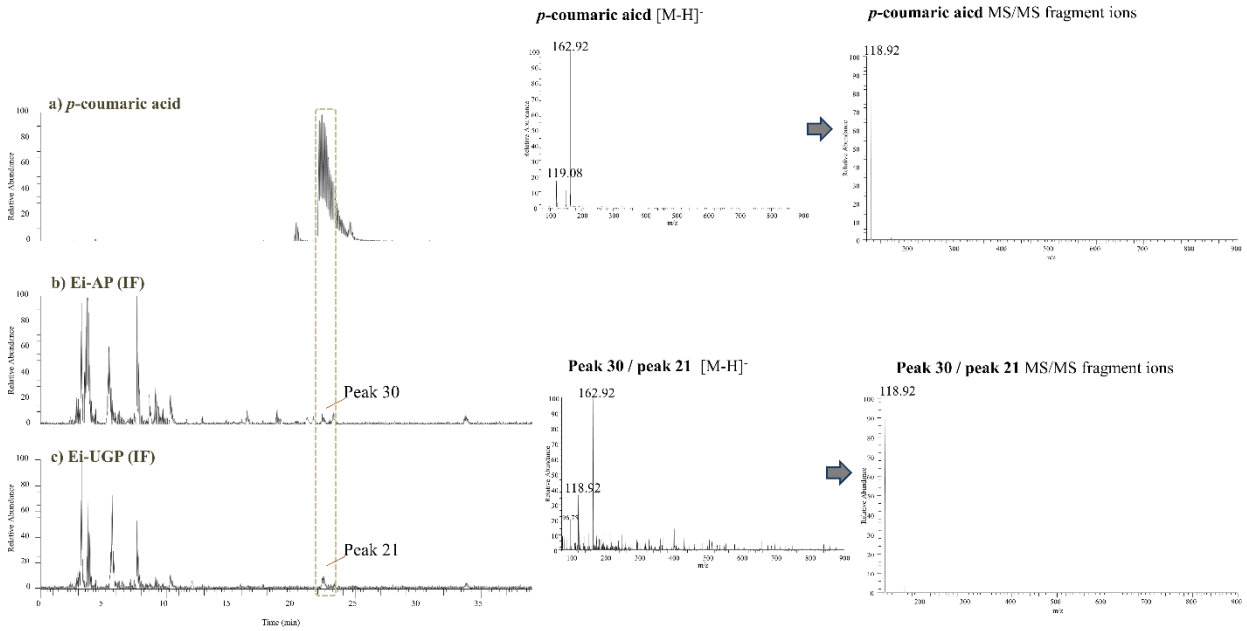


**Figure S5.** UPLC-DAD-MS/MS chromatograms and spectra: a) reference standard of *p*-coumaric acid; (b, c) peaks 30 and 21 in the lyophilized extracts from *Eleusine indica* aerial and underground parts (Ei-AP and Ei-UGP, respectively). Locality: Ilha do Fundão (IF).

