**Supplementary Materials**

**Structural characterization of chlorogenic acid-metal complexes derived from the aqueous extracts of medicinal plants and their DNA cleavage and antibacterial activities**

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**Supplementary Tables**

**Table S1**. HPLC results of DZ aqueous and methanol extracts dissolved in two solvents (, n = 3).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample | Peak no. | Methanol | | FA-methanol | | Peak area increase (%) |
| Retention time (min) | Peak area | Retention time (min) | Peak area |
| aqueous extracts | 1 | 19.43 ± 0.45 (CGA) | 562.60 ± 44.21 | 20.23 ± 0.78 | 1,057.16 ± 67.42 | 87.91 |
| 2 | 20.75 ± 0.66 | 148.18 ± 18.92 | 21.62 ± 0.84 | 297.81 ± 24.42 | 100.97 |
| 3 | 22.14 ± 0.21 | 39.05 ± 9.75 | 23.14 ± 5.64 | 54.40 ± 8.53 | 39.31 |
| 4 | 24.00 ± 0.85 | 51.54 ± 13.41 | 24.81 ± 0.91 | 55.47 ± 12.73 | 7.63 |
| 5 | 32.03 ± 0.46 | 70.42 ± 18.64 | 32.95 ± 0.55 | 99.13 ± 16.55 | 40.76 |
| 6 | 34.03 ± 0.25 | 52.18 ± 9.98 | 34.79 ± 0.43 | 87.28 ± 16.21 | 67.27 |
| 7 | 35.29 ± 0.51 | 62.95 ± 5.65 | 36.35 ± 0.56 | 101.41 ± 9.42 | 61.10 |
| 8 | 39.48 ± 0.63 | 60.98 ± 9.31 | 40.55 ± 0.98 | 125.88 ± 11.39 | 106.45 |
| methanol  extracts | 1 | 13.63 ± 0.47 | 222.80 ± 19.63 | 14.49 ± 0.31 | 207.20 ± 9.77 | -7.00 |
| 2 | 15.82 ± 0.69 | 125.46 ± 11.59 | 15.95 ± 0.43 | 135.10 ± 13.21 | 7.68 |
| 3 | 20.01 ± 0.54 (CGA) | 1,201.84 ± 79.21 | 20.12 ± 0.39 | 1,114.50 ± 58.23 | -7.27 |
| 4 | 21.42 ± 0.28 | 302.74 ± 17.94 | 21.51 ± 0.31 | 236.09 ± 19.88 | -22.02 |
| 5 | 22.87 ± 0.37 | 125.06 ± 8.94 | 23.00 ± 0.65 | 126.97 ± 10.65 | 1.53 |
| 6 | 24.57 ± 0.32 | 271.25 ± 11.89 | 24.67 ± 0.44 | 297.80 ± 9.34 | 9.79 |
| 7 | 26.47 ± 0.48 | 124.01 ± 9.79 | 26.56 ± 0.37 | 127.84 ± 10.43 | 3.10 |
| 8 | 32.70 ± 0.47 | 604.96 ± 21.85 | 32.74 ± 0.29 | 666.66 ± 18.30 | 10.20 |
| 9 | 33.08 ± 0.64 | 326.71 ± 31.92 | 33.14 ± 0.59 | 367.52 ± 28.77 | 12.49 |
| 10 | 36.04 ± 0.51 | 163.16 ± 18.34 | 36.06 ± 0.36 | 168.91 ± 23.61 | 3.52 |
| 11 | 40.21 ± 0.98 | 166.06 ± 11.49 | 40.22 ± 0.76 | 170.47 ± 13.58 | 2.66 |
| 12 | 51.47 ± 0.49 | 304.62 ± 27.49 | 51.46 ± 0.91 | 164.57 ± 19.86 | -45.98 |
| 13 | 89.02 ± 0.41 | 111.77 ± 22.49 | 88.77 ± 0.38 | 106.98 ± 18.67 | -4.28 |

