***Supporting information of***

**Toxicological and genotoxicological assessment of sewage sludge and other biogenic wastes water extracts: a piece of the SLURP jigsaw puzzle**

**Figure S1**.Linear relation between the number of HepG2 cells and the optical density readings at l = 490 nm (OD490).

**Table S1**. Chemical characterization of aqueous extracts of biogenic wastes (expressed as mg/L).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **S1** | **S2** | **S3** | **S4** | **L** | **M1** | **M2** | **D** | **C** |
| **Ammonia nitrogen** | 87.9 | 20.3 | 17.7 | 266.2 | 189.5 | 92.8 | 78.8 | 198.4 | 186.0 |
| **Chloride** | 26.6 | 41.6 | 53.9 | 55.9 | 54.3 | 64.6 | 66.3 | 295.5 | 1381.7 |
| **Nitrate** | < LOD | 20 | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| **Sulphate** | 23.6 | 138.3 | 415.1 | 42.0 | 1069.5 | 10.0 | < LOD | 31.8 | 146.3 |
| **Total Phosphorus** | 10.7 | 6.0 | 11.8 | 8.3 | 9.8 | 7.1 | 24.6 | 32.4 | 39.7 |
| **Calcium** | 14.6 | 19.4 | 80.1 | 76.6 | 648.9 | 18.6 | 75.9 | 77.7 | 252.3 |
| **Magnesium** | < LOD | < LOD | 26 | 11.9 | 38.2 | < LOD | 39.3 | 36.8 | 101.5 |
| **Potassium** | 15.1 | 8.3 | 71.6 | 44.1 | 57.1 | 112.8 | 249.1 | 611.9 | 1653.6 |
| **Sodium** | 19.5 | 43.2 | 78.4 | 50.8 | 56.1 | 33 | 106.9 | 168.9 | 1457.9 |
| **Aluminium** | 4.68 | 0.28 | 3.95 | 2.09 | 3.97 | 0.17 | 1.24 | 1.53 | 16.68 |
| **Antimony** | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| **Arsenic** | 0.01 | < LOD | < LOD | 0.03 | 0.02 | < LOD | 0.01 | 0.02 | 0.04 |
| **Beryllium**  | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| **Boron**  | 0.13 | 0.16 | 0.38 | 0.24 | 0.2 | 0.19 | 0.30 | 0.42 | 0.86 |
| **Cadmium**  | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| **Copper**  | 0.04 | 0.02 | 0.1 | 0.08 | 0.09 | 0.17 | 0.16 | 0.28 | 0.46 |
| **Iron** | 0.60 | 2.34 | 9.86 | 2.04 | 2.99 | 1.09 | 3.06 | 20.04 | 14.72 |
| **Lead**  | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | 0.02 |
| **Manganese** | 0.06 | 0.06 | 0.21 | 0.09 | 0.10 | 0.11 | 0.71 | 0.80 | 0.86 |
| **Mercury**  | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| **Nichel** | 0.01 | < LOD | 0.01 | 0.03 | 0.07 | < LOD | 0.01 | 0.04 | 0.11 |
| **Total Chromium** | < LOD | 0.16 | 0.02 | < LOD | 0.01 | < LOD | 0.01 | 0.02 | 0.09 |
| **Vanadium**  | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | 0.03 |
| **Zinc** | 0.33 | 0.07 | 0.42 | 0.17 | 0.20 | 1.47 | 1.21 | 0.99 | 1.48 |
| LOD: limit of detection |

**Table S2**. Germination index (GI) expressed as a percentage in *Allium cepa*, *Cucumis sativus* and *Lepidium sativum* seeds, treated with different doses of aqueous extracts of biogenic wastes. Data are expressed as mean GI% ± SE.