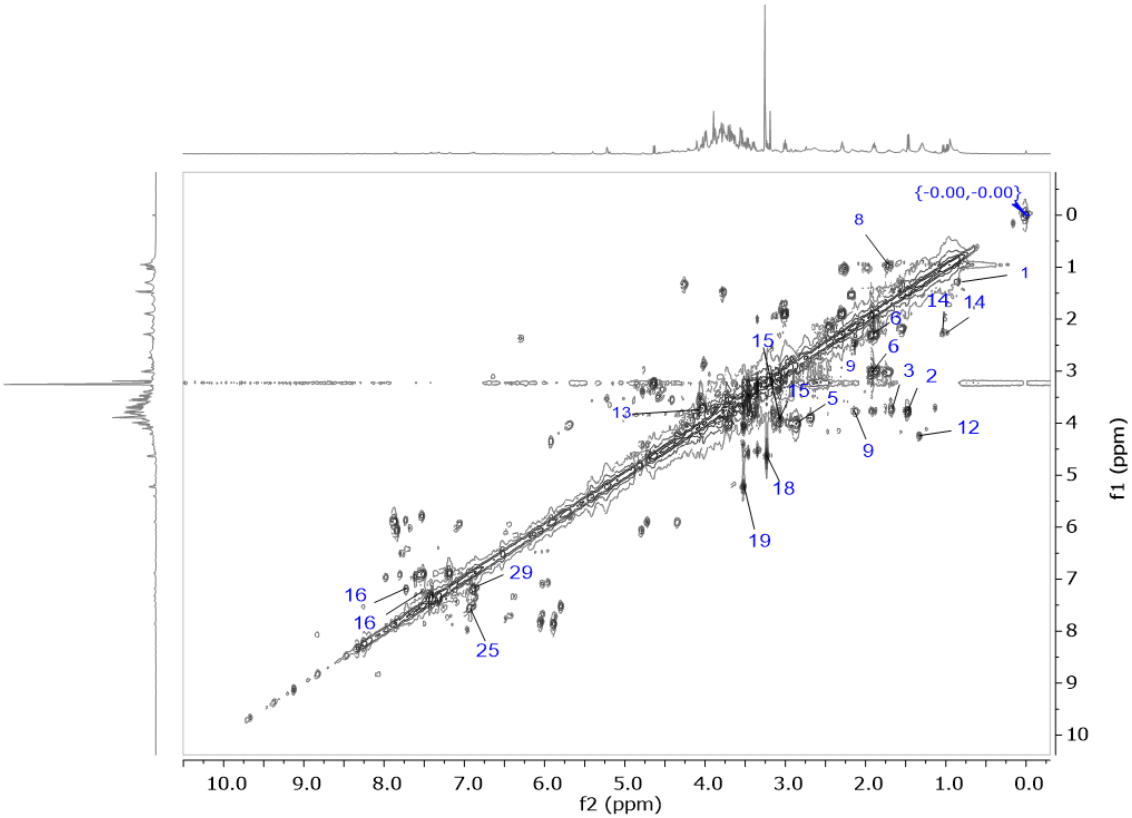


**Figure S6.** 2D-NMR COSY spectra of lyophilized extract from *Eleusine indica* aerial parts. Locality: Ilha do Fundão (IF). 1: fatty acid derivatives; 2: alanine; 3: arginine; 5: asparagine; 6: γ-aminobutyrate; 8: leucine; 9: glutamine; 12: threonine; 13: L-serine; 14: valine; 15: tyrosine; 16: tryptophan; 18: β-glucose; 19: α-glucose; 25: *p*-coumaric acid; 29: tyrosol.

