Supplementary Materials

Comparative Metabolomics of Ligulate and Tubular Flowers of Two Cultivars of *Calendula officinalis* L.

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**Table S1.** Some characteristics of the two cultivars of *Calendula officinalis,* ‘Paradise Garden’ and ‘Golden Sea’, used in the study

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |   |
|   | Paradise Garden (PG) | Golden Sea (GS) | PC vs GS |
| Characteristics of cultivar | Mean | Standard | Mean | Standard | t-test |
|   | value | error | value | error | *p* |
|   |   |   |   |   |  |
|   |   |   |   |   |   |
| Plant height, cm | 57.3 | 2.03 | 56.2 | 2.6 | n.s. |
| Number of branches | 6.6 | 0.43 | 6.38 | 0.3 | n.s. |
| Number of leaves | 28.9 | 1.26 | 31.6 | 1.5 | \* |
| Seed yield, kg/ha | 798.3 | 52.0 | 887.6 | 52.6 | \* |
| Inflorescences characteristics: |   |   |   |   |   |
| Diameter, cm | 6.68 | 0.38 | 6.6 | 0.40 | n.s. |
| Weight (raw), g | 3.12 | 0.09 | 3.17 | 0.10 | n.s. |
| Productivity, kg/ha | 1853.9 | 163.7 | 2001.5 | 164.1 | n.s. |
| Inflorescence parts (%): |   |   |   |   |   |
| Receptacle | 42.0 | 7 | 34.6 | 15 | n.s. |
| Ligulate flowers | 35.6 | 9 | 34.6 | 22 | n.s. |
| Tubular flowers | 22.4 | 9 | 30.8 | 10 | \*\* |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
| n.s., not significant; \*, *p* < 0.05; \*\*, *p* < 0.01 |

**Table S2.** Additional information to MS/MS fragmentation of lipids found in *Calendula officinalis* flowers

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | RT | [M+H]+ | [M+Na]+ | MS/MS fragmentation of parent ion [M+H]+ : |
| Code | (min) | (*m/z*) | (*m/z*) | fragment (*m/z*), intensity (%), [ion]+ |
| L1 | Trihydroxyoctadecadienoic acid | 329.2325 | 351.2134 | 311.2210 (75) [M-H2O+H]+, 293.2106 (100) [M-2H2O+H]+, 275.2004 (9.5) [M-3H2O+H]+ |
| L2 | Octadecatrienoic acid, isomer 1 | 279.2319 |   | 261.2213 (40), 243.2108 (19) |
| L3 | Dehydrophytosphingosine | 316.2844 |   |  298.2736 (15) [M-H2O+H]+, 280.2826 (10) [M-2H2O+H]+, 60.0453 (100) |
| L4 | Oxooctadecadienoic acid, isomer 1 | 295.2274 |   | 277.2155 (100) [Octadecadienoic acid+H]+, 259.2052 (13) [Octadecapentenone+H]+ |
| L5 | Oxooctadecadienoic acid, isomer 2 | 295.2274 |   | 277.2155 (100) [Octadecadienoic acid+H]+, 259.2052 (13) [Octadecapentenone+H]+ |
| L6 | Octadecatrienoic acid, isomer 2 | 279.2317 |   | 261.2213 (43), 243.2108 (17) |
| L7 | Dimethyl-pentyl-furandecanoic acid, isomer 1 | 337.2738 | 359.2555 | 319.2632 (16) [M-H2O+H]+, 301.2518 (37) [M-2H2O+H]+, 247.2953 (9) |
| L8 | Dimethyl-pentyl-furandecanoic acid, isomer 2 | 337.2738 | 359.2555 | 319.2632 (16) [M-H2O+H]+, 301.2518 (32) [M-2H2O+H]+, 247.2059 (11) |
| L9 | Octadecatrienoic acid, isomer 3 | 279.2316 |   | 261.2213 (39), 243.2108 (18)  |
| L10 | Hydroxyoctadecatrienoyl-carnitine | 438.3220 |   | 379.2468 (11) [Trihydroxydocosapentaenoic acid+H]+ |