|  |  |
| --- | --- |
|  | ***GI%\_Allium cepa*** |
| **Sample** | **undiluted** | **1:2** | **1:10** | **1:20** | **1:100** | **1:200** | **1:1000** |
| **S1** | 112.5±14.2 | 131.7±17.2 | 91.7±9.2 | 122.5±19.1 | 127.5±11.8 | 116.7±22.4 | 130.0±24.6 |
| **S2** | 116.2±10.6 | 124.9±5.3 | 112.2±17.5 | 143.0±17.7 | 153.6±12.1 | 140.3±22.8 | 106.2±16.8 |
| **S3** | 122.8±28.2 | 175.4±104.7 | 112.8±44.0 | 220.6±32.9 | 157.9±24.2 | 157.5±24.3 | 152.6±37.8 |
| **S4** | 12.1±3.4 | 40.2±5.5 | 74.1±8.5 | 104.2±14.8 | 95.1±14.5 | 97.4±3.5 | 102.1±10.4 |
| **L** | 21.7±3.5 | 50.9±6.1 | 68.2±8.0 | 71.7±11.0 | 79.2±8.7 | 81.8±7.9 | 94.4±11.8 |
| **M1** | 137.8±42.2 | 185.5±77.9 | 162.9±32.6 | 142.6±27.9 | 205.5±78.8 | 105.3±30.4 | 167.9±21.8 |
| **M2** | 125.0±6.3 | 112.5±6.3 | 89.2±26.7 | 105.8±35.5 | 100.8±15.4 | 103.3±21.7 | 97.5±13.8 |
| **D** | 32.1±2.3 | 85.5±19.4 | 127.6±13.9 | 94.9±14.7 | 75.5±13.1 | 151.0±15.7 | 132.9±15.3 |
| **C** | 3.3±3.3 | 13.3±10.8 | 71.7±17.8 | 74.2±14.2 | 105.0±16.6 | 71.7±26.0 | 56.7±7.1 |
|  | ***GI%\_Cucumis sativus*** |
| **Sample** | **undiluted** | **1:2** | **1:10** | **1:20** | **1:100** | **1:200** | **1:1000** |
| **S1** | 104.7±3.3 | 104.5±4.7 | 96.9±3.7 | 109.3±1.7 | 103.1±7.1 | 106.9±2.7 | 106.4±4.0 |
| **S2** | 85.5±3.5 | 107.7±3.3 | 119.6±6.2 | 102.8±5.2 | 95.3±1.4 | 101.5±3.6 | 111.0±6.2 |
| **S3** | 86.6±3.3 | 90.4±4.5 | 86.4±5.5 | 80.7±2.0 | 85.9±2.9 | 86.0±3.4 | 87.2±5.1 |
| **S4** | 70.2±3.2 | 91.3±0.7 | 97.4±7.2 | 96.7±10.8 | 104.3±3.4 | 108.7±1.4 | 87.0±3.2 |
| **L** | 56.6±4.2 | 69.1±1.2 | 87.1±9.0 | 85.8±6.3 | 93.5±7.4 | 97.2±0.5 | 83.4±8.9 |
| **M1** | 108.2±2.9 | 102.0±1.8 | 90.2±4.9 | 90.9±2.6 | 93.0±1.3 | 102.4±2.6 | 93.6±3.7 |
| **M2** | 94.6±4.3 | 99.1±1.9 | 87.7±1.3 | 89.5±3.7 | 87.6±4.6 | 82.5±4.1 | 90.5±8.7 |
| **D** | 78.4±3.1 | 116.6±2.9 | 113.1±5.0 | 116.7±6.6 | 123.0±5.7 | 112.7±3.1 | 105.6±7.7 |
| **C** | 67.7±2.3 | 98.2±5.0 | 118.2±3.0 | 101.5±6.7 | 90.4±0.8 | 90.7±8.0 | 103.0±3.4 |
|  | ***GI%\_Lepidium sativum*** |
| **Sample** | **undiluted** | **1:2** | **1:10** | **1:20** | **1:100** | **1:200** | **1:1000** |
| **S1** | 97.3±9.4 | 58.7±29.2 | 61.5±23.4 | 83.4±20.9 | 61.5±9.8 | 80.0±15.1 | 65.7±2.9 |
| **S2** | 91.2±4.6 | 106.0±50.0 | 92.1±10.6 | 121.7±29.7 | 110.5±32.1 | 105.8±19.7 | 76.2±22.7 |
| **S3** | 98.6±4.2 | 120.7±2.5 | 112.0±11.0 | 105.1±4.7 | 92.2±8.2 | 104.3±3.7 | 116.8±3.6 |
| **S4** | 0.0±0.0 | 59.1±3.9 | 83.4±6.4 | 78.8±6.8 | 94.7±2.1 | 100.0±2.5 | 90.5±8.6 |
| **L** | 6.1±0.8 | 29.9±6.3 | 52.1±1.4 | 57.3±6.8 | 98.9±6.1 | 93.1±1.8 | 99.2±1.6 |
| **M1** | 80.6±9.4 | 136.2±4.7 | 129.6±5.5 | 93.2±5.6 | 134.0±5.5 | 122.8±7.5 | 100.9±5.0 |
| **M2** | 103.4±12.8 | 76.5±27.9 | 96.3±9.9 | 73.3±11.3 | 98.2±27.0 | 96.6±19.3 | 70.1±3.5 |
| **D** | 2.4±0.3 | 63.1±8.4 | 72.7±3.5 | 59.4±0.8 | 82.8±3.7 | 78.0±6.5 | 84.8±7.6 |
| **C** | 1.2±0.4 | 30.3±5.0 | 94.1±4.9 | 98.2±1.9 | 92.0±7.4 | 100.6±4.8 | 91.4±4.8 |

