**Electronic Supplementary Information for:**

# Exploring the Anti-Chagas Activity of *Zanthoxylum chiloperone*'s Seedlings Through Metabolomics and Protein–Ligand Docking

**Table S1.** Protocols for seedlings production.

| **Protocol 1: *Z. chiloperone* seeds sown in the month of October 2016** | | | |
|---|---|---|---|
| **Substrate** | **Number of seeds sown** | **Number of germinated seeds** | **Germination (%)** |
| Bovine manure | 200 | 9 | 4.5 |
| Mulch | 200 | 4 | 2% |
| Commercial fertilizer | 200 | 10 | 5 % |
| Sawdust | 200 | 3 | 1.5 % |
| Rice husk | 200 | 0 | 0 % |
| Washed sand | 200 | 13 | 6.5 % |
| **Total** | **1200** | **39** | **3.25 %** |
| **Protocol 2: *Z. chiloperone* seeds sown in the month of January 2017** | | | |
| **Substrate** | **Number of seeds sown** | **Number of germinated seeds** | **Germination (%)** |
| Washed sand | 200 | 13 | 6.5 % |
| Washed sand | 200 | 0 | 0 % |
| Manure | 200 | 2 | 1 % |
| Manure | 200 | 5 | 2.5 % |
| Commercial fertilizer | 200 | 1 | 0.5 % |
| Commercial fertilizer and manure (50:50) | 200 | 2 | 1 % |

| | | | |
|---|---|---|---|
| **Total** | **1200** | **23** | **1.9 %** |
| **Protocol 3: *Z. chiloperone* seeds sown in the month of January 2017** | | | |
| **Substrate** | **Number of seeds sown** | **Number of germinated seeds** | **Germination (%)** |
| Washed sand | 300 | 2 | 0.6 % |
| Washed sand | 300 | 6 | 2 % |
| **Total** | **600** | **8** | **1.3 %** |

Note: The difference between the three protocols is the number of seeds planted and the planting season.

**Table S2.** Cytotoxicity evaluation of 12,18 and 24-month-old (ZC12, ZC18 and ZC24 respectively) *Z. chiloperone* seedling extracts.

| **Percentage ± SD of mouse peritoneal macrophage lysis.** | | | | |
|---|---|---|---|---|
| **Conc. Extract 100 µg/mL** | **Conc. Extract 50 µg/mL** | **Conc. Extract 25 µg/mL** | **Control Pos.*** | **Control Neg.*** |
| **Cytotoxicity evaluation of extracts of 12-month-old *Z .chiloperone* seedlings (ZC12)** | | | | |
| Alive* : 97± 1 | A*: 100 | A*:100 | A*: 0 | A*: 100 |
| Dead*3 ± 1 | D*: 0 | D*: 0 | D*:100 | D*: 0 |
| **Cytotoxicity evaluation of extracts of 18-month-old *Z .chiloperone* seedlings (ZC18)** | | | | |
| Alive* : 96± 1 | A*: 99 | A*:99 | A*: 1 | A*: 99 |
| Dead*4 ± 1 | D*:1 | D*: 1 | D*:99 | D*: 1 |
| **Cytotoxicity evaluation of extracts of 24-month-old *Z.chiloperone* seedlings (ZC24)** | | | | |
| Alive* : 97± 1 | A*: 99 | A*:99 | A*: 1 | A*: 98 |
| Dead*3 ± 1 | D*:1 | D*: 1 | D*:99 | D*: 2 |

Control Neg*:Macrophage+RPMI medium Control Pos*:Macrophage+DMSO A: alive D:dead

**Table S3.** Tentatively identified compounds derivation protocol scripting and their retention indices (RI) as examples of metabolites present in *Z. chiloperone* seedlings**.**