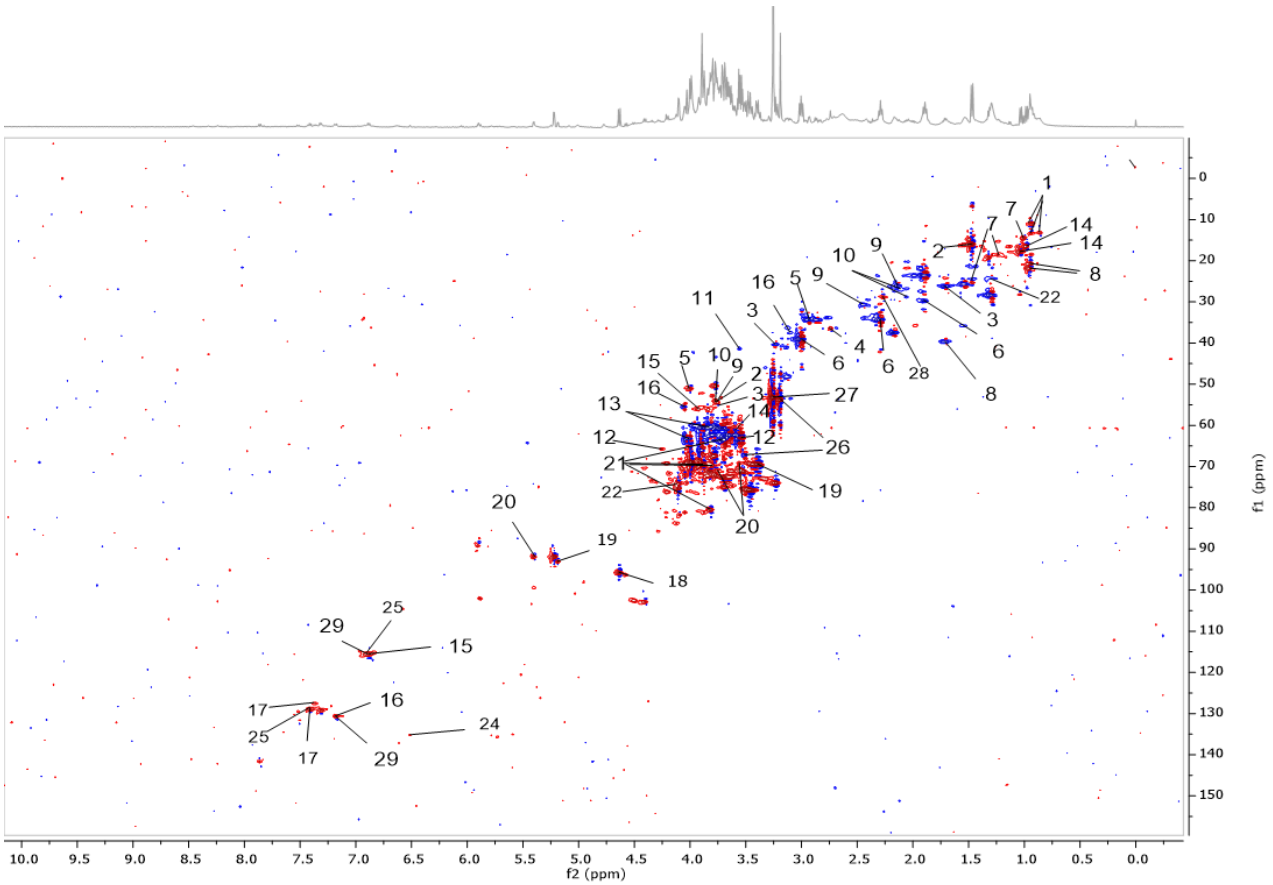


**Figure S7.** 2D-NMR HSQC spectra of lyophilized extract from *Eleusine indica* aerial parts. Locality: Ilha do Fundão (IF). 1: fatty acid derivatives; 2: alanine; 3: arginine; 4: aspartate; 5: asparagine; 6: γ-aminobutyrate; 7: isoleucine; 8: leucine; 9: glutamine; 10: glutamic acid; 11: glycine; 12: threonine; 13: L-serine; 14: valine; 15: tyrosine; 16: tryptophan; 17: phenylalanine; 18: β-glucose; 19: α-glucose; 20: sucrose; 21: fructose; 22: lactic acid; 23: formic acid; 24: fumaric acid; 25: *p*-coumaric acid; 26: choline; 27: betaine; 28: pyruvic acid; 29: tyrosol.