**Table S3**. EC50, EC20 and EC10 values (geq/L) with relative confidence limits (95%) of aqueous extracts of biogenic wastes for *Lepidium sativum.*

|  |  |  |  |
| --- | --- | --- | --- |
|  | **EC50**  | **EC20** | **EC10** |
| **S1** | n.d. | n.d. | n.d. |
| **S2** | *119.6* (104.3-1000) | *106.6* (0.0-601.7) | 99.7 (1.2-571.7) |
| **S3** | n.d. | n.d. | n.d. |
| **S4** | 53.6 (51.9-55.2) | 45.9 (44.7-48.0) | 41.8 (37.6-41.5) |
| **L** | 10.0 (4.6-16.0) | 1.9 (0.4-4.2) | 0.7 (0.1-1.9) |
| **M1** | *105.2* (100.9-111.5) | 96.4 (93.4-100.6) | 91.6 (84.6-92.4) |
| **M2** | n.d. | n.d. | n.d. |
| **D** | 33.6 (16.6-69.9) | 5.1 (0.7-61.1) | 1.7 (0.1-56.5) |
| **C** | 47.0 (45.8-47.9) | 42.2 (36.5-42.8) | 39.6 (31.5-40.2) |
| n.d.: not determined; Values in *italics*: for the genotoxicity test was considered the undiluted sample. |

**Table S4**. Macroscopic parameters of *Allium cepa* bulb roots after 72 hours of exposure to aqueous extracts of biogenic wastes, expressed as the number bulbs positive to each alteration per condition.