**Table S2**. HPLC results of SY aqueous and methanol extracts dissolved in two solvents(, n = 3).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Samples | Peak no. | Methanol | | FA-methanol | | Peak area increase (%) |
| Retention time (min) | Peak area | Retention time (min) | Peak area |
| aqueous extracts | 1 | 12.88 ± 0.61 | 951.41 ± 34.99 | 12.90 ± 0.59 | 1,316.41 ± 28.41 | 38.36 |
| 2 | 14.99 ± 0.49 | 153.63 ± 18,64 | 15.04 ± 0.62 | 260.81 ± 15.38 | 69.76 |
| 3 | 19.07 ± 0.69 | 581.14 ± 22,76 | 19.09 ± 0.54 | 2,994.63 ± 42.59 | 415.30 |
| 4 | 20.35 ± 0.53 (CGA) | 870.39 ± 78.03 | 20.37 ± 0.29 | 3,495.44 ± 91.24 | 301.59 |
| 5 | 21.71 ± 0.16 | 366.97 ± 16.65 | 21.74 ± 0.42 | 779.56 ± 17.80 | 112.43 |
| 6 | 23.52 ± 0.84 | 156.75 ± 14.35 | 23.41 ± 0.67 | 185.17 ± 17.71 | 18.13 |
| 7 | 26.26 ± 0.62 | 140.18 ± 13.10 | 26.29 ± 0.90 | 364.19 ± 18,53 | 159.80 |
| 8 | 30.36 ± 0.17 | 71.42 ± 9.52 | 30.39 ± 0.58 | 199.17 ± 13.09 | 178.87 |
| 9 | 33.83 ± 0.26 | 112.82 ± 9.98 | 33.82 ± 0.54 | 239.67 ± 14.99 | 112.44 |
| 10 | 34.90 ± 0.30 | 123.39 ± 12.28 | 34.93 ± 0.25 | 232.17 ± 17.46 | 88.16 |
| 11 | 37.34 ± 0.41 | 401.57 ± 56.57 | 37.37 ± 0.52 | 1,094.90 ± 45.33 | 172.65 |
| 12 | 41.71 ± 0.25 | 512.33 ± 32.09 | 41.71 ± 0.47 | 851.81 ± 28.91 | 66.26 |
| methanol extracts | 1 | 13.05 ± 0.63 | 383.22 ± 32.89 | 13.74 ± 0.42 | 425.31 ± 47.51 | 10.98 |
| 2 | 19.36 ± 0.50 | 4,415.14 ± 87.42 | 20.14 ± 0.48 | 4,547.59 ± 99.28 | 3.00 |
| 3 | 20.74 ± 0.81 (CGA) | 618.16 ± 53.11 | 21.53 ± 0.74 | 679.85 ± 49.80 | 9.98 |
| 4 | 26.91 ± 0.62 | 213.83 ± 23.54 | 27.42 ± 0.70 | 233.61 ± 18.70 | 9.25 |
| 5 | 32.61 ± 0.39 | 228.05 ± 31.38 | 33.07 ± 0.51 | 237.40 ± 39.48 | 4.10 |
| 6 | 34.82 ± 0.33 | 207.75 ± 21.55 | 35.32 ± 0.27 | 220.99 ± 26.31 | 6.38 |
| 7 | 35.79 ± 0.44 | 334.12 ± 38.54 | 36.20 ± 0.38 | 346.74 ± 27.64 | 3.78 |
| 8 | 38.41 ± 0.27 | 2,538.56 ± 89.70 | 38.90 ± 0.41 | 2,540.80 ± 95.76 | 0.09 |
| 9 | 42.73 ± 0.42 | 1,887.97 ± 69.76 | 43.17 ± 0.59 | 1,911.66 ± 78.59 | 1.25 |
| 10 | 78.24 ± 0.87 | 4,18.32 ± 35.62 | 78.41 ± 0.90 | 410.98 ± 27.93 | -1.76 |