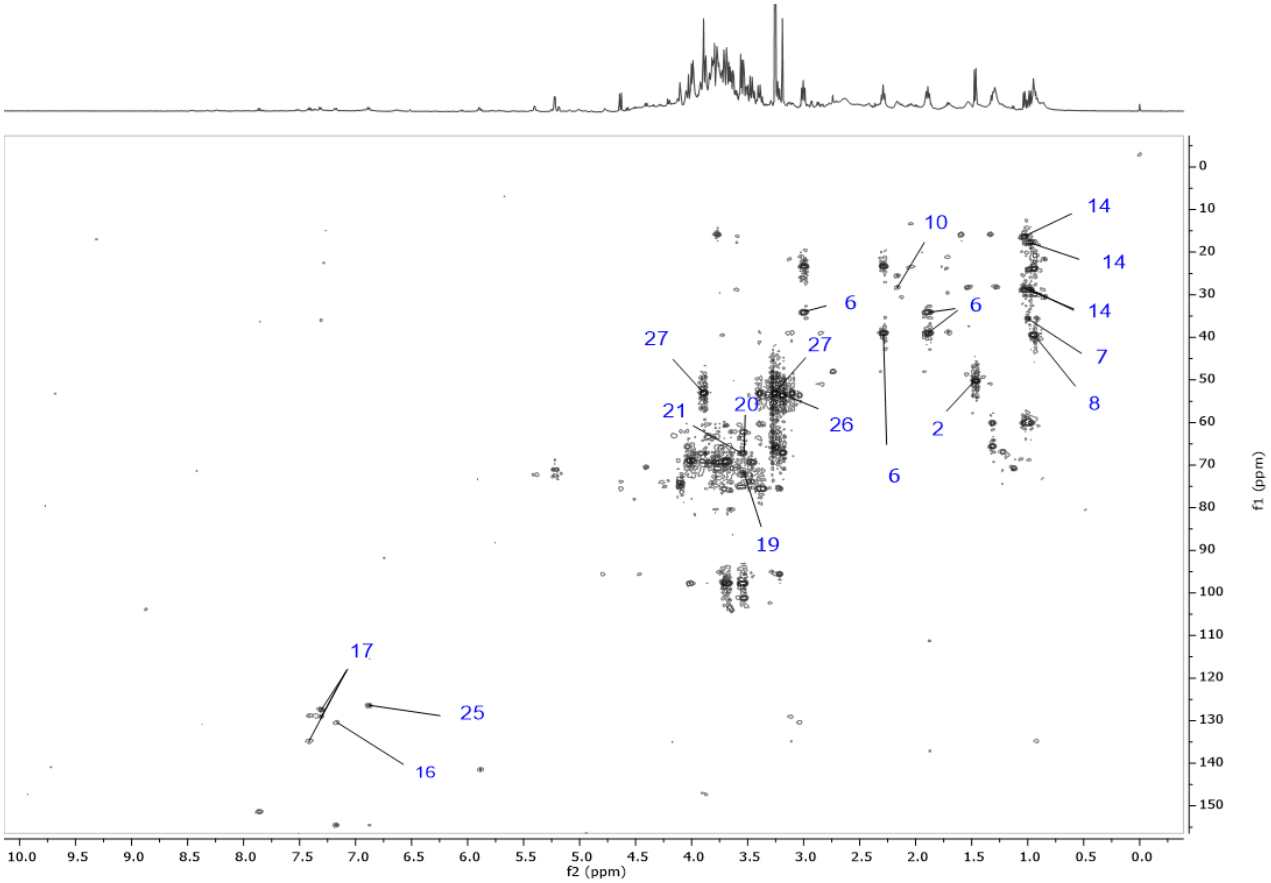


**Figure S8.** 2D-NMR HMBC spectra of lyophilized extract from *Eleusine indica* aerial parts. Locality: Ilha do Fundão (IF). 2: alanine; 6: γ-aminobutyrate; 7: isoleucine; 8: leucine; 10: glutamic acid; 14: valine; 16: tryptophan; 17: phenylalanine; 19: α-glucose; 20: sucrose; 21: fructose; 25: *p*-coumaric acid; 26: choline; 27: betaine.