|  |  |
| --- | --- |
| **Sample** | **Doses** |
| **S1** | **undiluted** | **1:2** | **1:10** | **1:20** | **1:100** | **1:200** | **1:1000** |
| Hooks | 12\*\*\* | 12\*\*\* | 1 | 4 | 5 | 6\* | 5 |
| C-tumors | 0 | 0 | 0 | 2 | 2 | 3 | 3 |
| Broken tips | 0 | 0 | 1 | 1 | 2 | 1 | 1 |
| Color change | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Turgidity  | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **S2** |  |  |  |  |  |  |  |
| Hooks | 3 | 4 | 4 | 6 | 2 | 2 | 7 |
| C-tumors | 3 | 3 | 5 | 2 | 4 | 4 | 0 |
| Broken tips | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Color change | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Turgidity  | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **S3** |  |  |  |  |  |  |  |
| Hooks | 5\* | 7\*\* | 7\*\* | 6\*\* | 2 | 0 | 2 |
| C-tumors | 7 | 6 | 3 | 6 | 7 | 4 | 2 |
| Broken tips | 5 | 5 | 4 | 1 | 1 | 5 | 4 |
| Color change | 5\* | 1 | 0 | 0 | 0 | 0 | 0 |
| Turgidity  | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **S4** |  |  |  |  |  |  |  |
| Hooks | r.n.e. | r.n.e. | 7 | 5 | 2 | 7 | 5 |
| C-tumors | r.n.e. | r.n.e. | 1 | 1 | 6\* | 3 | 1 |
| Broken tips | r.n.e. | r.n.e. | 0 | 2 | 0 | 1 | 2 |
| Color change | r.n.e. | r.n.e. | 0 | 0 | 0 | 0 | 0 |
| Turgidity  | r.n.e. | r.n.e. | 0 | 0 | 0 | 0 | 0 |
| **L** |  |  |  |  |  |  |  |
| Hooks | r.n.e. | r.n.e. | r.n.e. | r.n.e. | 3 | 5 | 8 |
| C-tumors | r.n.e. | r.n.e. | r.n.e. | r.n.e. | 3 | 0 | 0 |
| Broken tips | r.n.e. | r.n.e. | r.n.e. | r.n.e. | 1 | 1 | 2 |
| Color change | r.n.e. | r.n.e. | r.n.e. | r.n.e. | 0 | 0 | 0 |
| Turgidity  | r.n.e. | r.n.e. | r.n.e. | r.n.e. | 0 | 0 | 0 |
| **M1** |  |  |  |  |  |  |  |
| Hooks | 12\*\*\* | 5 | 9\* | 8 | 4 | 9\* | 4 |
| C-tumors | 2 | 8\* | 0 | 1 | 2 | 3 | 4 |
| Broken tips | 2 | 2 | 3 | 1 | 2 | 0 | 3 |
| Color change | 9\*\*\* | 4\* | 0 | 0 | 0 | 0 | 0 |
| Turgidity  | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M2** |  |  |  |  |  |  |  |
| Hooks | 12\*\*\* | 12\*\*\* | 1 | 0 | 4 | 1 | 0 |
| C-tumors | 0 | 0 | 3 | 4 | 4 | 7 | 6 |
| Broken tips | 0 | 0 | 5 | 3 | 1 | 0 | 1 |
| Color change | 12\*\*\* | 12\*\*\* | 1 | 2 | 0 | 0 | 0 |
| Turgidity  | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **D** |  |  |  |  |  |  |  |
| Hooks | 12\*\*\* | 10\* | 2 | 8 | 1 | 4 | 8 |
| C-tumors | 0 | 5 | 7\* | 2 | 6\* | 3 | 2 |
| Broken tips | 0 | 5 | 4 | 6 | 4 | 1 | 2 |
| Color change | 12\*\*\* | 12\*\*\* | 0 | 0 | 0 | 0 | 0 |
| Turgidity  | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **C** |  |  |  |  |  |  |  |
| Hooks | 12\*\*\* | 7 | 2 | 2 | 7 | 3 | 5 |
| C-tumors | 0 | 0 | 4 | 3 | 2 | 2 | 3 |
| Broken tips | 0 | 1 | 5 | 6\* | 7\* | 6\* | 0 |
| Color change | 2 | 3 | 0 | 0 | 0 | 0 | 0 |
| Turgidity  | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| r.n.e.: roots not evaluable; Statistically significant versus negative control according to χ2 test: \*p< 0.05, \*\*p<0.01, \*\*\*p<0.001 |

**Table S5**. EC50, EC20 and EC10 values (geq/L) with relative confidence limits (95%) of aqueous extracts of biogenic wastes for *Allium cepa* bulbs

|  |  |  |  |
| --- | --- | --- | --- |
|  | **EC50** | **EC20** | **EC10** |
| **S1** | 4.8 (2.6-8.5) | 0.6 (0.2-1.8) | 0.2 (0.1-0.7) |
| **S2** | *193.9* (128.1-289.2) | 94.6 (75.7-126.7) | 62.2 (45.4-90.3) |
| **S3** | *134.1* (104.7-654.2) | 75.15 (17.4-96.0) | 53.57 (2.3-90.7) |
| **S4** | 0.8 (0.6-1.0) | 0.2 (0.1-0.3) | 0.1 (0.0-0.1) |
| **L** | 0.6 (0.4-0.9) | 0.1 (0.0-0.3) | 0.1 (0.0-0.2) |
| **M1** | *133.1* (91.1-269.0) | 47.3 (29.5-74.6) | 28.8 (13.1-47.4) |
| **M2** | 9.9 (6.9-12.1) | 2.1 (0.9-3.3) | 0.9 (0.3-1.6) |
| **D** | 50.5 (42.7-57.9) | 27.5 (21.6-34.7) | 19.0 (14.0-25.9) |
| **C** | 3.1 (2.2-4.3) | 0.6 (0.3-0.9) | 0.2 (0.1-0.4) |
| Values in *italics*: for the genotoxicity test was considered the undiluted sample. |

