SUPPLEMENTARY MATERIAL

Novel Illudalane Sesquiterpenes from Encephalartos villosus Lehm. 
Antimicrobial activity.

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Abstract

Phytochemical investigation of Encephalartos villosus Lehm. leaves afford two new illudalane sesquiterpenes namely Encephaldiene 1 and Encephaldiene 2 together with four known flavone glycosides, Luteolin-7-rutinoside, Luteolin-7-glucoside, Luteolin-7-rhamnoside and Apigenin-7-glucoside. The structures of the isolated compounds were elucidated by means of spectroscopic methods including 1D and 2D NMR experiments along with HRESIMS spectrometry.

Antimicrobial activity of CHCl$_3$ and MeOH extracts was investigated. Both extracts showed antibacterial activity against Gram-positive bacteria Streptococcus pneumonia and Bacillus subtilis, and antifungal activity against Aspergillus fumigatus. While CHCl$_3$ extract showed additional activity against Gram-negative bacteria Escherichia coli.

Keywords: Encephalartos villosus, illudalane sesquiterpenes, encephaldiene, antimicrobial activity.
Table S1. $^1$H and $^{13}$C NMR data of compounds 1–2 (CDCl$_3$, 600 MHz)$^a$.

| Position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
|----------|------------|------------|------------|------------|
| 1a       | 1.30 br d (18.4) | 23         | 1.3 br d (18.4) | 23         |
| 1b       | 1.43 br d (18.4) |            | 1.45 br d (18.4) |            |
| 2        |             | 132        |            | 127        |
| 3        |             | 32         | 1.25 m     | 32         |
| 4        | 4.25 dd (7, 12) | 68         | 4.20 dd (7.1, 12 ) | 68         |
| 5        | 2.33 ddd(7, 7.5, 12) | 34         | 2.33 ddd(7.1, 7.7, 12) | 30         |
| 6        | 1.68 m     | 39         | 1.68 m     | 39         |
| 7        | 7.70 dd(12 ) | 129        | 7.70 d(12 ) | 129        |
| 8        | 7.55 dd ( 12) | 131        | 7.50 d (12 ) | 131        |
| 9        |             | 167        |            | 168        |
| 10a      | 1.26 br d (16.6) | 29         | 1.25 br d (16.6) | 29         |
| 10b      | 1.31 br d (16.6) |            | 1.30 br d (16.6) |            |
| 11       |             | 38         |            | 39         |
| 12       | 0.92 S     | 14         | 0.91       | 11         |
| 13       | 0.89 S     | 14.5       | 0.92       | 14         |
| 14       | 0.90 S     | 11         | 0.89 d (7.8 ) | 14         |
| 15       | 0.93 S     | 12         | -          | -          |

$^a$J values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, HSQC, and HMBC experiments.
Figure S1. Diagnostic HMBC NMR correlations of compounds 1 & 2.
Figure S2: $^1$H NMR of compounds 1.
Figure S3: $^{13}$C NMR of compounds 1.
Figure S4: HSQC correlations of compounds 1.
Figure S5: HMBC correlations of compounds 1.
Figure S6: COSY correlations of compounds 1.
Figure S7: $^1\text{H}$ NMR of compounds 2.
Figure S8: $^{13}$C NMR of compounds 2.
Figure S9: HSQC correlations of compounds 2.
Figure S10: HMBC correlations of compounds 2.
Figure S11: COSY correlations of compounds 2.
Figure S12. Antimicrobial activity of CHCl₃ and MeOH extracts of *Encephalartos villosus* as compared to reference antibiotics.