**Table S3**. HPLC results of YXC aqueous and methanol extracts dissolved in two solvents(, n = 3).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Samples | Peak no. | Methanol | | FA-methanol | | Peak area increase (%) |
| Retention time (min) | Peak area | Retention time (min) | Peak area |
| aqueous extracts | 1 | 7.98 ± 0.72 | 871.76 ± 69.54 | 7.84 ± 0.58 | 2,300.81 ± 87.94 | 163.93 |
| 2 | 13.23 ± 0.39 (CGA) | 599.68 ± 74.22 | 13.10 ± 0.41 | 2,346.57 ± 87.59 | 291.31 |
| 3 | 14.01 ± 0.27 | 1,123.80 ± 45.68 | 13.89 ± 0.34 | 3,787.79 ± 69.36 | 237.05 |
| 4 | 15.82 ± 0.50 | 397.57 ± 47.53 | 15.67 ± 0.48 | 976.30 ± 52.47 | 145.57 |
| 5 | 30.34 ± 0.39 | 374.90 ± 38.68 | 30.31 ± 0.44 | 1,790.73 ± 49.74 | 377.65 |
| 6 | 30.73 ± 0.54 | 235.26 ± 36.90 | 30.72 ± 0.43 | 1,242.02 ± 49.71 | 427.93 |
| 7 | 33.97 ± 0.59 | 1,705.05 ± 67.38 | 33.99 ± 0.70 | 5,769.25 ± 5.99 | 238.36 |
| 8 | 37.98 ± 0.61 | 101.89 ± 27.09 | 38.00 ± 0.58 | 293.00 ± 30.07 | 187.57 |
| methanol extracts | 1 | 7.95 ± 0.73 | 783.45 ± 65.74 | 7.94 ± 0.59 | 414.00 ± 38.92 | -47.16 |
| 2 | 13.20 ± 0.62 (CGA) | 140.22 ± 27.41 | 13.47 ± 0.49 | 144.18 ± 19.99 | 2.83 |
| 3 | 13.99 ± 0.58 | 156.56 ± 29.54 | 14.28 ± 0.63 | 163.98 ± 17.93 | 4.74 |
| 4 | 30.43 ± 0.28 | 466.94 ± 31.45 | 30.72 ± 0.36 | 487.93 ± 42.53 | 4.50 |
| 5 | 30.82 ± 0.45 | 192.56 ± 17.65 | 31.09 ± 0.52 | 202.21 ± 15.96 | 5.01 |
| 6 | 34.04 ± 0.39 | 1,235.26 ± 86.94 | 34.23 ± 0.68 | 1,288.38 ± 94.05 | 4.30 |