**Table S6**. Genotoxicity of aqueous extracts of biogenic wastes in *Allium cepa* bulbs, expressed as distribution of each type of chromosomal aberration within the three main categories: direct DNA damage (DDD), mitotic spindle defect (MSD, and genic amplification (GA).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **DDD** | **MSD** | **GA** |
| **Sample** | **Dose** | **Frag** | **Ring** | **SticC** | **Brid** | **Lagg** | **Binuc** | **PolS** | **MultiP** | **C-mit** | **Buds** |
| S1 | EC50 | 4 | 1 | 59 | 16 | 62 | 0 | 10 | 16 | 1 | 31 |
| EC20 | 2 | 0 | 20 | 0 | 31 | 0 | 9 | 43 | 3 | 40 |
| EC10 | 3 | 1 | 15 | 1 | 32 | 0 | 6 | 14 | 0 | 19 |
| Neg control | 3 | 0 | 33 | 1 | 19 | 0 | 4 | 7 | 0 | 17 |
| S2 | EC50 | 1 | 1 | 7 | 0 | 25 | 0 | 3 | 27 | 1 | 30 |
| EC20 | 0 | 0 | 5 | 1 | 24 | 0 | 5 | 23 | 0 | 37 |
| EC10 | 0 | 0 | 6 | 0 | 31 | 0 | 4 | 16 | 5 | 38 |
| Neg control | 1 | 0 | 7 | 0 | 18 | 0 | 1 | 17 | 0 | 27 |
| S3 | EC50 | 3 | 2 | 8 | 2 | 33 | 0 | 14 | 34 | 16 | 21 |
| EC20 | 1 | 0 | 16 | 0 | 40 | 0 | 4 | 24 | 1 | 31 |
| EC10 | 2 | 0 | 10 | 1 | 40 | 0 | 0 | 24 | 2 | 16 |
| Neg control | 2 | 0 | 20 | 1 | 13 | 0 | 2 | 6 | 1 | 13 |
| S4 | EC50 | 1 | 1 | 84 | 1 | 51 | 0 | 10 | 9 | 1 | 75 |
| EC20 | 2 | 3 | 51 | 1 | 50 | 0 | 10 | 24 | 6 | 43 |
| EC10 | 0 | 1 | 26 | 1 | 46 | 0 | 1 | 26 | 1 | 49 |
| Neg control | 1 | 3 | 3 | 1 | 28 | 0 | 4 | 12 | 2 | 20 |
| L | EC50 | 3 | 3 | 136 | 7 | 39 | 0 | 8 | 16 | 0 | 72 |
| EC20 | 5 | 3 | 3 | 3 | 64 | 0 | 4 | 22 | 3 | 34 |
| EC10 | 0 | 3 | 23 | 1 | 43 | 0 | 2 | 30 | 5 | 53 |
| Neg control | 1 | 3 | 3 | 1 | 28 | 0 | 4 | 12 | 2 | 20 |
| M1 | EC50 | 0 | 2 | 30 | 2 | 22 | 0 | 14 | 27 | 1 | 25 |
| EC20 | 1 | 3 | 23 | 3 | 39 | 0 | 7 | 40 | 6 | 25 |
| EC10 | 0 | 1 | 18 | 1 | 19 | 0 | 4 | 35 | 3 | 37 |
| Neg control | 1 | 0 | 6 | 0 | 34 | 0 | 6 | 11 | 1 | 6 |
| M2 | EC50 | 1 | 2 | 20 | 0 | 39 | 0 | 21 | 54 | 36 | 25 |
| EC20 | 2 | 2 | 27 | 0 | 31 | 0 | 14 | 35 | 1 | 35 |
| EC10 | 2 | 3 | 25 | 3 | 36 | 0 | 3 | 12 | 0 | 37 |
| Neg control | 2 | 1 | 1 | 1 | 32 | 0 | 0 | 6 | 7 | 6 |
| D | EC50 | 4 | 1 | 8 | 1 | 28 | 0 | 18 | 53 | 11 | 16 |
| EC20 | 1 | 0 | 5 | 1 | 31 | 0 | 6 | 13 | 1 | 33 |
| EC10 | 1 | 2 | 2 | 0 | 18 | 0 | 5 | 22 | 5 | 22 |
| Neg control | 0 | 1 | 6 | 0 | 27 | 0 | 3 | 15 | 1 | 11 |
| C | EC50 | 1 | 2 | 37 | 3 | 36 | 0 | 5 | 29 | 2 | 23 |
| EC20 | 0 | 3 | 26 | 3 | 32 | 0 | 11 | 47 | 1 | 41 |
| EC10 | 1 | 2 | 11 | 4 | 39 | 0 | 3 | 38 | 0 | 30 |
| Neg control | 0 | 1 | 3 | 0 | 27 | 0 | 4 | 13 | 1 | 3 |
| Frag: fragments; SticC: sticky chains; Brid: bridges; Lagg: laggards; Binuc: binucleation; PolS: polar slip; MultiP: multipoles; C-mit: c-mitosis |

