## Description of novel bacterial taxa

### Description of *Candidatus* Caballeronia ardisicola sp. nov.

Caballeronia ardisicola [ar.di.si.i'co.la N.L. fem. n. Ardisia a plant genus; L. suff. -cola (from L. n. incola) a dweller, inhabitant; N.L. fem. n. ardisicola a dweller of Ardisia]. Not cultivated. Obtains energy by respiration. Detected as a symbiont in leaf galls of *Ardisia cornudentata* and *Ardisia mamillata* from the Ghent University Botanical Garden (Belgium) in 2018. Represented by the draft genome (GenBank GCA_940588625).

### Description of *Candidatus* Caballeronia hochstetteri sp. nov.

Caballeronia hochstetteri [hochstetteri, name based on the specific epithet of the host plant]. Not cultivated. Obtains energy by respiration. Detected as a symbiont in leaf galls of *Pavetta hochstetteri* from the Royal Botanic Garden Edinburgh (United Kingdom) in 2019. Represented by the draft genome (GenBank GCA_940590175).

### Description of *Candidatus* Paraburkholderia dryadicola sp. nov.

Paraburkholderia dryadicola [dry.a.di'co.la dryadum specific epithet of a host plant species; L. suff. -cola (from L. n. incola) a dweller, inhabitant; N.L. fem. n. dryadicola a dweller of (*Vangueria*) *dryadum*]. Not cultivated. Obtains energy by respiration. Detected as a symbiont in leaves of *Vangueria dryadum* and *Vangueria macrocalyx* from the Lowveld National Botanic Gardens (South Africa) in 2019. Represented by the draft genome (GenBank GCA_940590165).

### Description of *Candidatus* Paraburkholderia soutpansbergensis sp. nov.

Paraburkholderia soutpansbergensis [sout.pans.ber.gen.si's N.L. fem. adj. soutpansbergensis, name based on the specific epithet of the host plant]. Not cultivated. Rod-shaped. Obtains energy by respiration. Detected as a symbiont in leaves of *Vangueria soutpansbergensis* from the Lowveld National Botanic Gardens (South Africa) in 2019. Represented by the draft genome (GenBank GCA_940746715).

## Plant collection and extraction of kirkamide and streptol metabolites

Fresh leaves of *Fadogia homblei*, *Vangueria macrocalyx, V. pygmaea, V. dryadum, V. infausta, V. lasiantha, V. madagascariensis, V. randii and V. soutpansbergensis* were collected (Table SI-1) and stored at 5 °C. Leaves of *Psychotria kirkii* were collected from the Botanical Garden of Zurich, Switzerland and used as positive control for kirkamide and streptol-glucoside (Hsiao et al., 2019; Pinto-Carbó et al., 2016).

The extraction method of Sieber et al. (2015) was adjusted as follows: Dried leaf material (3 g of 5x5 mm pieces) was extracted with 80% distilled methanol (Merck Ltd) in a speed-extractor (Büchi E-91) at 50 °C and 100 bar. The speed-extractor was set to 5 cycles with a heating phase of 1 min each, a solvent holding phase of 9 min and a discharge phase of 5 min. The extracts were dried using a Büchi Genevac plus centrifugal evaporator (EZ-2 Plus) at 45 °C and set to low boiling point. After extraction and drying, all the extracts were stored at approximately 5 °C.

**Table SI-1**: Plant species analysed for the production of kirkamide, streptol and streptol glucoside. *Psychotria kirkii* was collected from the Botanical Garden of Zurich, Switzerland and used as positive control. PRU: H.G.W.J. Schweickerdt Herbarium, University of Pretoria, South Africa.

| **Plant species** | **Coordinates** | **Location of collection** | **Pant tissue collected** | **Voucher number** |
|---|---|---|---|---|
| ***Fadogia homblei*** | S 25° 34' 20,5''<br>E 28° 25' 58,4'' | Roodeplaat | Leaf tissue | PRU 128010 |
| ***Vangueria dryadum*** | S 25° 26' 35,5''<br>E 30° 58' 9,3'' | Lowveld National Botanical Gardens | Leaf tissue | PRU 128005 |
| ***Vangueria infausta*** | S 25° 26' 35,2''<br>E 30° 58' 16,4'' | Lowveld National Botanical Gardens | Leaf tissue | PRU 128003 |
| ***Vangueria lasiantha*** | S 25° 26' 36,5''<br>E 30° 58' 9,4'' | Lowveld National Botanical Gardens | Leaf tissue | PRU 128007 |
| ***Vangueria macrocalyx*** | S 25° 26' 34,6''<br>E 30° 58' 15,6'' | Lowveld National Botanical Gardens | Leaf tissue | PRU 128006 |
| ***Vangueria madagascariensis*** | S 25° 26' 34,7''<br>E 30° 58' 16'' | Lowveld National Botanical Gardens | Leaf tissue | PRU 128004 |
| ***Vangueria pygmaea*** | S 25° 44' 10,2''<br>E 28° 31' 59,3'' | Cullinan | Leaf tissue | PRU 126008 |
| ***Vangueria randii*** | S 25° 26' 41,4''<br>E 30° 58' 7,7'' | Lowveld National Botanical Gardens | Leaf tissue | PRU 128008 |
| ***Vangueria soutpansbergensis*** | S 25° 26' 34,5''<br>E 30° 58' 15,6'' | Lowveld National Botanical Gardens | Leaf tissue | PRU 128002 |

**Analytical Chemistry - Results**

Derivatised samples of the Rubiaceae species, *Psychotria kirkii* (positive control), *Fadogia homblei*, *Vangueria dryadum*, *V. infausta*, *V. lasiantha*, *V. macrocalyx*, *V. madagascariensis*, *V. pygmaea V. randii*, and *V. soutpansbergensis* were analysed for the presence of kirkamide by GC-MS. The ion fragments 73, 147, 282, 332, 415, 431, 490 and 505 *m/z* were used to confirm the presence of kirkamide in the crude derivatised extracts). All the fragments were present in the extract of *P. kirkii* at rt 27.830, confirming presence of kirkamide in this species, but these were not detected in any of the other plant species (Figures SI-1 to SI-10).

An initial UPLC-QToF-MS analysis showed the presence of streptol and streptol glucoside in *P. kirkii* and in some of the gousiekte causing species (Figure SI-11), but this needs further confirmation with plant material that will be collected during the spring season (Sept-Oct). The streptol (m/z = 175.0607, $[M-H]^-$, calculated MF = $C_7H_{12}O_5$ at 2.8 ppm) ion fragments 85, 109, 111, 121 and 175 *m/z* and the streptol glucoside (m/z = 337.1126, [M-H]-, calculated MF = $C_{13}H_{22}O_{10}$ at 4.2 ppm) ones of 112, 139, 175 and 337 *m/z* were used in the analyses. Streptol eluted at 0.716 min and streptol glucoside at 0.774 min.