**Table S4**. Contents of the eight metal ions in the aqueous extracts of four medicinal plants dissolved in different solvents (, n = 3).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Samples | | Ca | Cu | Fe | Mg | Mn | Ni | Sr | Zn |
| JYH aqueous extract | dissolved in methanol | 5.02 ± 0.34 | 17.8 ± 1.09 | 0.65 ± 0.04 | 1.48 ± 0.10 | 0.09 ± 0.01 | 0.07 ± 0.004 | 0.16 ± 0.01 | 0.06 ± 0.004 |
| dissolved in 15% FA-methanol | 49.82 ± 2.49 | 78.92 ± 4.42 | 0.73 ± 0.05 | 1.98 ± 0.12 | 0.22 ± 0.01 | 0.98 ± 0.06 | 0.26 ± 0.01 | 0.18 ± 0.01 |
| Content increase % | 892.43 | 343.37 | 12.31 | 33.78 | 144.44 | 1,300 | 62.5 | 200 |
| DZ aqueous extract | dissolved in methanol | 550.85 ± 31.34 | 0.81 ± 0.07 | 5.09 ± 0.41 | 310.61 ± 18.69 | 10.02 ± 1.74 | 0.09 ± 0.008 | 3.06 ± 0.37 | 2.06 ± 0.07 |
| dissolved in 15% FA-methanol | 884.80 ± 75.86 | 1.36 ± 0.12 | 5.10 ± 0.39 | 482.85 ± 17.99 | 18.78 ± 1.93 | 0.13 ± 0.01 | 6.67 ± 0.78 | 2.21 ± 0.15 |
| Content increase % | 60.62 | 67.9 | 0.2 | 55.45 | 87.43 | 44.44 | 117.97 | 7.28 |
| SY aqueous extract | dissolved in methanol | 106.17 ± 11.45 | 0.34 ± 0.02 | 1.56 ± 0.25 | 50.80 ± 5.48 | 0.61 ± 0.07 | 0.08 ± 0.005 | 0.11 ± 0.01 | 1.05 ± 0.11 |
| dissolved in 15% FA-methanol | 497.14 ± 23.46 | 0.56 ± 0.01 | 3.23 ± 0.28 | 272.41 ± 32.09 | 4.26 ± 0.24 | 0.25 ± 0.01 | 0.64 ± 0.05 | 2.14 ± 0.20 |
| Content increase % | 368.25 | 64.71 | 107.05 | 436.24 | 598.36 | 212.5 | 481.82 | 103.81 |
| YXC aqueous extract | dissolved in methanol | 9.32 ± 0.76 | 0.06 ± 0.008 | 0.19 ± 0.01 | 4.33 ± 0.37 | 0.45 ± 0.02 | 0.02 ± 0.005 | 0.02 ± 0.001 | 0.10 ± 0.03 |
| dissolved in 15% FA-methanol | 41.70 ± 2.08 | 0.15 ± 0.001 | 0.56 ± 0.06 | 16.15 ± 1.12 | 0.61 ± 0.03 | 0.06 ± 0.005 | 0.08 ± 0.001 | 0.35 ± 0.02 |
| Content increase % | 347.42 | 150 | 194.74 | 272.98 | 35.56 | 200 | 300 | 250 |

**Supplementary Figures**

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**Fig. S1 Chemical structure of chlorogenic acid.**



**Fig. S2** HPLC spectra of DZ aqueous extracts dissolved in two solvents (327 nm). **a**: Samples dissolved in methanol. **b:** Samples dissolved in 15% FA-methanol.



**Fig. S3** HPLC spectra of DZ methanol extracts dissolved in two solvents (327 nm). **a**: Samples dissolved in methanol. **b**: Samples dissolved in 15% FA-methanol.



**Fig. S4** HPLC spectra of SY aqueous extracts dissolved in two solvents (327 nm). **a**: Samples dissolved in methanol. **b**: Samples dissolved in 15% FA-methanol.



**Fig. S5** HPLC spectra of SY methanol extracts dissolved in two solvents (327 nm). **a**: Samples dissolved in methanol. **b**: Samples dissolved in 15% FA-methanol.



**Fig. S6** HPLC spectra of YXC aqueous extracts dissolved in two solvents (327 nm). **a**: Samples dissolved in methanol. **b**: Samples dissolved in 15% FA-methanol.



**Fig. S7** HPLC spectra of YXC methanol extracts dissolved in two solvents (327 nm). **a:** Samples dissolved in methanol. **b**: Samples dissolved in 15% FA-methanol.



**Fig. S8** UV-Vis spectra of chlorogenic acid and chlorogenic acid–Ca2+ reaction liquid (**a**), chlorogenic acid–Mg2+ reaction liquid (**b**), chlorogenic acid–Zn2+ reaction liquid (**c**), chlorogenic acid–Fe3+ reaction liquid (**d**), chlorogenic acid–Ni2+ reaction liquid (**e**), chlorogenic acid–Mn2+ reaction liquid (**f**), and chlorogenic acid–Sr2+ reaction liquid (**g**) in water or 15% formic acid



**Fig. S9** Theoretical (**a**) and measured (**b**) isotopic distributions of C32H35O18Ca.Theoretical (**c**) and measured (**d**) isotopic distributions of C48H53O27Ca.Theoretical (**e**) and measured (**f**) isotopic distributions of C48H51O27Ca2.