| L11 | Dimethyl-pentyl-furandodecanoic acid | 365.3046 | 387.2864 | 347.2946 (100) [M-H2O+H]+, 329.2472 (30) |
| L12 | Octadecatrienoyl-sn-glycerol | 353.2686 |   | 335.2579 (60) [M-H2O+H]+, 261.2210 (83) |
| L13 | Aminolipid, isomer 1 | 473.2395 |   | 225.1846 (26) [Tetradecadienoic acid+H]+, 207.1740 (21) [Tetradecatrienal+H]+, 189.1634 (29) [Diaminononanoic acid+H]+, 159.0288 (10) [Hydroxy-hexadienedioic acid+H]+, 145.0493 (15) [Hexenedioic acid+H]+, 133.1011 (13) [Diaminopentanoic acid+H]+, 85.0654 (100) [Pentenone+H]+  |
| L14 | Aminolipid, isomer 2 | 473.2397 |   | 225.1846 (26) [Tetradecadienoic acid+H]+, 207.1740 (21) [Tetradecatrienal+H]+, 189.1634 (29) [Diaminononanoic acid+H]+, 159.0288 (10) [Hydroxy-hexadienedioic acid+H]+, 145.0493 (15) [Hexenedioic acid+H]+, 133.1011 (13) [Diaminopentanoic acid+H]+, 85.0654 (100) [Pentenone+H]+ |
| L15 | Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester | 437.2906 | 461.2872 |  247.1692 (15) [Hexadecendiynoic acid+H]+, 233.1534 (11) [Pentadecendiynoic acid+H]+, 203.1793 (74), 191.1793 (54) [Trimethylundecatetraene+H]+, 119.0857 (94), 95.0860 (100) [Heptatriene+H]+ |
| L16 | Octadecatrienoic acid, isomer 4 | 279.2317 |   | 261.2213 (45), 243.2108 (22) |
| L17 | Octacosanedioic acid | 455.4093 | 477.3910 | 419.3877 (62) [Octacosatrienoic acid+H]+, 365.3410 (40) [Tetracosadienoic acid+H]+, 195.0860 (100), 81.0705 (90)+, 67.0550 (77)  |
| L18 | Tricosatrienoic acid | 349.3095 | 371.2919 | 331.2990 (21) [M-H2O+H]+, 261.2209 (51) [Trimethylpentadecatetraenone+H]+, 95.0860 (67) [Heptatriene+H]+, 81.0705 (90), 67.0550 (100) |
| L19 | Dioxooctacosanoic acid, isomer 1 | 453.3935 |   | 435.3820 [M-H2O+H]+, 349.3094 (56), 295.2628 (72) [Methyloctadecadienoic acid+H]+, 113.0600 (48) [Hexadienoic acid+H]+, 109.1015 (44) [Octatriene+H]+, 95.0859 (92) [Heptatriene+H]+, 81.0705 (100) [C6H9]+ |
| L20 | Pentadecenyl-phenol | 303.2680 |   |  285.2573 (62) [M-H2O+H]+, 221.1898 (35) [Nonylphenol+H]+, 163.1479 (73) [Hexylbenzene+H]+ |
| L21 | Dimethyloctacosanedioic acid | 483.4409 | 505.4217 | 465.4303 (84) [M-H2O+H]+, 393.3723 (12) [Hexacosadienoic acid+H]+ |
| L22 | Phenolic lipid 1 | 407.3153 |   | 207.1377 (95) [Phenyl-heptanoic acid+H]+, 179.1064 (100) [Phenyl-pentanoic acid+H]+, 161.0959 (20) [Phenyl-pentenal+H]+, 133.1012 (55) |
| L23 | Butenedioic acid, ditridecyl ester, isomer 1 | 481.4245 | 503.4062 | 463.4138 (71) [M-H2O+H]+, 409.3671 (32) [Oxo-hexacosenoic acid+H]+, 377.3409 (52) [Trimethyldocosatrienoic acid+H]+, 323.2939 (50) [Methyleicosadienoic acid+H]+, 309.2780 (33) [Eicosadienoic acid+H]+, 113.0599 (44) [Hexadienoic acid+H]+, 81.0705 (100) |
| L24 | Heptadecenyl-phenol | 331.2992 |   | 313.2887 (90) [M-H2O+H]+, 239.2366 (38) [Hexadecadienol+H]+, 109.1015 (22) [Octatriene+H]+, 95.0880 (49) [Heptatriene+H]+, 81.0705 (37), 67.0550 (33), 57.0708 (100) |