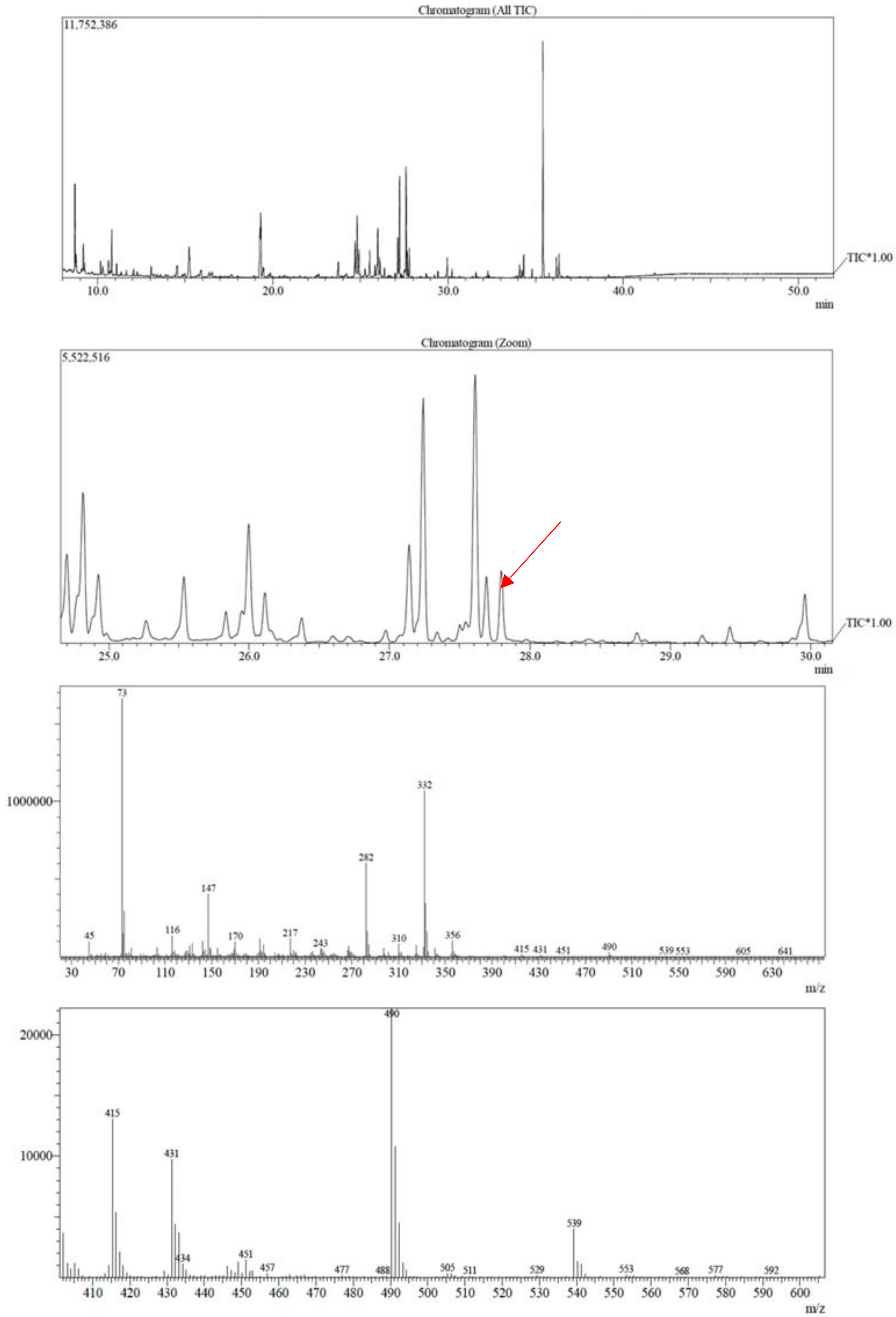


**Figure SI-1**: GC-MS results of *Psychotria kirkii* (endosymbiont = *Ca*. B. kirkii) showing the presence of kirkamide in the chromatograms and the mass spectra with ion fragments 73, 147, 282, 332, 415, 431, 490 and 505 *m/z* (middle panel) and the enlarged *m/z* region of kirkamide from 410 to 600 *m/z* (bottom panel).

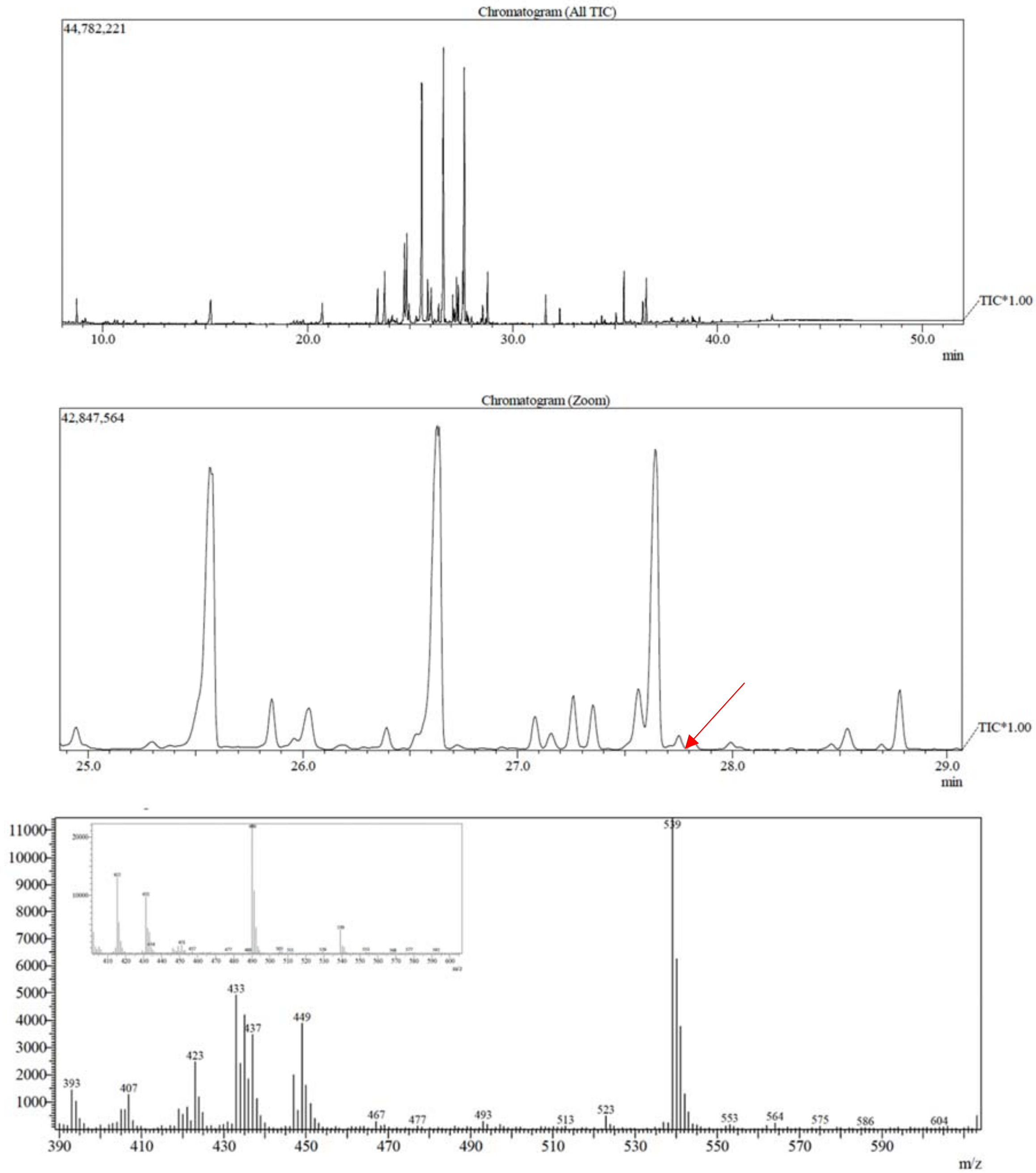


**Figure SI-2**: GC-MS result of *Fadogia homblei* (endosymbiont = *Paraburkholderia caledonica*) showing the absence of kirkamide in the chromatograms and the enlarged *m/z* region from 400 to 600 *m/z*. Top panel: total ion chromatogram. Middle panel: total ion chromatogram, focused around the expected retention time of kirkamide. Bottom panel: m/z spectrum at the expected retention time of kirkamide with the reference kirkamide spectrum inserted. The red arrow points to the expected retention time of kirkamide.