**Fig. S10** Theoretical (**a**) and measured (**b**) isotopic distributions of C48H53O27Mg.Theoretical (**c**) and measured (**d**) isotopic distributions of C48H51O27Mg2.



**Fig. S11** Theoretical (**a**) and measured (**b**) isotopic distributions of C16H17O9Zn.Theoretical (**c**) and measured (**d**) isotopic distributions of C32H35O18Zn.



**Fig. S12** Theoretical (**a**) and measured (**b**) isotopic distributions of C16H17O9Cu.Theoretical (**c**) and measured (**d**) isotopic distributions of C32H35O18Cu.Theoretical (**e**) and measured (**f**) isotopic distributions of C32H33O18Cu2.



**Fig. S13** Theoretical (**a**) and measured (**b**) isotopic distributions of C16H17O9Ni. Theoretical (**c**) and measured (**d**) isotopic distributions of C32H35O18Ni. Theoretical (**e**) and measured (**f**) isotopic distributions of C32H33O18Ni2. Theoretical (**g**) and measured (**h**) isotopic distributions of C48H51O27Ni2.



**Fig. S14** Theoretical (**a**) and measured (**b**) isotopic distributions of C16H17O9Sr. Theoretical (**c**) and measured (**d**) isotopic distributions of C32H35O18Sr. Theoretical (**e**) and measured (**f**) isotopic distributions of C32H33O18Sr2. Theoretical (**g**) and measured (**h**) isotopic distributions of C48H53O27Sr.



**Fig. S15** Theoretical isotopic distribution of C32H34O18Fe(III) (**a**). Theoretical (**b**) and measured (**c**) isotopic distributions of C32H35O18Fe(II). Theoretical isotopic distribution of C48H52O27Fe(III) (**d**). Theoretical (**e**) and measured (**f**) isotopic distributions of C48H53O27Fe(II).

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**Fig. S16** Chromogenic assays of the different samples using 1,10-phenanthroline.

(1: Fe2+ solution; 2: Fe2+ solution + 1,10-phenanthroline; 3: Fe3+ solution; 4: Fe3+ solution + 1,10-phenanthroline; 5: CGA solution; 6: CGA solution + 1,10-phenanthroline; 7: reaction supernatant for CGA and Fe3+ solution; 8: reaction supernatant for CGA and Fe3+ solution + 1,10-phenanthroline).

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**Fig. S17** Effect of DNA cleavage of the experimental samples (incubation time: 24 h). Lane 1, DNA blank control; lane 2, DMSO with a final concentration of 5%; lanes 3‒10, Ca(II), Mg(II), Zn(II), Fe(III), Cu(II), Ni(II), Mn(II), and Sr(II), respectively, with a final concentration of 50 μmol/L; lane 11, CGA with a final concentration of 100 μmol/L; lanes 12–19, reaction products of CGA with Ca(II), Mg(II), Zn(II), Fe(III), Cu(II), Ni(II), Mn(II), and Sr(II), respectively; final concentration of CGA: 100 μmol/L, final concentration of the metal ions: 50 μmol/L.

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**Fig. S18** Effect of time on the DNA cleavage activity of the CGA and Cu(II) reaction products. Lanes 1‒8, incubation with CGA and Cu(II) reaction products for 0 h, 5 min, 10 min, 15 min, 0.5 h, 1 h, 1.5 h, and 2 h, respectively; lane 9, DNA blank control (CGA final concentration: 100 μmol/L, Cu(II) final concentration: 50 μmol/L).

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**Fig. S19.** Effect of concentration on DNA cleavage activity of the CGA and Cu(II) reaction products (incubation time: 2 h). Lanes 1‒7, final concentrations of CGA in the reaction system were 10, 20, 30, 40, 50, 60, and 70 μmol/L, respectively; lane 8, DNA blank control (molar ratio of CGA to Cu(II) is 2:1).