| Class | Compound | Experimental RI | Library RI |
|---|---|---|---|
| Alcohols | 1-Dodecanol[#] | 1567.3 | 1559.6 |
| | 1-Tridecanol[#] | 1664.3 | 1661.4 |
| | 1-Tetradecanol[#] | 1762.3 | 1760 |
| | 1-Pentadecanol[#] | 1860.7 | 1856.4 |
| | 1-Hexadecanol[#] | 1958.4 | 1955.1 |
| | 1-Heptadecanol[#] | 2050.7 | 2054 |
| | 1-Pentacosanol[#] | 2822.1 | 2826 |
| | 1-Triacontanol[#] | 3330.4 | 3333.9 |
| Aldehydes | Nonanal | 1107.6 | 1106 |
| | Benzaldehyde | 984.42 | 980 |
| | Decanal | 1221.7 | 1227 |
| | Syringaldehyde[#] | 1759.7 | N/A |
| n-Alkanes[*] | C10-30 | N/A | N/A |
| Amides | Neoherculin | 2277.0 | N/A |
| Amino acids | Serine[#] | 1355.7 | 1368 |
| | Proline[#] | 1292.9 | 1293.8 |
| | Alanine[#] | 1098.2 | 1095.3 |
| | Tyrosine[#] | 1934.3 | 1928.5 |
| Aromatic | *o*-Cymene | 1023.5 | 1023 |

| | | | |
|---|---|---|---|
| Carbohydrates | Fructose[#] | 1875.0 | 1875 |
| | Galactose[#] | 1889.3 | 1897.6 |
| | Glucose[#] | 1908.4 | 1926 |
| | Turanose[#] | 2788.4 | N/A |
| | Melibiose[#] | 3227.9 | N/A |
| | Maltose[#] | 3322.7 | N/A |
| | Ribitol[#] | 1714.4 | 1710 |
| Free Fatty Acids[*,#] | C6-C24 | N/A | N/A |
| Fatty Acid Methyl Esters[*] | C6-C24 | N/A | N/A |
| Fatty Acid Ethyl Esters | Ethyl octadecanoate | 2214.8 | 2207 |
| Isopropyl Esters | Isopropyl tetradecanoate | 1823.7 | 1823 |
| Ketones | 2-Heptadecanone | 1902.5 | 1902 |
| | Hexahydropseudoionone | 1404.4 | 1404 |
| | 6-Methyl-2-heptanone | 967.95 | 965 |
| Sterols | Campesterol[#] | 3686.2 | N/A |
| | β-Sitosterol[#] | 3775.7 | N/A |
| | Lupeol[#] | 3696.7 | N/A |
| | Stigmasterol[#] | 3709.8 | N/A |
| Terpenoids | D-Limonene | 1033.5 | 1035 |
| | Nerolidol[#] | 1677.3 | 1691 |
| | Neral | 1243.2 | 1244 |

| | | | |
|---|---|---|---|
| | Linalool[#] | 1230.6 | 1227.4 |
| | *trans*-β-Ionone | 1484.5 | 1486 |
| | Phytol[#] | 2147.9 | 2162.5 |
| | β-Eudesmol[#] | 1752.8 | 1760 |
| | γ-Terpinene | 1061.4 | 1061 |
| | Farnesyl acetone | 1913.1 | 1913 |
| | *trans*-β-Ocimene | 1047.5 | 1044 |
| | β-Caryophyllene oxide | 1593.6 | 1581 |
| | α-Ylangene | 1352.3 | 1352 |
| | β-Myrcene | 993.03 | 993 |
| Tocopherols | α-Tocopherol[#] | 3551.7 | N/A |
| | γ-Tocopherol[#] | 3391.1 | N/A |
| | δ-Tocopherol[#] | 3275.3 | N/A |

[*] Compounds presented as a range of incrementally increasing carbon number since the homologous series was detected.

[#] PubChem and the NIST Chemistry WebBook were consulted to obtain library retention indices. Retention indices of denoted compounds listed herein may refer to their O-methyloxime and/or TMS derivatives due to the sample derivatization protocol.