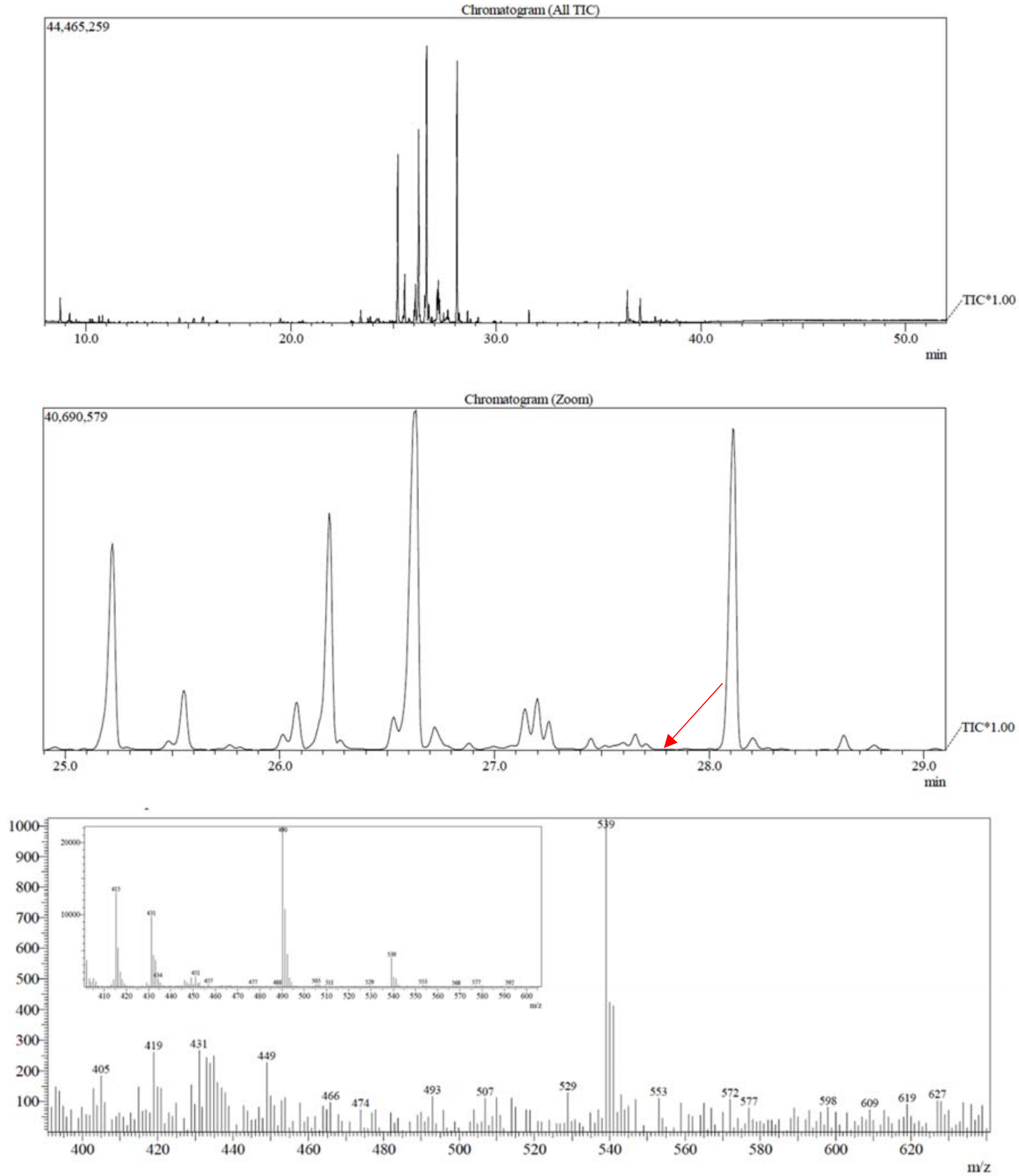


**Figure SI-3**: GC-MS result of *Vangueria dryadum* (endosymbiont = *Paraburkholderia dryadicola*) showing the absence of kirkamide in the chromatograms and the enlarged *m/z* region from 400 to 600 *m/z*. Top panel: total ion chromatogram. Middle panel: total ion chromatogram, focused around the expected retention time of kirkamide. Bottom panel: m/z spectrum at the expected retention time of kirkamide with the reference kirkamide spectrum inserted. The red arrow points to the expected retention time of kirkamide.

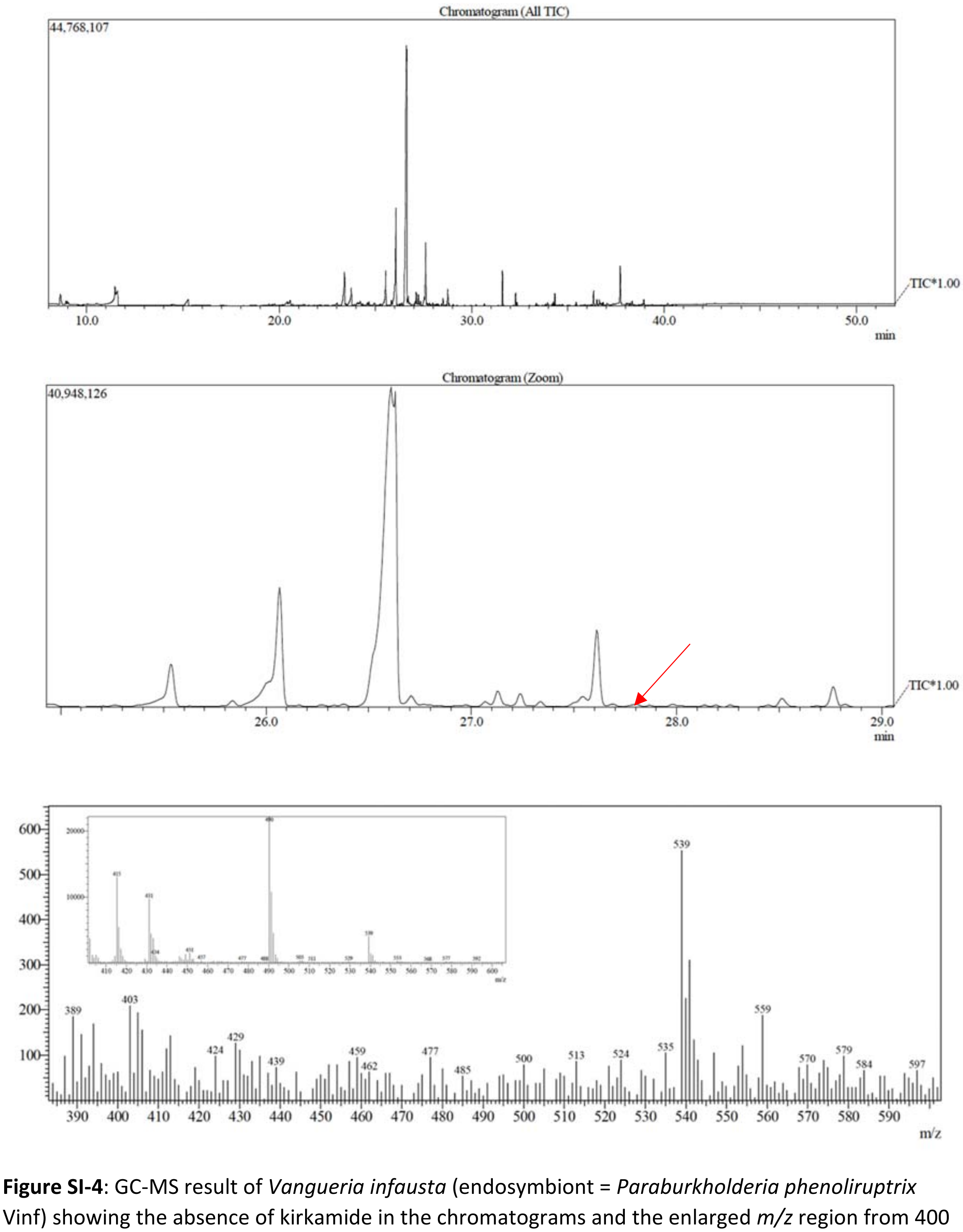


**Figure SI-4**: GC-MS result of *Vangueria infausta* (endosymbiont = *Paraburkholderia phenoliruptrix* Vinf) showing the absence of kirkamide in the chromatograms and the enlarged *m/z* region from 400 to 600 *m/z*. Top panel: total ion chromatogram. Middle panel: total ion chromatogram, focused around the expected retention time of kirkamide. Bottom panel: m/z spectrum at the expected retention time of kirkamide with the reference kirkamide spectrum inserted. The red arrow points to the expected retention time of kirkamide.