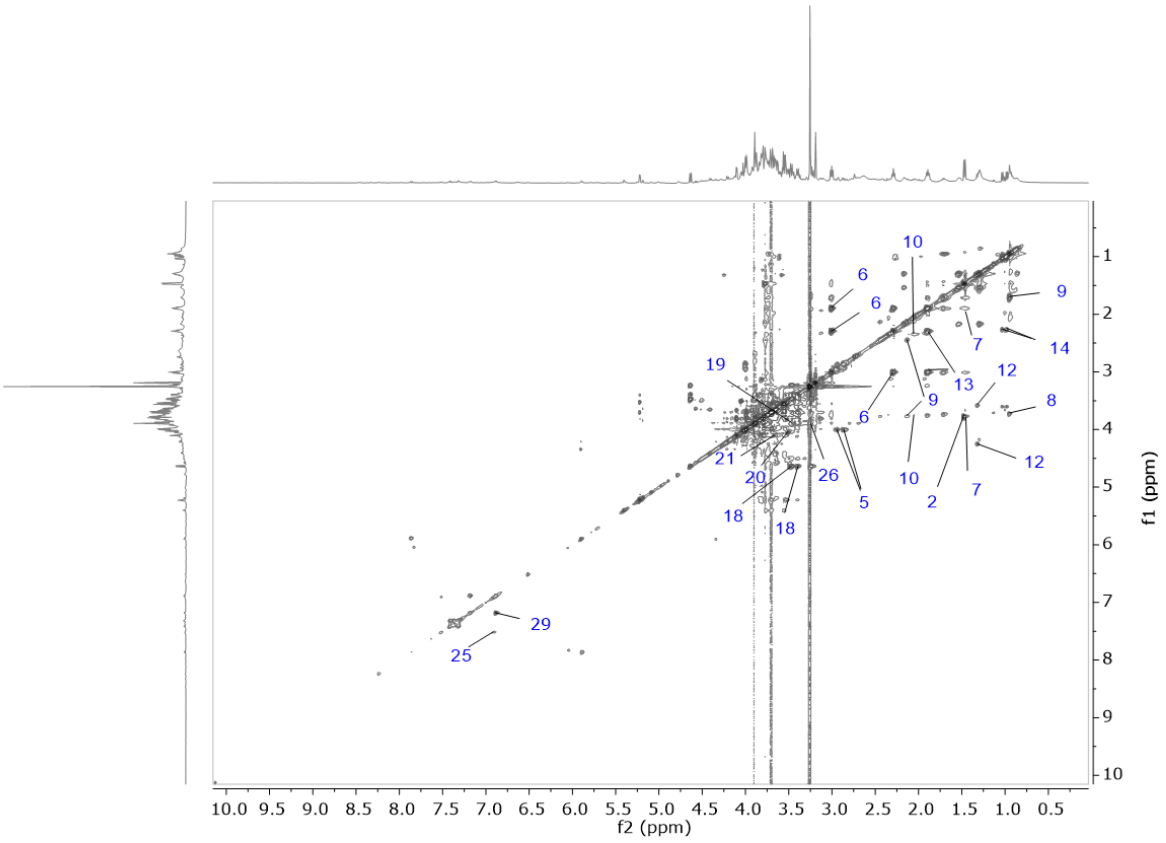


**Figure S9.** 2D-NMR TOCSY spectra of lyophilized extract from *Eleusine indica* aerial parts. Locality: Ilha do Fundão (IF). 2: alanine; 5: asparagine; 6: ɣ-aminobutyrate; 7: isoleucine; 8: leucine; 9: glutamine; 10: glutamic acid; 12: threonine; 13: L-serine; 14: valine; 18: β-glucose; 19: α-glucose; 20: sucrose; 21: fructose; 25: *p*-coumaric acid; 26: choline; 29: tyrosol.

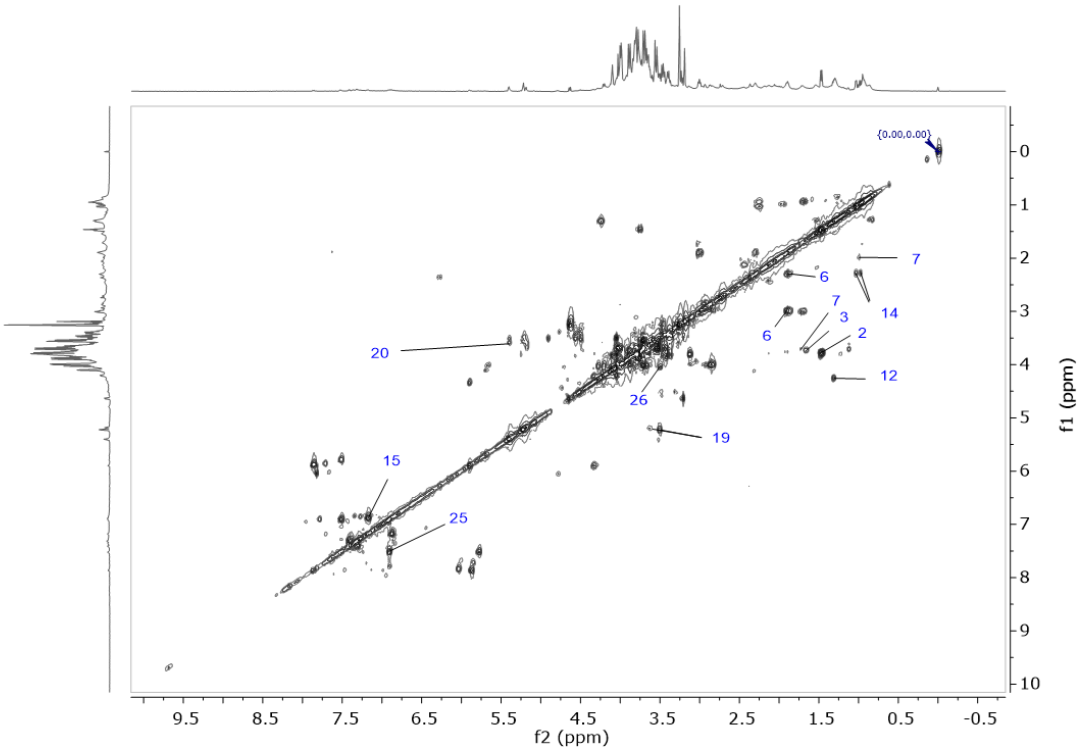


**Figure S10.** 2D-NMR COSY spectra of lyophilized extract from *Eleusine indica* underground parts. Locality: Ilha do Fundão (IF). 2: alanine; 3: arginine; 6: γ-aminobutyrate; 7: isoleucine; 12: threonine; 14: valine; 15: tyrosine; 19: α-glucose; 20: sucrose; 25: *p*-coumaric acid; 26: choline.