| L25 | Dioxooctacosanoic acid, isomer 2 | 453.3935 | 475.3752 | 435.3821 (46) [M-H2O+H]+, 349.3094 (53), 295.2630 (29), 113.0602 (44) [Hexadienoic acid+H]+, 109.1014 (40) [Octatriene+H]+, 95.0857 (90) [Heptatriene+H]+, 81.0705 (100), 67.0550 (93) |
| L26 | Phenolic lipid 2 | 473.3627 |   | 273.1848 (100) [Octadecatrienoic acid+H]+, 247.1324 (59) [Hydroxypentadecadien-diynoic acid+H]+, 245.1532 (62) [Hexadecatetraenynoic acid+H]+, 189.1272 (88) [Tridecendiynal+H]+, 179.1063 (34) [Hydroxy-phenylpentanone+H]+ |
| L27 | Oxidized phosphatidylcholine | 758.5676 |   | 429.3724 (100), 184.0731(54), 124.9999 (35), 86.0970 (48) |
| L28 | Butenedioic acid, ditridecyl ester, isomer 2 | 481.4247 | 503.4066 | 463.4139 (77) [M-H2O+H]+, 409.3671 (32) [Oxo-hexacosenoic acid+H]+, 377.3409 (57) [Trimethyldocosatrienoic acid+H]+, 323.2937 (67) [Methyleicosadienoic acid+H]+, 95.0860 (90) [Heptatriene+H]+, 81.0705 (100), 67.0550 (91) |
| L29 | Unknown lipid 1 | 465.3934 | 487.3748 | 379.3198 (35), 309.2785 (100) [Eicosadienoic acid+H]+, 295.2626 (100) [Methyloctadecadienoic acid+H]+, 113.0599 (56) Hexadienoic acid+H]+, 95.0860 (44) [Heptatriene+H]+, 81.0705 (49), 71.0499 (48) |
| L30 | Unknown lipid 2, isomer 1 | 479.4086 | 501.3907 | 393.3354 (20) [Hexacosadienoic acid+H]+, 375.3250 (44), 323.2941 (20) [Methyleicosadienoic acid+H]+, 295.2626 (100) [Methyloctadecadienoic acid+H]+, 95.0859 (62) [Heptatriene+H]+, 81.0705 (63), 67.0550 (54) |
| L31 | Unknown lipid 3, isomer 1 | 429.3723 |   | 191.1063 (12) [Dodecapentaenoic acid+H]+; 165.0908 (100) [Decadiyonic acid+H]+ |
| L32 | Unknown lipid 2, isomer 2 | 479.4098 | 501.3903 | 393.3354 (30) [Hexacosadienoic acid+H]+, 375.3250 (44), 323.2940 (100) [Methyleicosadienoic acid+H]+, 295.2626 (100) [Methyloctadecadienoic acid+H]+, 113.0599 (60) [Hexadienoic acid+H]+, 95.0859 (42) [Heptatriene+H]+, 71.0499 (51), 67.0550 (49) |
| L33 | Unknown lipid 4, isomer 1 | 493.4247 | 515.4068 | 407.3511 (47), 389.3404 (39), 379.3193 (25), 337.3093 (73) [Dimethyleicosadienoic acid+H]+, 309.2783 (100) [Eicosadienoic acid+H]+, 113.0600 (70) [Hexadienoic acid+H]+, 95.0860 (69) [Heptatriene+H]+ |
| L34 | Unknown lipid 3, isomer 2 | 429.3722 |   |  191.1068 (12) [Dodecapentaenoic acid+H]+, 165.0909 (100) [Decadiyonic acid+H]+  |
| L35 | Unknown lipid 2, isomer 3 | 479.4096 | 501.3913 | 393.3355 (47) [Hexacosadienoic acid+H]+, 375.3250 (44), 323.2942 (100) [Methyleicosadienoic acid+H]+, 295.2631 (100) [Methyloctadecadienoic acid+H]+, 113.0597 (67) [Hexadienoic acid+H]+, 95.0859 (46) [Heptatriene+H]+ |
| L36 | Unknown lipid 4, isomer 2 | 493.4248 |   | 407.3512 (44), 389.3404 (39), 379.3193 (25), 337.3095 (100) [Dimethyl-eicosadienoic acid+H]+, 309.2784 (97) [Eicosadienoic acid+H]+, 295.2631 (52), 113.0601 (72) [Hexadienoic acid+H]+, 95.0860 (65) [Heptatriene+H]+ |

