**Acetone crude extracts** (32.15 g)



**Vacuum Liquid Chromatography (VLC)**

hexane:chloroform (8:2, 6:4); 100% chloroform; 100% methanol



|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| F1 | F2 | F3 | F4 | **F5** | F6 |
| (2.85 g) | (2.56 g) | (2.69 g) | (3.22 g) | (2.01 g) | (18.58 g) |
|  |  |  |  |  |  |



**Radial Chromatography (RC)** hexane:chloroform (9:1)



|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  | F1 |  | F2 |  | **F3** |  |  |  | F4 |
|  |  |  |  |  |  | (0.31 g) |  | (0.41 g) |  | (0.52g) |  |  | (0.76 g) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  **RC** |
|  |  |  |  |  |  |  |  |  |  | hexane:chloroform (2:8) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F1 | F2 | F3 | F4 |  | F5 | F6 |  |  | **F7** |  |
| (0.07 g) | (0.12 g) | (0.03 g) | (0.11 g) | (0.07 g) (0.08 g) |  | (0.04 g) |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  **RC** |
|  |  |  |  |  |  |  |  |  | hexane:chloroform:methanol (7:2:1) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | **F1** |  | F2 |  | F3 |  |  |  | F4 |
|  |  |  |  |  |  | (19 mg) | (11 mg) | (29 mg) |  | (30 mg) |
|  |  |  |  **Preparative TLC** |  |  |  |  |  |
|  |  |  |  |  | Hexane:ethyl acetate (3:7) |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  | **F1** |  | F2 |  | F3 |  | F4 |  |  |  |  |  |
|  |  |  | (0.8 mg) |  | (5.4 mg) |  | (7.1 mg) | (5.6 mg) |  |  |  |  |



**High Performance Liquid Chromatography (HPLC)**

Gradient; methanol:water



**AF53611**

(0.5 mg)

Figure S1. Flow chart of the isolation of an active compound AF53611 (later recognised as HCPCA (6-((2-hydroxy-4-metoxyphenoxy) carbonyl) phenazine-1-carboxylic acid)) from *S. kebangsaanensis* crude extract.