**Table S7**. Results of Ames test using *Salmonella typhimurium* TA98 strain treated with increasing doses (0.01, 0.1, 1, 10 mgeq/plate) of sample extracts without and with metabolic activation (± S9 mix), expressed as mean revertant number ± SD.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **S1** | **S2** | **S3** | **S4** | **L** | **M1** | **M2** | **D** | **C** |
| Dose(mgeq/plate) | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| **0.01** | 22.0 ± 2.8 | 44.5 ± 0.7 | 22.0 ± 9.9 | 38.5 ± 4.9 | 19.0 ± 1.4 | 40.5 ± 0.7 | 21.5 ± 0.7 | 53.5 ± 9.2 | 23.5 ± 2.1 | 60.5 ± 2.1 | 24.5 ± 9.2 | 63.0 ± 2.8 | 22.0 ± 1.4 | 56.0 ± 1.4 | 21.5 ± 4.9 | 56.0 ± 2.8 | 27.5 ± 10.6 | 42.5 ± 0.7 |
| **0.1** | 30.0 ± 1.4 | 39.0 ± 9.9 | 28.5 ± 0.7 | 43.0 ± 4.2 | 28.0 ± 2.8 | 41.0 ± 5.7 | 27.0 ± 7.1 | 60.5 ± 10.6 | 20.5 ± 2.1 | 45.5 ± 9.2 | 21.5 ± 13.4 | 65.0 ± 2.8 | 27.0 ± 5.7 | 59.0 ± 1.4 | 26.0 ± 5.7 | 60.0 ± 1.4 | 26.0 ± 5.7 | 56.0 ± 1.4 |
| **1** | 29.5 ± 6.4 | 40.0 ± 7.1 | 33.5 ± 3.5 | 41.0 ± 12.7 | 22.0 ± 5.7 | 41.3 ± 5.1 | 26.5 ± 2.1 | 59.0 ± 4.2 | 28.5 ± 4.9 | 53.0 ± 2.8 | 24.0 ± 4.2 | 59.0 ± 4.2 | 27.0 ± 4.2 | 51.0 ± 8.5 | 30.0 ± 14.1 | 56.5 ± 7.8 | 23.5 ± 0.7 | 48.5 ± 2.1 |
| **10** | 20.5 ± 3.5 | 47.5 ± 10.6 | 23.5 ± 6.4 | 36.0 ± 1.4 | 18.5 ± 2.1 | 45.0 ± 8.5 | 23.5 ± 7.8 | 54.5 ± 7.8 | 25.5 ± 0.7 | 38.5 ± 10.6 | 24.5 ± 0.7 | 55.5 ± 10.6 | 27.5 ± 4.9 | 53.0 ± 9.9 | 27.0 ± 8.5 | 52.0 ± 1.4 | 18.5 ± 0.7 | 52.5 ± 3.5 |
| **Negative** | 25.5 ± 3.7 | 36.0 ± 8.1 | 24.5 ± 2.9 | 43.0 ± 8.6 | 25.5 ± 3.7 | 36.0 ± 8.1 | 25.5 ± 3.7 | 53.5 ± 4.7 | 25.5 ± 3.7 | 53.5 ± 4.7 | 25.5 ± 3.7 | 53.5 ± 4.7 | 25.5 ± 3.7 | 53.5 ± 4.7 | 25.5 ± 3.7 | 53.5 ± 4.7 | 25.5 ± 3.7 | 53.5 ± 4.7 |
| Positive controls TA98 -S9 > 1000 revertant/plate (TA98 –S9: 100 µL 2NF); TA98 +S9 > 1000 revertant/plate (200 µL 2AF) |