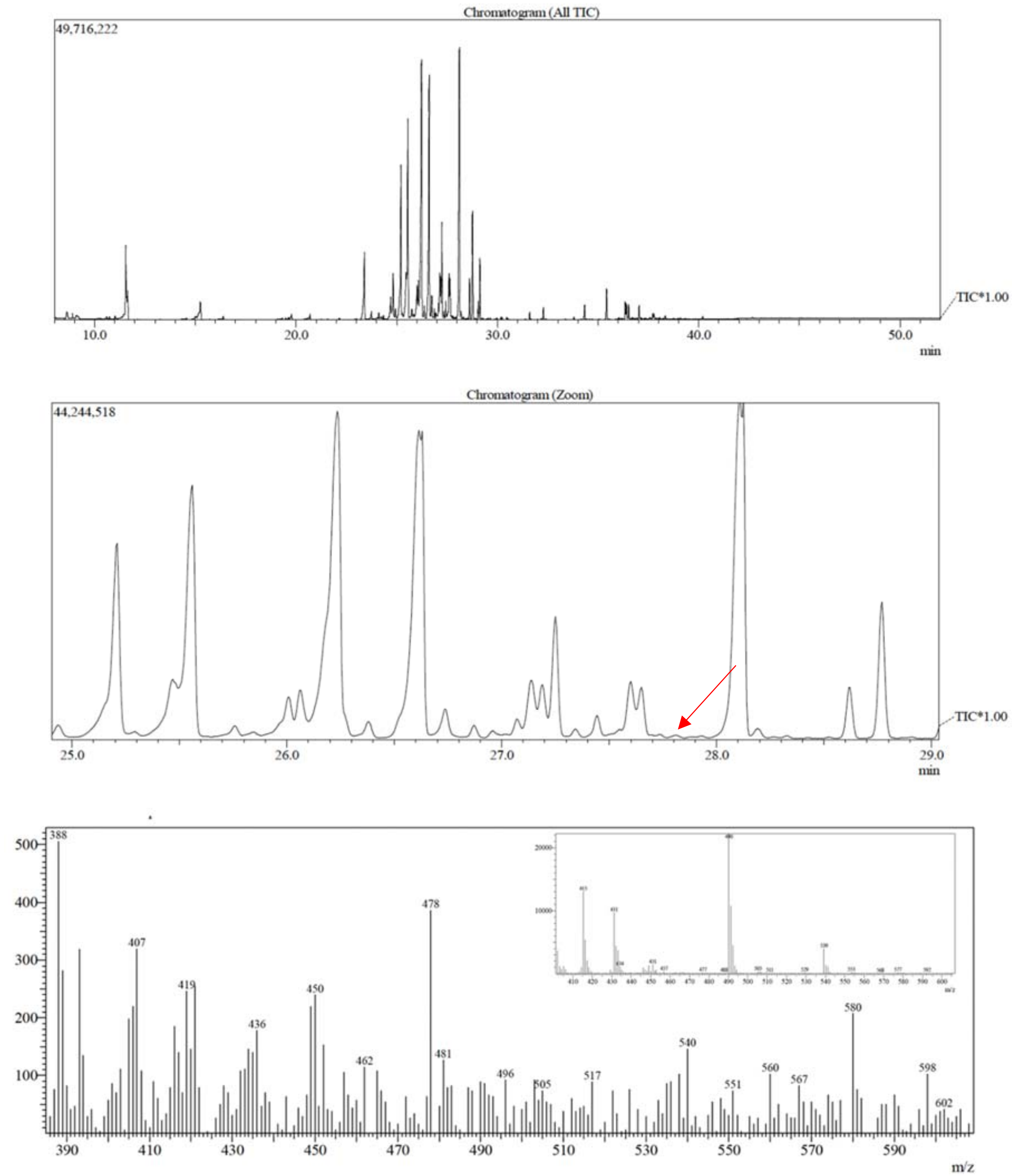


**Figure SI-5**: GC-MS result of *Vangueria lasianta* showing the absence of kirkamide in the chromatograms and the enlarged *m/z* region from 400 to 600 *m/z*. Top panel: total ion chromatogram. Middle panel: total ion chromatogram, focused around the expected retention time of kirkamide. Bottom panel: m/z spectrum at the expected retention time of kirkamide with the reference kirkamide spectrum inserted. The red arrow points to the expected retention time of kirkamide.

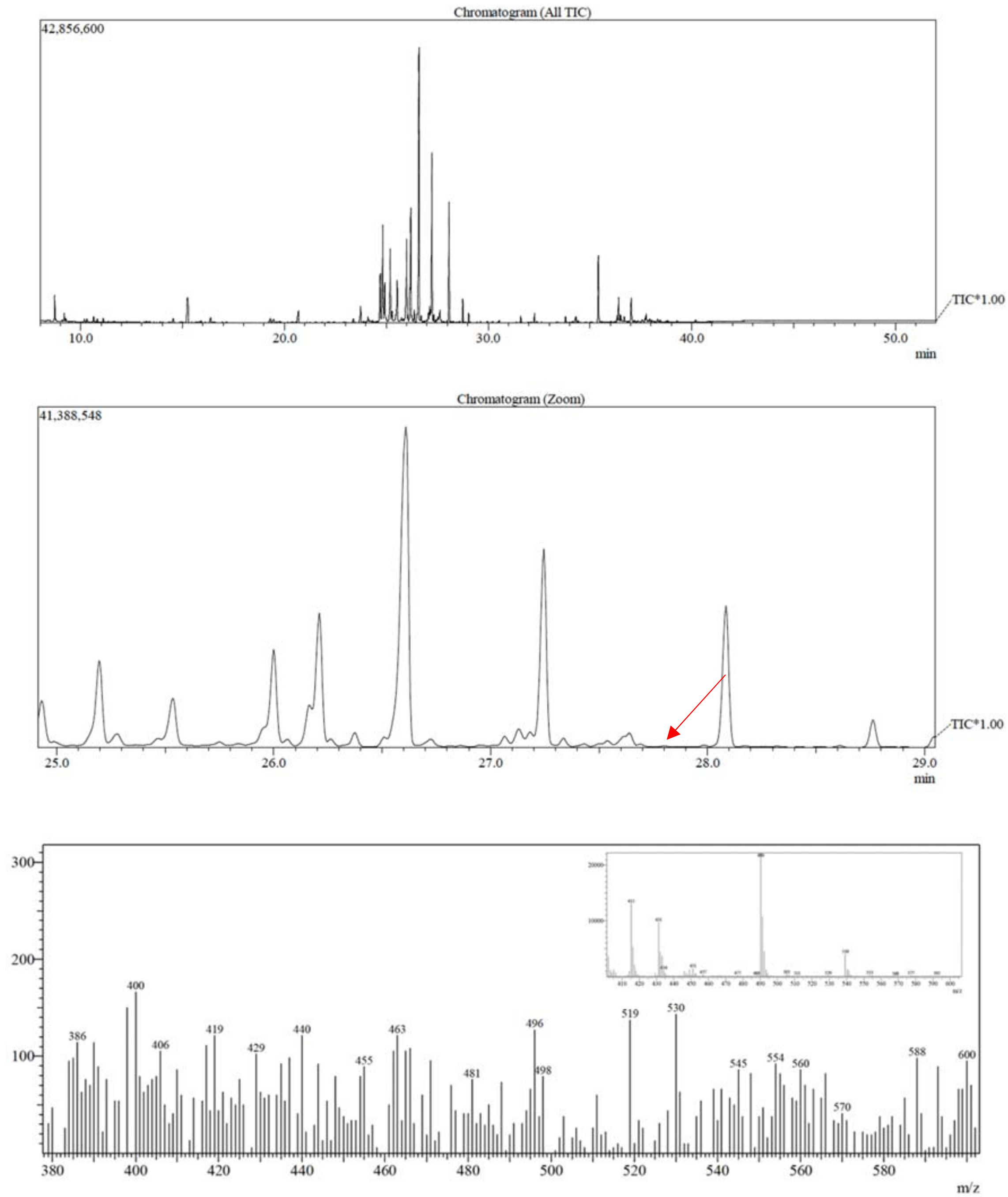


**Figure SI-6**: GC-MS result of *Vangueria macrocalyx* (endosymbiont = *Paraburkholderia dryadicola* Vmac) showing the absence of kirkamide in the chromatograms and the enlarged *m/z* region from 400 to 600 *m/z*. Top panel: total ion chromatogram. Middle panel: total ion chromatogram, focused around the expected retention time of kirkamide. Bottom panel: m/z spectrum at the expected retention time of kirkamide with the reference kirkamide spectrum inserted. The red arrow points to the expected retention time of kirkamide.