**Table S3.** Differences in the content of the metabolites between the two cultivars of *Calendula officinalis*, "Paradise Garden" and "Golden Sea", for the ligulate and tubular flowers.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |   |   |
|   | Ligulate flowers | Tubular flowers |
|   | ‘Golden Sea’ / ‘Paradise Garden’ | ‘Golden Sea’ / ‘Paradise Garden’ |
| Metabolite | Ratioa | t-testb | Correlationc | Ratioa | t-testb | Correlationc |
|   | (fold) | *р* | *r* | (fold) | *р* | *r* |
| 3-*O*-Caffeoylquinic acid (Neochlorogenic acid) | 1.60 | \*\* | 0.91 | 1.22 | \*\* | 0.85 |
| 5-*O*-Caffeoylquinic acid (Chlorogenic acid) | 2.65 | \*\*\* | 0.96 | 1.63 | \*\* | 0.87 |
| 4-*O*-Caffeoylquinic acid (Cryptochlorogenic acid) | 1.76 | \*\*\* | 0.88 | 1.06 | n.s. | 0.63 |
| Quercetin-3-*O*-rutinosyl-rhamnoside | -1.14 | n.s. | -0.69 | -1.43 | n.s. | -0.23 |
| Quercetin-3-*O*-β-D-rutinoside (Rutin) | -1.05 | n.s. | -0.81 | 1.25 | \* | -0.87 |
| Isorhamnetin-3-*O*-rutinosyl-rhamnoside | 1.07 | n.s. | 0.46 | -1.17 | n.s. | -0.86 |
| Kaempferol-3-*O*-rutinoside | 3.72 | \*\*\* | 0.80 | 1.97 | \*\*\* | 0.85 |
| Quercetin-3-*O*-glucoside (Isoquercitrin) | 2.08 | \*\*\* | 0.89 | -1.10 | n.s. | -0.66 |
| Quercetin-3-*O*-rhamnosyl-glucoside | 1.32 | \*\*\* | 0.95 | -1.24 | \*\* | -0.91 |
| Isorhamnetin-3-*O*-rutinoside (Narcissin) | 1.51 | \*\*\* | 0.65 | -1.09 | \* | -0.84 |
| Isorhamnetin 3-*O*-rhamnopyranosyl-glucopyranoside | 1.58 | \*\* | 0.82 | -1.10 | n.s. | -0.89 |
| 3,5-Di-*O*-caffeoylquinic acid | -1.17 | \* | -0.33 | 1.02 | n.s. | -0.20 |
| Tris-trans-p-coumaroyl-spermine | 1.14 | n.s. | 0.13 | 1.06 | n.s. | 0.63 |
| Isorhamnetin-malonyl-hexoside | 1.46 | \*\* | 0.85 | -1.14 | \*\* | -0.96 |
| Tetra-trans-p-coumaroyl-spermine | 1.08 | n.s. | 0.17 | 1.11 | n.s. | 0.73 |
| Calendulaglycoside A | 1.01 | n.s. | 0.11 | -1.11 | n.s. | -0.44 |
| Calendulaglycoside B | -1.03 | n.s. | -0.45 | -1.05 | n.s. | -0.41 |
| Calenduloside G | 1.15 | n.s. | 0.50 | -1.04 | n.s. | 0.41 |
| Acetyloleanolic acid-glucuronide-hexoside | 1.45 | \*\*\* | 0.84 | 1.76 | \*\*\* | 0.85 |
| Calendulaglycoside C | -1.89 | \*\*\* | -0.96 | -1.38 | \*\* | -0.85 |
| Calenduloside F | -1.68 | \*\* | -0.96 | -1.21 | n.s. | -0.80 |
| Calenduloside E (Oleanolic acid-glucuronide) | -2.15 | \*\*\* | -0.90 | -1.52 | \*\* | -0.83 |
| Trihydroxyoctadecadienoic acid | -2.22 | \*\*\* | -0.99 | -1.23 | \*\* | -0.96 |
| Octadecatrienoic acid, isomer 1 | 1.54 | \*\* | 0.86 | 1.38 | \*\* | 0.92 |
| Dehydrophytosphingosine | -3.60 | \*\*\* | -0.99 | -1.21 | \* | -0.87 |
| Oxooctadecadienoic acid, isomer 1 | -1.50 | \*\*\* | -0.99 | 1.24 | \* | 0.86 |
| Oxooctadecadienoic acid, isomer 2 | 1.24 | n.s. | 0.78 | 1.29 | \*\* | 0.89 |
| Octadecatrienoic acid, isomer 2 | 1.07 | n.s. | -0.58 | 1.66 | \*\* | 0.94 |
| Dimethyl-pentyl-furandecanoic acid, isomer 1 | -1.18 | n.s. | -0.61 | 1.15 | n.s. | 0.05 |