**Table S8**. Results of Ames test using *Salmonella typhimurium* TA98 strain treated with increasing doses (0.01, 0.1, 1, 10 mgeq/plate) of sample extracts without and with metabolic activation (± S9 mix), expressed as mutagenicity ratio.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **S1** | **S2** | **S3** | **S4** | **L** | **M1** | **M2** | **D** | **C** |
| Dose(mgeq/plate) | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| **0.01** | 0.9 | 1.2 | 0.9 | 0.9 | 0.7 | 1.1 | 1.0 | 0.9 | 0.9 | 1.1 | 1.0 | 1.2 | 0.9 | 1.0 | 0.8 | 1.0 | 1.1 | 0.8 |
| **0.1** | 1.2 | 1.1 | 1.2 | 1.0 | 1.1 | 1.1 | 1.2 | 1.1 | 0.8 | 0.9 | 0.8 | 1.2 | 1.1 | 1.1 | 1.0 | 1.1 | 1.0 | 1.0 |
| **1** | 1.2 | 1.1 | 1.4 | 1.0 | 0.9 | 1.1 | 1.1 | 1.0 | 1.1 | 1.0 | 0.9 | 1.1 | 1.1 | 1.0 | 1.2 | 1.1 | 0.9 | 0.9 |
| **10** | 0.8 | 1.3 | 1.0 | 0.8 | 0.7 | 1.3 | 1.1 | 1.1 | 1.0 | 0.7 | 1.0 | 1.0 | 1.1 | 1.0 | 1.1 | 1.0 | 0.7 | 1.0 |
| Positive controls TA98 -S9 > 1000 revertant/plate (TA98 –S9: 100 µL 2NF); TA98 +S9 > 1000 revertant/plate (200 µL 2AF) |

**Table S9**. Results of Ames test using *Salmonella typhimurium* TA100 strain treated with increasing doses (0.01, 0.1, 1, 10 mgeq/plate) of sample extracts without and with metabolic activation (± S9 mix), expressed as mean revertant number ± SD.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **S1** | **S2** | **S3** | **S4** | **L** | **M1** | **M2** | **D** | **C** |
| Dose(mgeq/plate) | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| **0.01** | 127.0 ± 17.0 | 144.5 ± 6.4 | 144.0 ± 8.5 | 144.0 ± 26.9 | 149.0 ± 1.4 | 158.5 ± 16.3 | 135.0 ± 7.1 | 163.5 ± 6.4 | 125.5 ± 3.5 | 154.0 ± 4.2 | 160.0 ± 5.7 | 138.5 ± 0.7 | 134.5 ± 2.1 | 143.5 ± 31.8 | 145.5 ± 23.3 | 134.5 ± 3.5 | 176.0 ± 0.0 | 148.5 ± 12.0 |
| **0.1** | 136.0 ± 1.4 | 150.5 ± 34.6 | 174.0 ± 1.4 | 122.5 ± 12.0 | 135.0 ± 18.4 | 165.5 ± 12.0 | 143.5 ± 7.8 | 152.0 ± 2.8 | 142.5 ± 6.4 | 153.0 ± 1.4 | 164.5 ± 3.5 | 136.0 ± 18.4 | 160.5 ± 7.8 | 147.5 ± 13.4 | 161.5 ± 0.7 | 141.0 ± 2.8 | 183.5 ± 10.6 | 131.5 ± 2.1 |
| **1** | 132.0 ± 9.9 | 169.5 ± 7.8 | 210.0 ± 12.7 | 148.0 ± 56.6 | 141.5 ± 17.7 | 170.5 ± 0.7 | 133.0 ± 14.1 | 139.5 ± 9.2 | 150.5 ± 12.0 | 137.0 ± 0.0 | 162.5 ± 6.4 | 122.5 ± 14.8 | 150.0 ± 2.8 | 142.0 ± 11.3 | 151.0 ± 7.1 | 140.0 ± 4.2 | 159.0 ± 2.8 | 147.0 ± 21.2 |
| **10** | 135.0 ± 29.7 | 173.5 ± 27.6 | 225.0 ± 106.1 | 126.0 ± 0.0 | 144.5 ± 14.8 | 171.0 ± 22.6 | 150.0 ± 5.7 | 155.0 ± 33.9 | 133.0 ± 8.5 | 156.5 ± 0.7 | 141.5 ± 24.7 | 162.5 ± 24.7 | 133.5 ± 10.6 | 130.0 ± 15.6 | 126.5 ± 30.4 | 140.0 ± 12.7 | 149.0 ± 19.8 | 160.0 ± 7.1 |
| **Negative** | 142.3 ± 25.5 | 144.5 ± 41.7 | 142.3 ± 25.5 | 144.5 ± 41.7 | 142.3 ± 25.5 | 144.5 ± 41.7 | 142.3 ± 25.5 | 144.5 ± 41.7 | 142.3 ± 25.5 | 144.5 ± 41.7 | 142.3 ± 25.5 | 144.5 ± 41.7 | 142.3 ± 25.5 | 144.5 ± 41.7 | 142.3 ± 25.5 | 144.5 ± 41.7 | 142.3 ± 25.5 | 144.5 ± 41.7 |
| Positive controls: TA100 –S9 >1000 revertant/plate (100 µL NaN3); TA100 +S9 >1000 revertant/plate (200 µL 2AF) |