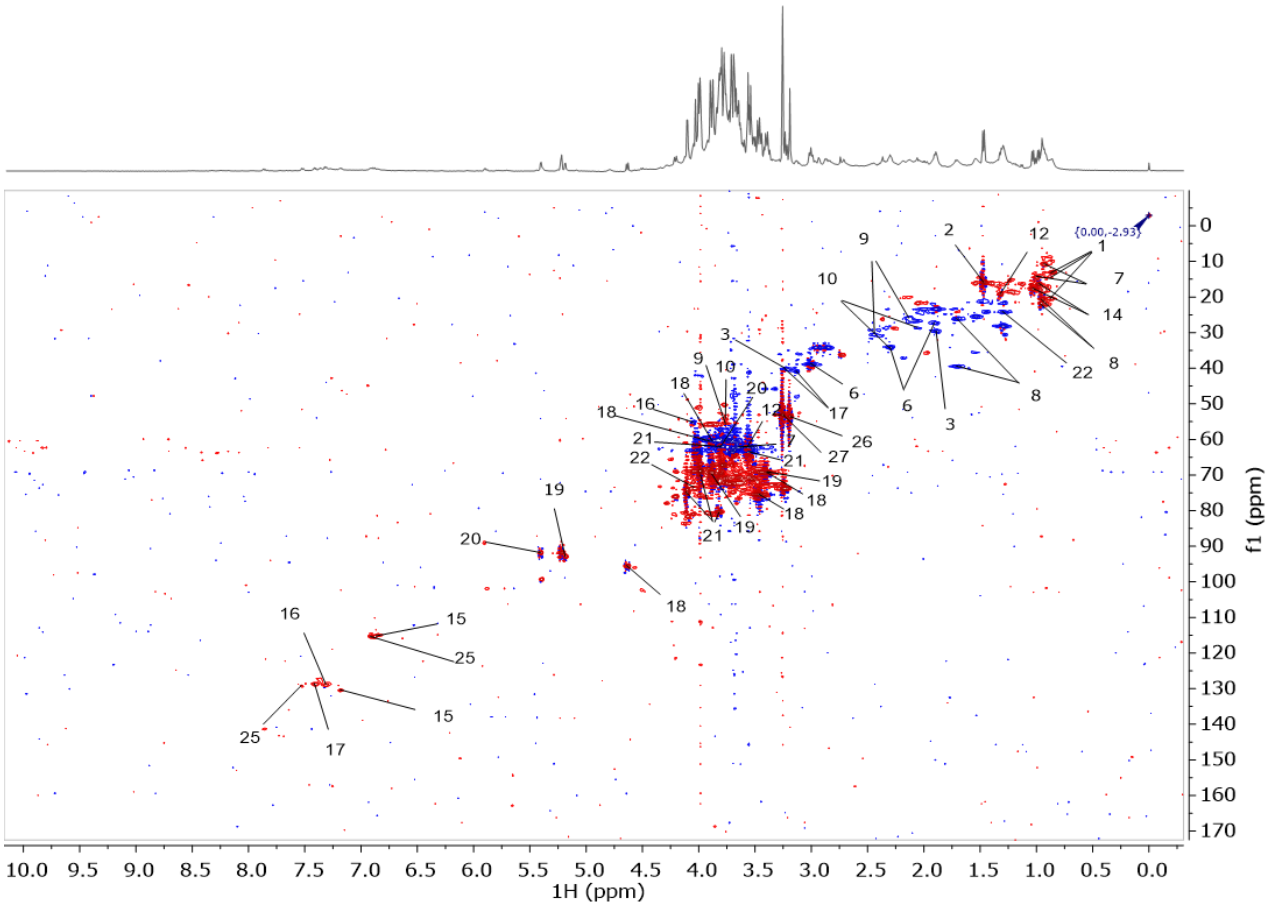


**Figure S11.** 2D-NMR HSQC spectra of lyophilized extract from *Eleusine indica* underground parts. Locality: Ilha do Fundão (IF). 1: fatty acid derivatives; 2: alanine; 3: arginine; 6: γ-aminobutyrate; 7: isoleucine; 8: leucine; 9: glutamine; 10: glutamic acid; 12: threonine; 14: valine; 15: tyrosine; 16: tryptophan; 17: phenylalanine; 18: β-glucose; 19: α-glucose; 20: sucrose; 21: fructose; 22: lactic acid; 27: *p*-coumaric acid; 26: choline; 27: betaine.