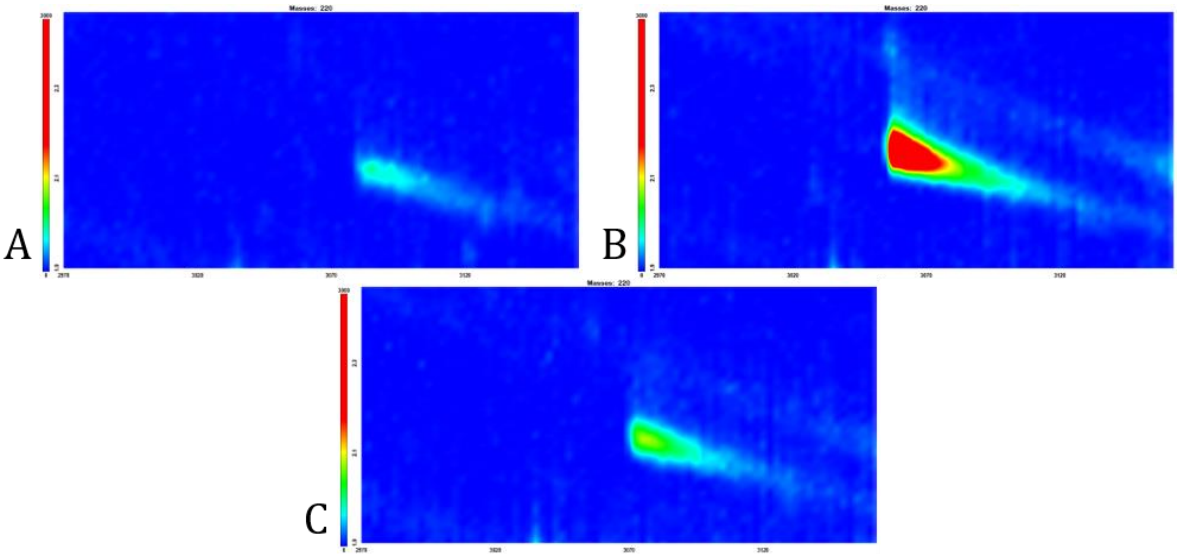


**Figure S1.** Chromatograms of canthin-6-one (**1**) using mass channel 220. A. ZC12, B. ZC18, C. ZC24. Horizontal axes represent first dimension retention time (s) and vertical axes represent second dimension retention time (2)**.**

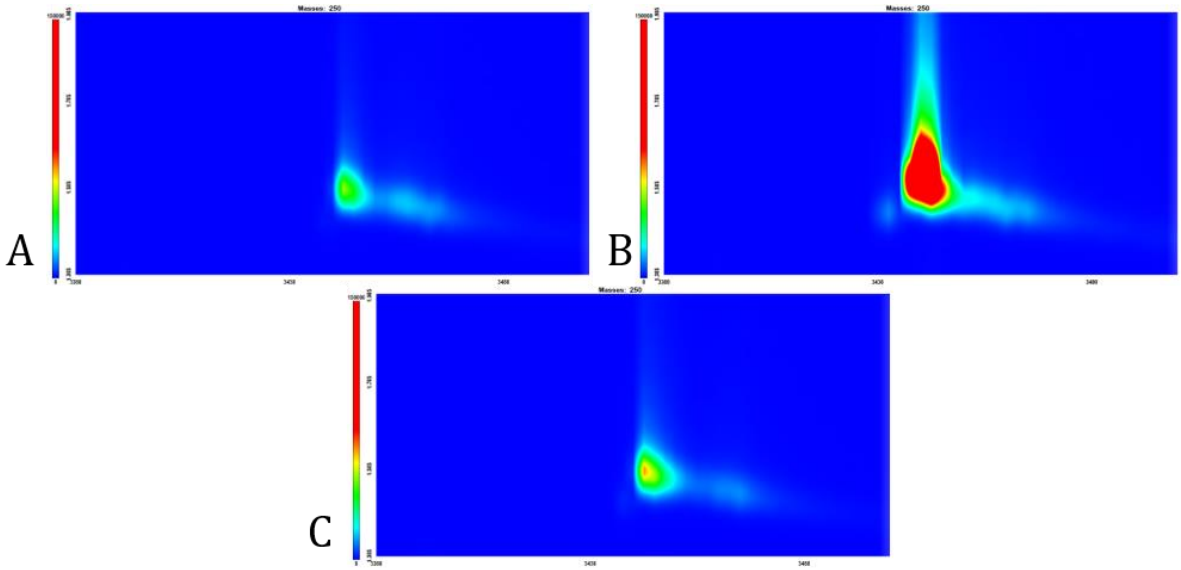


**Figure S2** Chromatograms of 5-methoxy-canthin-6-one (**2**) using mass channel 250. A. ZC12, B. ZC18, C. ZC24. Horizontal axes represent first dimension retention time (s) and vertical axes represent second dimension retention time (2)**.**