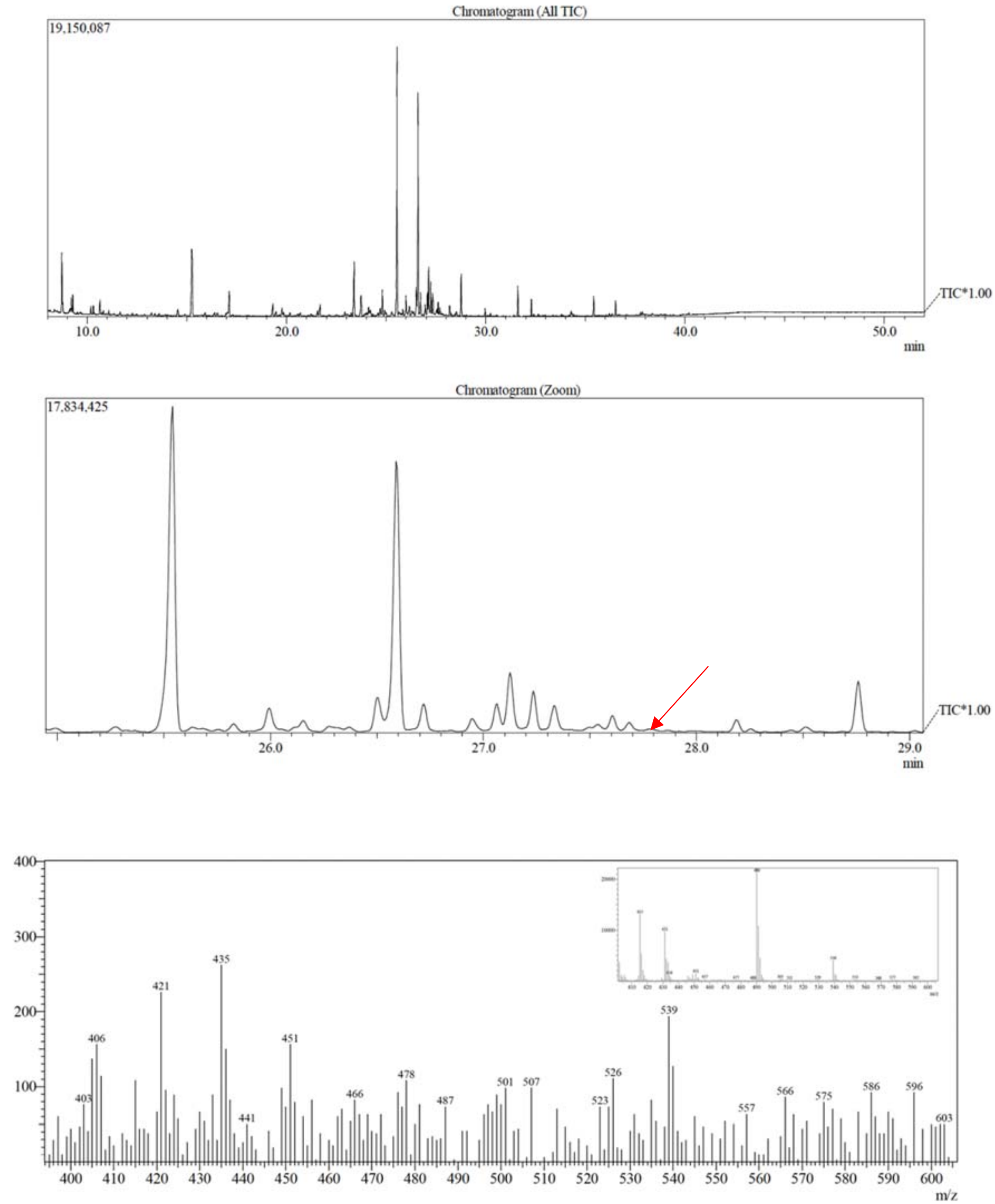


**Figure SI-7**: GC-MS result of *Vangueria madagascariensis* (endosymbiont = *Paraburkholderia phenoliruptrix* Vmad) showing the absence of kirkamide in the chromatograms and the enlarged *m/z* region from 400 to 600 *m/z*. Top panel: total ion chromatogram. Middle panel: total ion chromatogram, focused around the expected retention time of kirkamide. Bottom panel: m/z spectrum at the expected retention time of kirkamide with the reference kirkamide spectrum inserted The red arrow points to the expected retention time of kirkamide.