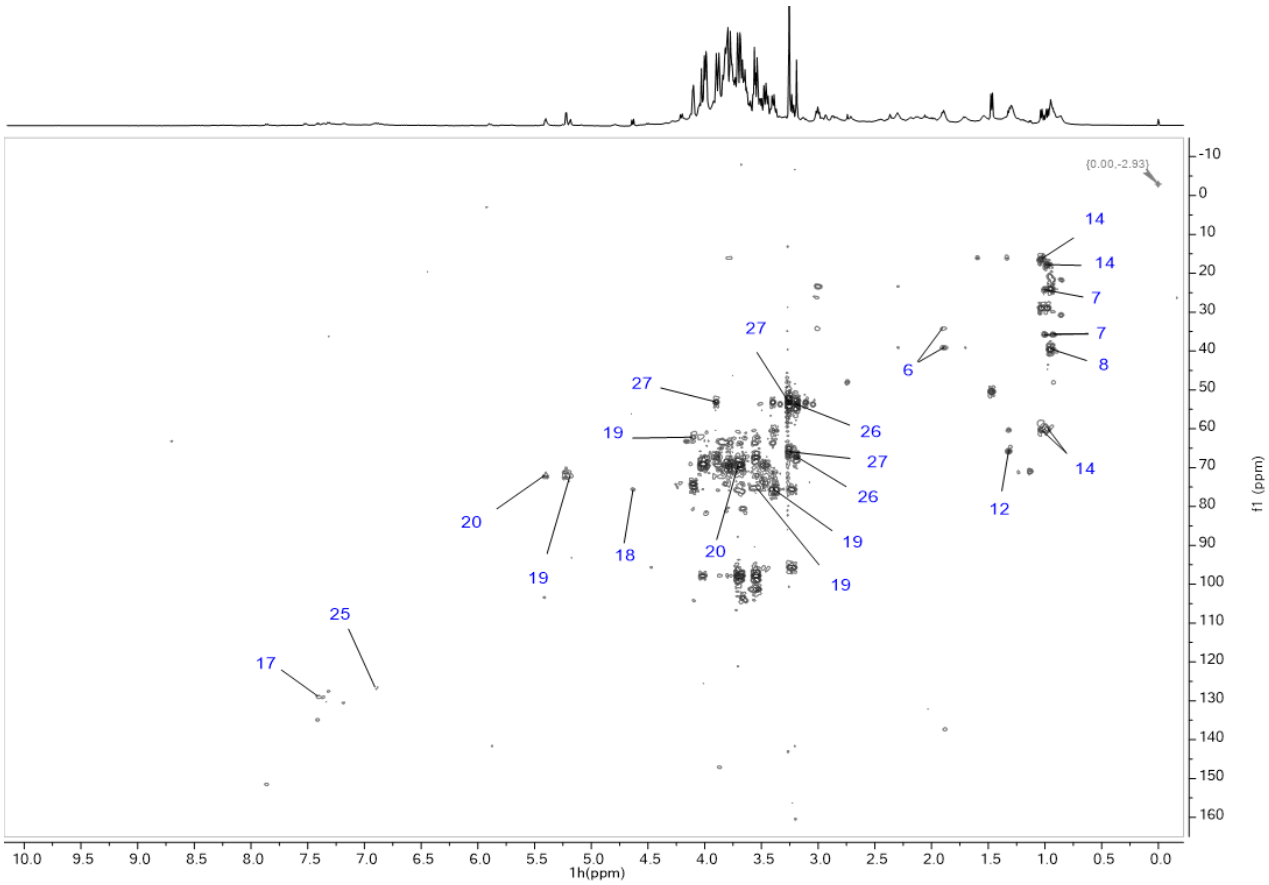


**Figure S12.** 2D-NMR HMBC spectra of lyophilized extract from *Eleusine indica* underground parts. Locality: Ilha do Fundão (IF). 6: γ-aminobutyrate; 7: isoleucine; 8: leucine; 12: threonine; 14: valine; 17: phenylalanine; 18: β-glucose; 19: α-glucose; 20: sucrose; 25: *p*-coumaric acid; 26: choline; 27: betaine.