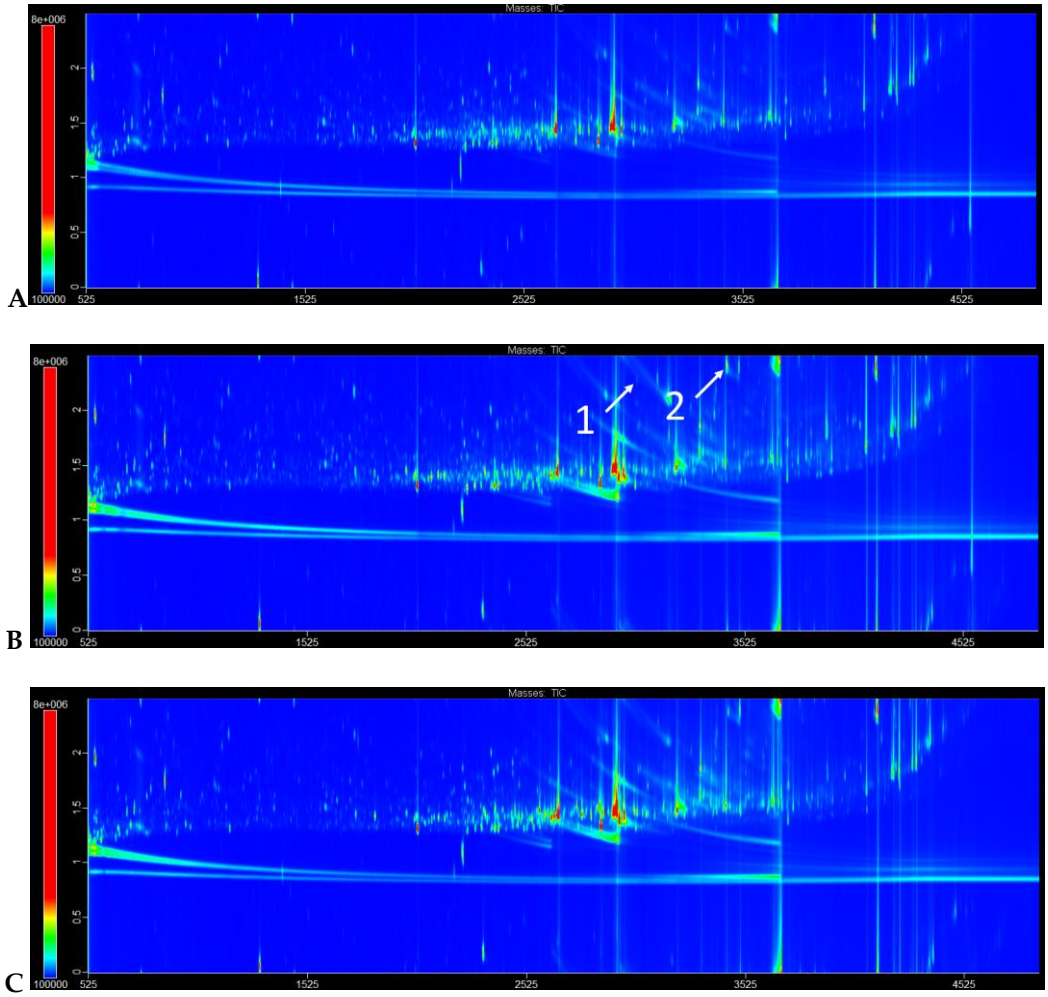


**Figure 3S**: GC×GC-TOFMS contour total ion chromatograms obtained from each sample using the same colour scale for z-axis. A. ZC12; B. ZC18; C. ZC24. The horizontal axes represent the first dimension retention times (s) and vertical axes represent the second dimension retention times (s). In B, alkaloids canthin-6-one (1) and 5-methoxy-canthin-6-one, 5-methoxy (2) have been labeled.