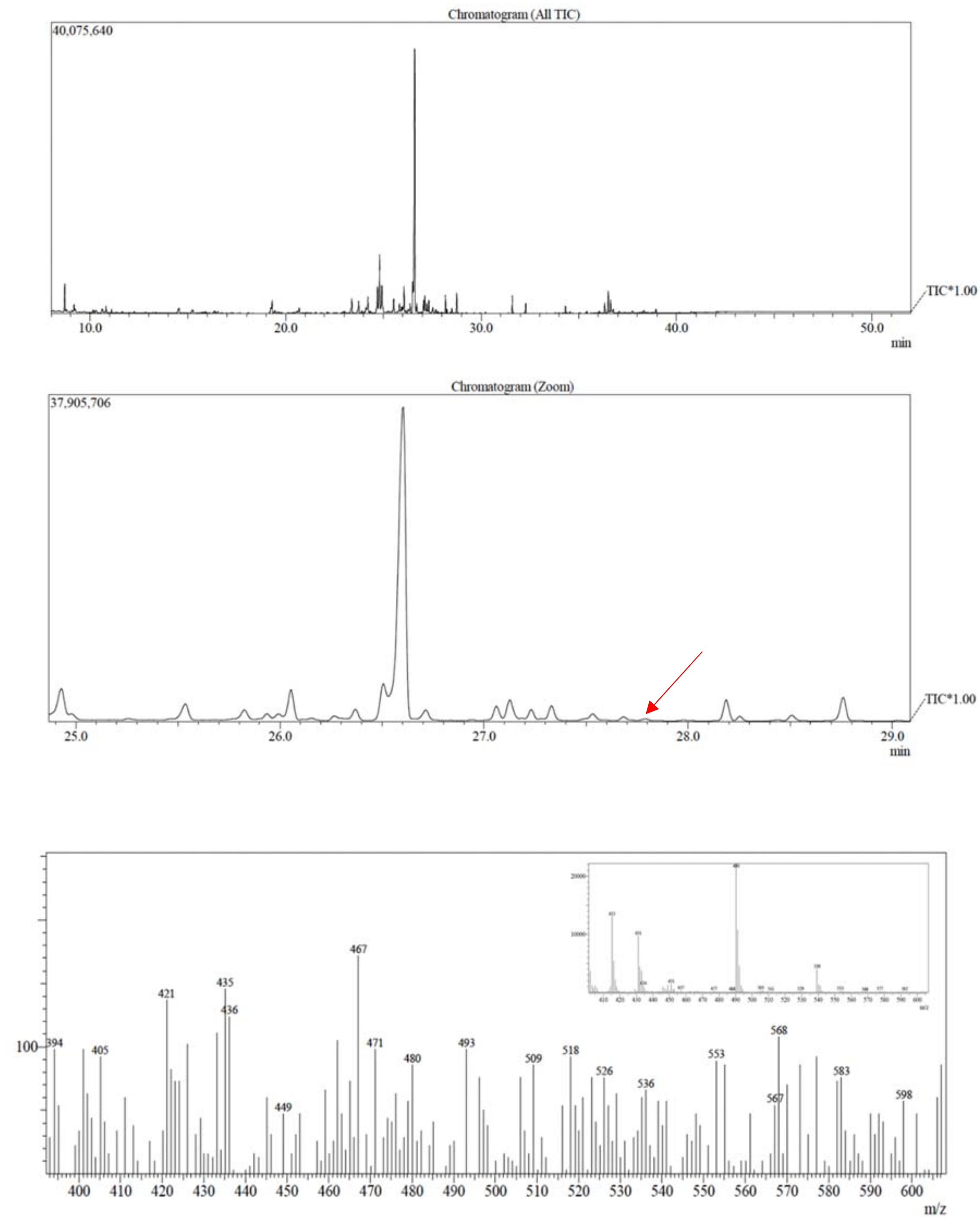


**Figure SI-8**: GC-MS result of *Vangueria pygmaea* (endosymbiont = *Paraburkholderia caledonica*) showing the absence of kirkamide in the chromatograms and the enlarged *m/z* region from 400 to 600 *m/z*. Top panel: total ion chromatogram. Middle panel: total ion chromatogram, focused around the expected retention time of kirkamide. Bottom panel: m/z spectrum at the expected retention time of kirkamide with the reference kirkamide spectrum inserted. The red arrow points to the expected retention time of kirkamide.

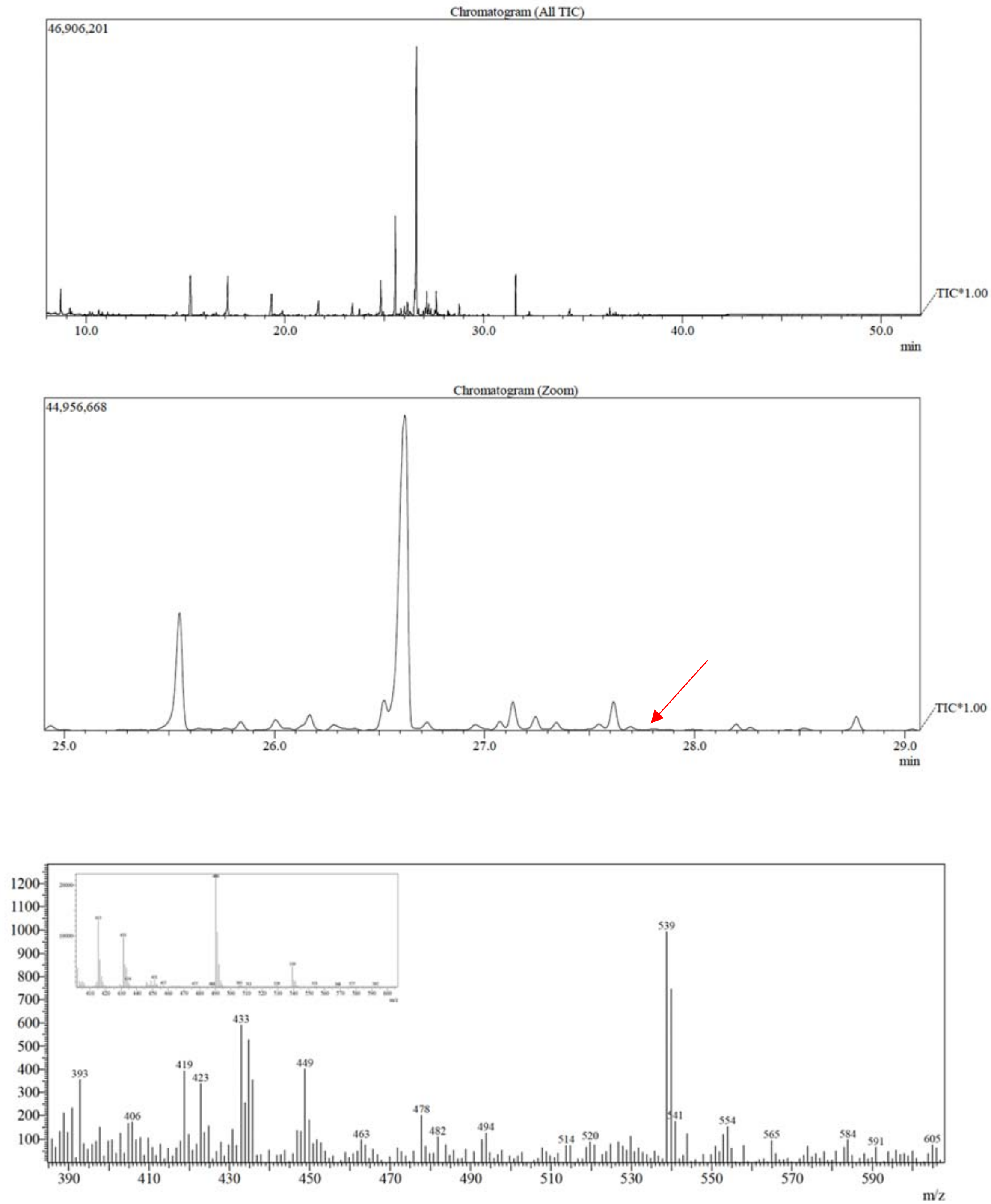


**Figure SI-9**: GC-MS result of *Vangueria randii* (endosymbiont = *Paraburkholderia phenoliruptrix* Vran) showing the absence of kirkamide in the chromatograms and the enlarged *m/z* region from 400 to 600 *m/z*. Top panel: total ion chromatogram. Middle panel: total ion chromatogram, focused around the expected retention time of kirkamide. Bottom panel: m/z spectrum at the expected retention time of kirkamide with the reference kirkamide spectrum inserted. The red arrow points to the expected retention time of kirkamide.