| Dimethyl-pentyl-furandecanoic acid, isomer 2 | -1.95 | \*\*\* | -0.82 | 1.16 | n.s. | 0.54 |
| Octadecatrienoic acid, isomer 3 | -1.06 | n.s. | 0.49 | 1.30 | n.s. | 0.71 |
| Hydroxyoctadecatrienoyl-carnitine | -1.36 | \* | -0.87 | 1.53 | \*\*\* | 0.90 |
| Dimethyl-pentyl-furandodecanoic acid | 1.32 | \*\* | 0.98 | 1.00 | n.s. | 0.48 |
| Octadecatrienoyl-sn-glycerol | -1.10 | n.s. | 0.51 | 1.04 | n.s. | 0.55 |
| Aminolipid, isomer 1 | -1.09 | n.s. | -0.66 | -1.08 | n.s. | -0.11 |
| Aminolipid, isomer 2 | -3.62 | \*\*\* | -0.82 | 1.09 | n.s. | 0.93 |
| Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester | -1.15 | n.s. | -0.61 | -1.62 | \*\*\* | -0.88 |
| Octadecatrienoic acid, isomer 4 | 1.22 | n.s. | 0.62 | 1.22 | \*\*\* | 0.84 |
| Octacosanedioic acid | -1.37 | \* | -0.86 | 1.60 | \*\* | 0.93 |
| Tricosatrienoic acid | 1.11 | n.s. | 0.25 | 1.54 | \*\*\* | 0.82 |
| Dioxooctacosanoic acid, isomer 1 | -1.38 | \* | -0.82 | 1.09 | n.s. | 0.35 |
| Pentadecenyl-phenol | -1.30 | n.s. | -0.48 | 1.15 | n.s. | 0.29 |
| Dimethyloctacosanedioic acid | -1.35 | \* | -0.81 | 1.62 | \*\*\* | 0.95 |
| Phenolic lipid 1 | 1.08 | n.s. | 0.07 | -1.30 | n.s. | -0.24 |
| Butenedioic acid, ditridecyl ester, isomer 1 | -1.02 | n.s. | -0.26 | 1.12 | n.s. | 0.79 |
| Heptadecenyl-phenol | 1.12 | n.s. | 0.32 | 1.75 | \*\*\* | 0.85 |
| Dioxooctacosanoic acid, isomer 2 | -1.85 | \*\* | -0.85 | 1.01 | n.s. | 0.10 |
| Phenolic lipid 2 | -2.28 | \*\* | -0.88 | -1.00 | n.s. | -0.08 |
| Oxidized phosphatidylcholine | 1.05 | n.s. | 0.55 | 1.06 | n.s. | 0.06 |
| Butenedioic acid, ditridecyl ester, isomer 2 | -1.09 | n.s. | -0.71 | 1.13 | n.s. | 0.71 |
| Unknown lipid 1 | -4.28 | \*\* | -0.85 | 1.05 | n.s. | 0.10 |
| Unknown lipid 2, isomer 1 | -1.01 | n.s. | -0.36 | 1.10 | n.s. | 0.35 |
| Unknown lipid 3, isomer 1 | 1.00 | n.s. | 0.27 | 1.46 | \*\* | 0.86 |
| Unknown lipid 2, isomer 2 | -4.23 | \*\*\* | -0.81 | 1.31 | \* | 0.81 |
| Unknown lipid 4, isomer 1 | -7.83 | \*\* | -0.83 | 1.25 | \*\* | 0.86 |
| Unknown lipid 3, isomer 2 | -1.07 | n.s. | -0.36 | 1.51 | \*\*\* | 0.95 |
| Unknown lipid 2, isomer 3 | -4.95 | \*\* | -0.80 | 1.11 | n.s. | 0.41 |
| Unknown lipid 4, isomer 2 | -6.18 | \*\*\* | -0.83 | 1.21 | \*\* | 0.83 |
| Sum of caffeoylquinic acids | 1.24 | \*\* |   | 1.16 | \* |   |
| Sum of flavonoids | 1.29 | \*\*\* |   | -1.09 | \*\* |   |
| Sum of phenolic compounds | 1.29 | \*\*\* |   | -1.00 | n.s. |   |
| Sum of triterpenoid glycosides | -1.01 | n.s. |   | -1.10 | n.s. |   |
| Sum of lipids | -1.27 | \*\*\* |   | 1.17 | \*\* |   |
| aRatio of the relative content of the metabolite, positive value – GS > PG, negative value – GS < PG. |
| bSignificance of differences: \* - *p* < 0.05, \*\* - *p* < 0.01, \*\*\* - *p* < 0.001; n.s., not significant |
| cCorrelation with orthogonal component from S-plot data of OPLS model |