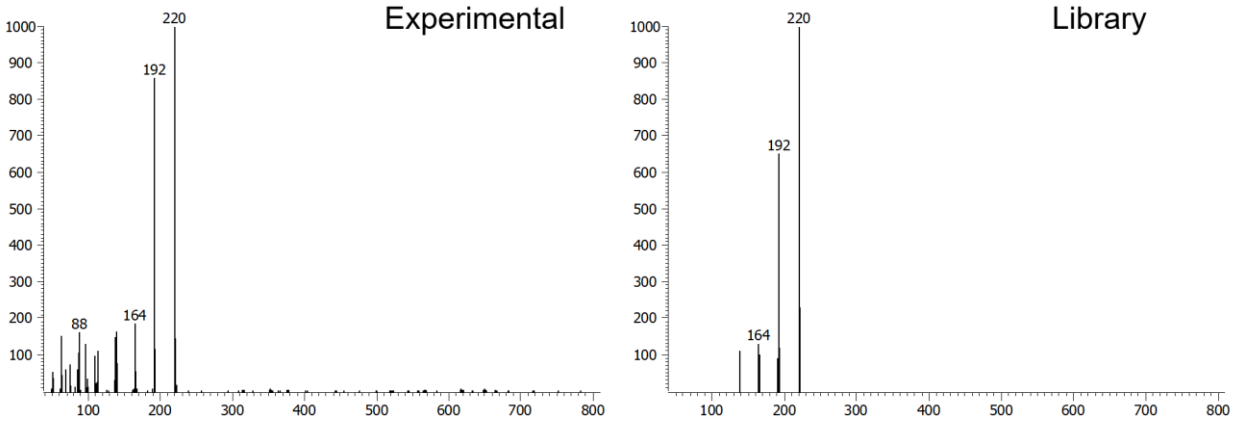


**Figure S4.** Experimental and library mass spectra of canthin-6-one (**1**). Vertical axis is relative abundance and horizontal axis is acquired *m/z* range.

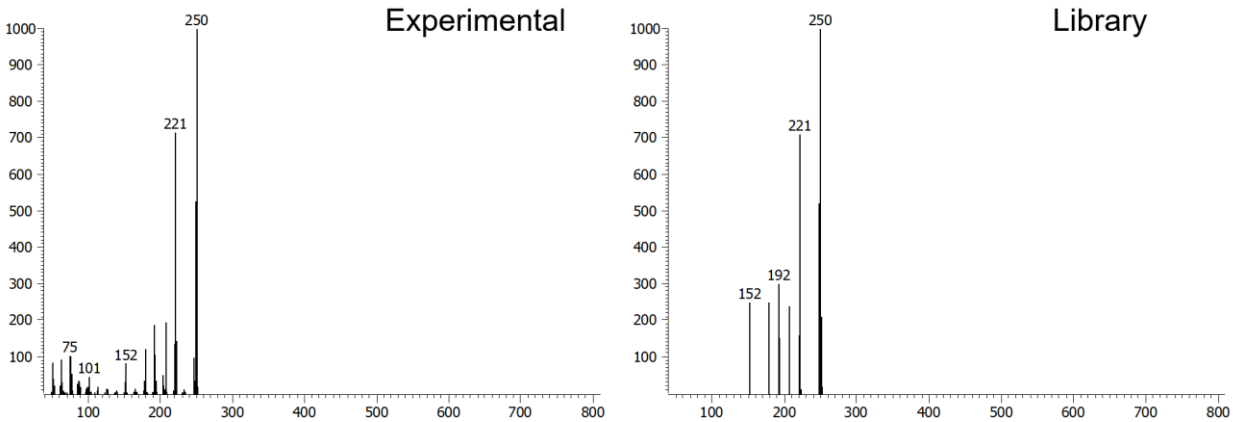


**Figure S5.** Experimental and library mass spectra of 5-methoxy-canthin-6-one (**2**). Vertical axis is relative abundance and horizontal axis is acquired *m/z* range.