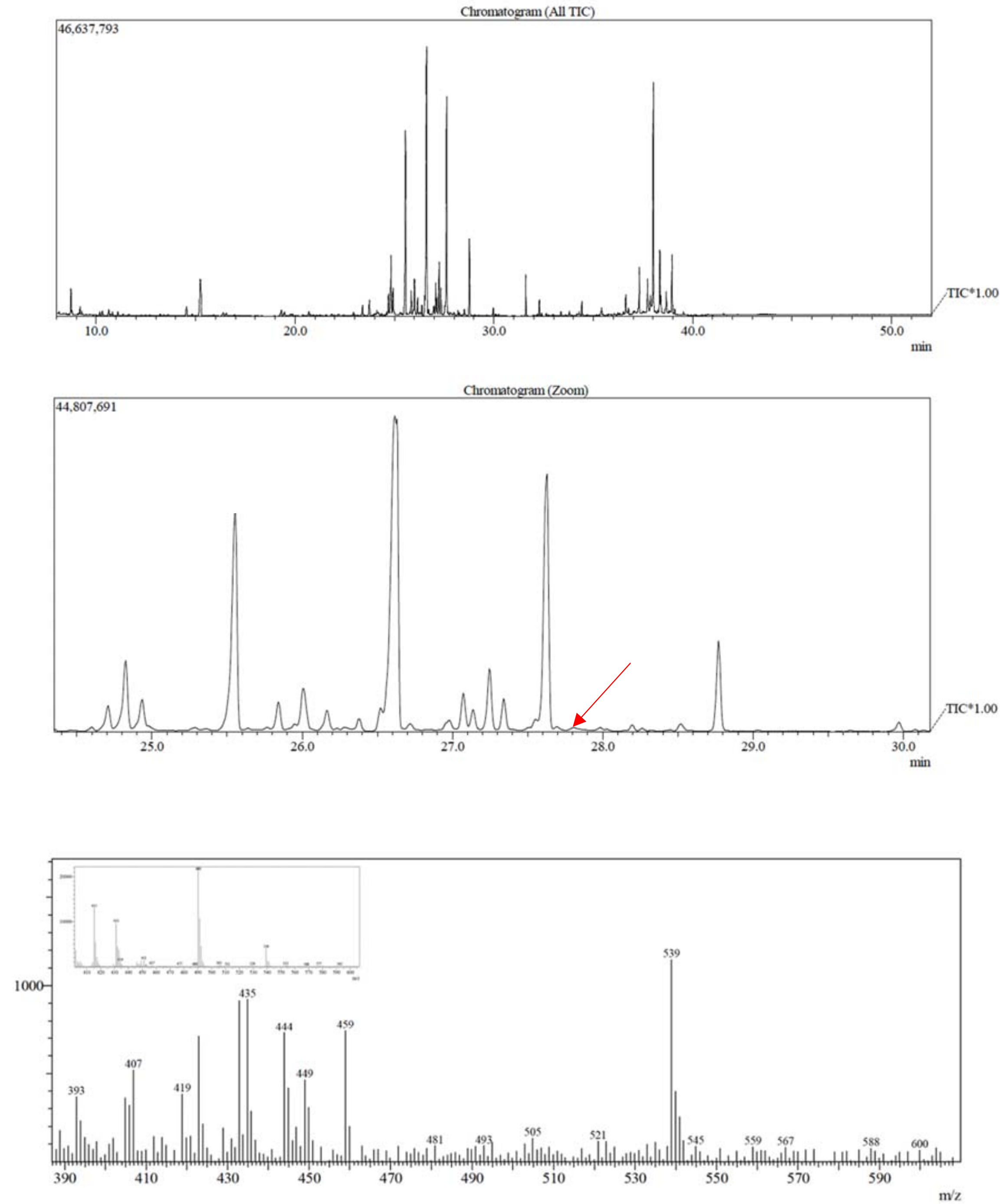


**Figure SI-10**: GC-MS result of *Vangueria soutpansbergensis* (endosymbiont = *Ca.* Paraburkholderia soutpansbergensis) showing the absence of kirkamide in the chromatograms and the enlarged *m/z* region from 400 to 600 *m/z*. Top panel: total ion chromatogram. Middle panel: total ion chromatogram, focused around the expected retention time of kirkamide. Bottom panel: m/z spectrum at the expected retention time of kirkamide with the reference kirkamide spectrum inserted. The red arrow points to the expected retention time of kirkamide.

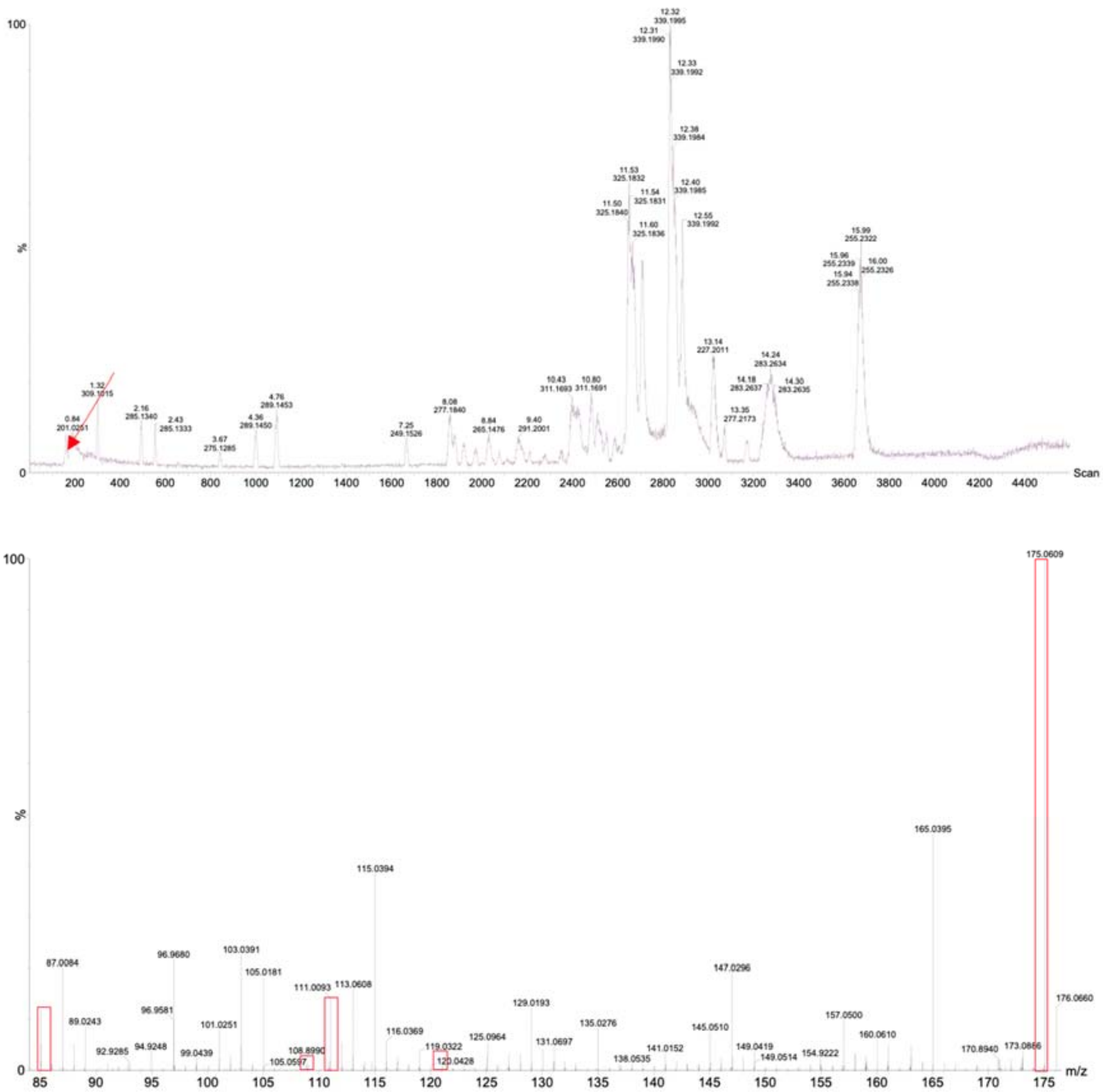

**Figure SI-11**: UPLC-QToF-MS results of streptol in *Psychotria kirkii* showing the chromatogram in the top panel and the presence of the ion fragments 85, 109, 111, 121 and 175 *m/z* at a retention time of 0.716 min in the bottom panel.