**Table S10**. Results of Ames test using *Salmonella typhimurium* TA100 strain treated with increasing doses (0.01, 0.1, 1, 10 mgeq/plate) of sample extracts without and with metabolic activation (± S9 mix), expressed as mutagenicity ratio.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **S1** | **S2** | **S3** | **S4** | **L** | **M1** | **M2** | **D** | **C** |
| Dose(mgeq/plate) | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| **0.01** | 0.9 | 1.0 | 1.0 | 1.0 | 1.0 | 1.1 | 1.0 | 1.1 | 0.9 | 1.1 | 1.1 | 1.0 | 0.9 | 1.0 | 1.0 | 0.9 | 1.2 | 1.0 |
| **0.1** | 1.0 | 1.0 | 1.2 | 0.8 | 0.9 | 1.1 | 1.0 | 1.1 | 1.0 | 1.1 | 1.2 | 0.9 | 1.1 | 1.0 | 1.1 | 1.0 | 1.3 | 0.9 |
| **1** | 0.9 | 1.2 | 1.5 | 1.0 | 1.0 | 1.2 | 1.1 | 1.0 | 1.1 | 0.9 | 1.1 | 0.8 | 1.1 | 1.0 | 1.1 | 1.0 | 1.1 | 1.0 |
| **10** | 0.9 | 1.2 | 1.6 | 0.9 | 1.0 | 1.2 | 1.6 | 1.0 | 0.9 | 1.1 | 1.0 | 1.1 | 0.9 | 0.9 | 0.9 | 1.0 | 1.0 | 1.1 |
| Positive controls: TA100 –S9 >1000 revertant/plate (100 µL NaN3); TA100 +S9 >1000 revertant/plate (200 µL 2AF) |

**Table S11**. Results of toxicity test on HepG2, expressed as mean cell number ± SD.

|  |  |
| --- | --- |
|  | **Cell number** |
| **Sample** | **50 mg/mL** | **5 mg/mL** | **0.5 mg/mL** | **0.05 mg/mL** |
| S1 | 11,158.2±1,022.7 | 14,513.0±937.5 | 16,803.1±3,153.4 | 10,575.7±823.9 |
| S2 | 13,428.2±1,051.1 | 12,986.3±2,698.9 | 15,778.6±454.5 | 11,399.3±1,079.6 |
| S3 | 9,330.2±4,289.8 | 9,732.1±369.3 | 11,700.6±1,676.1 | 9,350.3±2,102.3 |
| S4 | 16,481.6±2,187.5 | 15,216.1±1,136.4 | 18,550.8±113.6 | 12,504.1±937.5 |
| L | 15,858.9±738.6 | 15,959.4±1,789.8 | 9,551.2±3,295.5 | 12,544.3±198.9 |
| M1 | 11,961.8±2,215.9 | 14,472.8±1,562.5 | 11,821.1±85.2 | 12,182.7±937.5 |
| M2 | 10,776.5±198.9 | 8,305.7±227.3 | 11,238.6±284.1 | 11,178.3±255.7 |
| D | 13,910.3±3,664.8 | 11,158.2±1,477.3 | 12,765.3±3,693.2 | 12,263.1±2,073.9 |
| C | 9,631.5±795.5 | 16,180.3±2,386.4 | 11,801.1±2,840.9 | 12,624.7±198.9 |
| Negative control (cells treated with serum free DMEM) | 10,555.6±3,781.0 |
| Positive control (cells treated with Triton X-100 1%) | 350.7±511.7 |