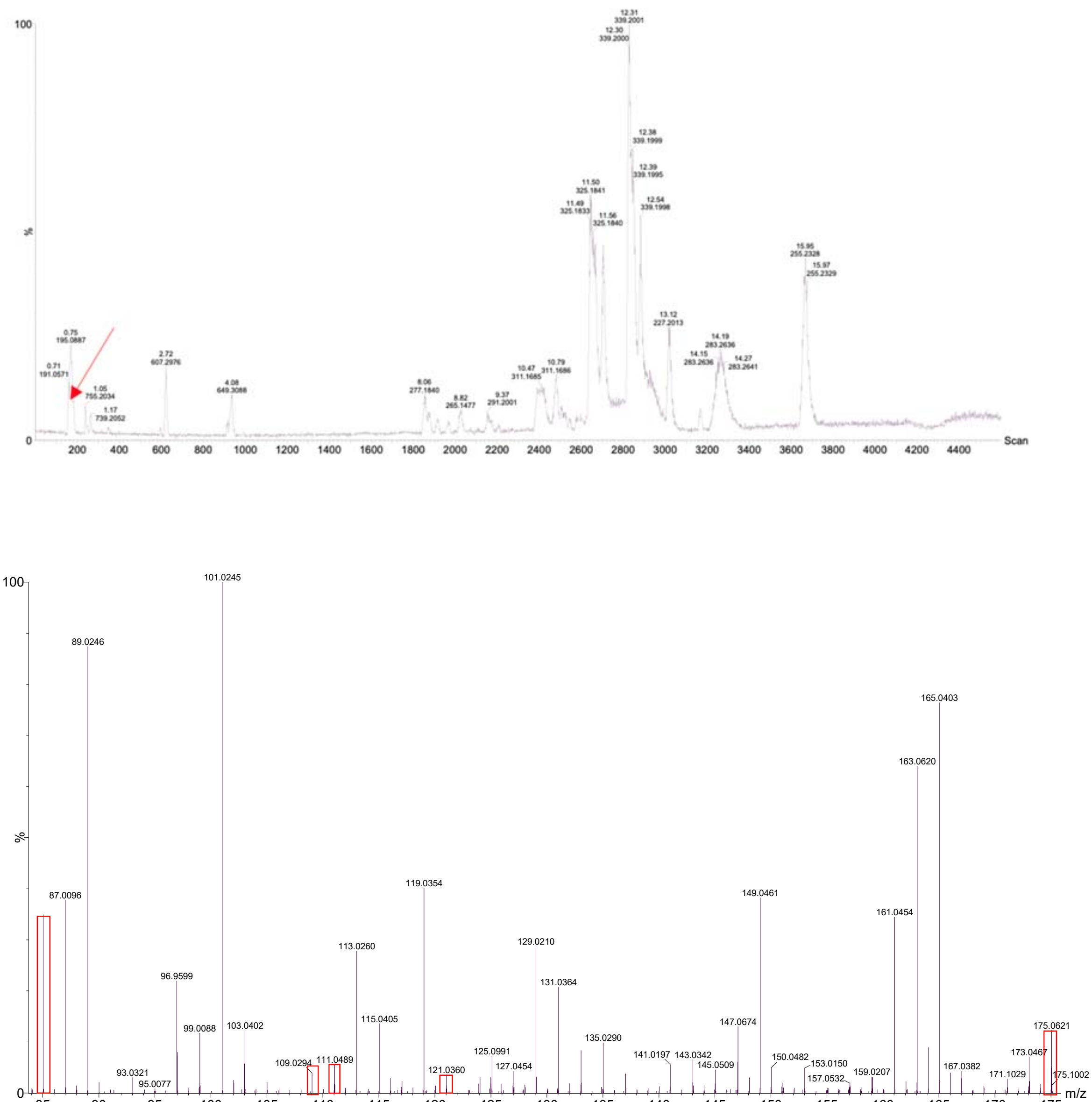


**Figure SI-12:** UPLC-QToF-MS results of streptol in *Vangueria dryadum* showing the chromatogram in the top panel and the presence of the ion fragments 85, 109, 111, 121 and 175 *m/z* at a retention time of 0.724 min in the bottom panel.

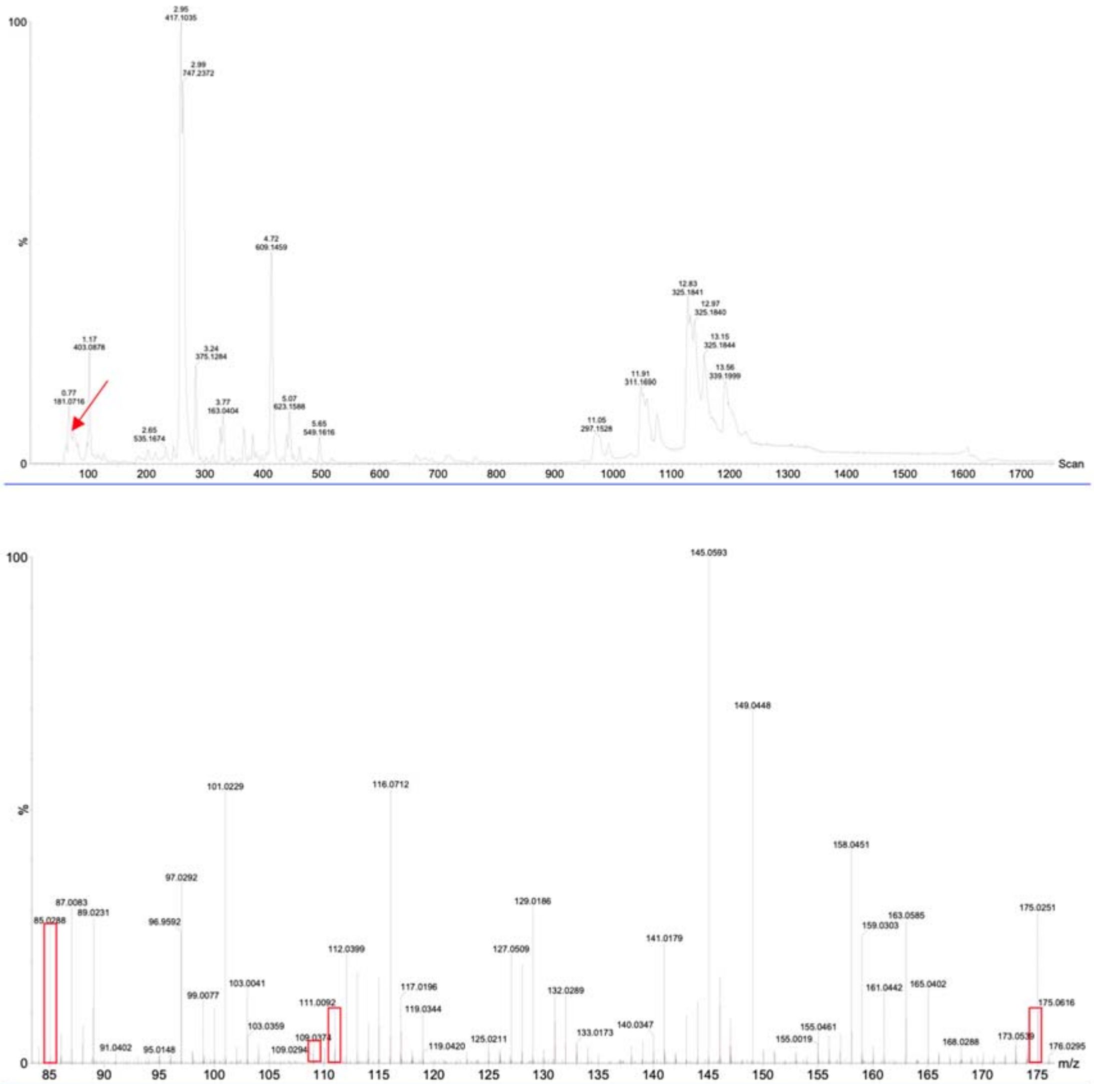


**Figure SI-13:** UPLC-QToF-MS results of streptol in *Vangueria pygmaea* showing the chromatogram in the top panel and the presence of the ion fragments 85, 109, 111 and 175 *m/z* at a retention time of 0.733 min in the bottom panel.

## References


Hsiao, C.C., Sieber, S., Georgiou, A., Bailly, A., Emmanouilidou, D., Carlier, A., Eberl, L., Gademann, K., 2019. Synthesis and biological evaluation of the novel growth inhibitor streptol glucoside, isolated from an obligate plant symbiont. Chemistry - A European Journal 25, 1722–1726. https://doi.org/10.1002/chem.201805693

Georgiou, A., Sieber, S., Hsiao, C.C., Grayfer, T., Gorenflos López, J.L., Gademann, K., Eberl, L., Bailly, A., 2021. Leaf nodule endosymbiotic *Burkholderia* confer targeted allelopathy to their *Psychotria* hosts. Scientific Reports 11, 1–15. https://doi.org/10.1038/s41598-021-01867-2

Pinto-Carbó, M., Sieber, S., Dessein, S., Wicker, T., Verstraete, B., Gademann, K., Eberl, L., Carlier, A., 2016. Evidence of horizontal gene transfer between obligate leaf nodule symbionts. ISME Journal 10, 2092–2105. https://doi.org/10.1038/ismej.2016.27

Sieber, S., Carlier, A., Neuburger, M., Grabenweger, G., Eberl, L., and Gademann, K. 2015. Isolation and Total Synthesis of Kirkamide, an Aminocyclitol from an Obligate Leaf Nodule Symbiont. Angewandte Chemie International 54, 7968–7970.