SUPPLEMENTARY MATERIAL

Novel Illudalane Sesquiterpenes from *Encephalartos villosus* Lehm. Antimicrobial activity.

Abeer Temraz

*Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura
University, Makkah, Saudi Arabia.*

*Department of Pharmacognosy, Faculty of Pharmacy (Girls), Al-Azhar
University, Nasr-City, Cairo, Egypt.*

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 CHCl₃ 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 CHCl₃ extract showed additional activity against Gram-negative bacteria *Escherichia coli*.

Keywords: *Encephalartos villosus* , illudalane sesquiterpenes, encephaldiene, antimicrobial activity.

Table S1. ^1H and ^{13}C NMR data of compounds 1–2 (CDCl_3 , 600 MHz)^a.

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{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.

Dr.Obaid Sameer
Sample: EV-11-6 (11)
@PROTON_NSI_KAAU CDCl₃ (D:\Umm ElQura) jaber 13

NAME Obaid EV-11-6 04-09-2014
EXPNO 1
PROCNO 1
Date_ 20140906
Time 15:01
INSTRUM spect
PROBHD 5 mm PAIXI 1H/
PULPROG zg30
TD 65536
SOLVENT CDCl₃
NS 64
DS 2
SWH 12335.534 Hz
FIDRES 0.188225 Hz
AQ 2.6564426 sec
RG 263
DM 40.533 umsec
DE 12.00 umsec
TE 128.1 K
D1 1.00000000 sec
TD0 1
----- CHANNEL f1 -----
NUC1 1H
P1 7.80 umsec
PL1 1.10 dB
PL12 12.96246630 W
SPOL 600.0842000 MHz
SI 32768
SF 600.080075 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 2.00

Figure S2: ¹H NMR of compounds 1.

Dr.Obaid Sameer
Sample: EV-11-6 (11)
@C13_NS256_KAAU CDC13 {D:\Umm ElQura} jaber 13

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NAME      Obaid  EV-11-6  04-09-2014
EXPNO     11
PROCNO    1
DATA_     20140907
Time      0.25
INSTRUM   spect
PROBHD    5 mm PAKT-13H
PULPROG   zgpg30
TD        131072
SOLVENT   CDCl3
NS        10240
DS        4
SWH        37678.788 Hz
FIDRES     0.288992 Hz
AQ         1.7302004 sec
RG         203
CW         13.105 usec
DE         70.92 usec
TE         128.7 K
D1         1.5000000 sec
D11        0.0300000 sec
TD0        1

===== CHANNEL f1 =====
NUC1       13C
P1         11.80 usec
PL1        4.90 dB
PL12       223.15917358 W
SFO1       100.6261804 MHz

===== CHANNEL f2 =====
CPDPRG2   waltz16
NUC2       1H
PCPD2      80.00 usec
PL2        0.80 dB
PL12       21.02 dB
PL13       23.02 dB
PL12W     13.89381886 W
PL13W     0.15207529 W
PL13W     0.08333388 W
SFO2       400.0824003 MHz
T1         1.31072
SF         150.8902360 MHz
WDW        EM
SSB        0
LB         1.50 Hz
GB         0
PC         1.40

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Figure S3: ^{13}C NMR of compounds 1.

Dr.Abeer Sameer
Sample: EV-11-6 (11)
@HSQCEDsp_NS2_KAAU CDCl3 {D:\Umm ElQura} jaber 13

Figure S4: HSQC correlations of compounds 1.

Figure S5: HMBC correlations of compounds 1.

Dr.Abeer
Sample: EV-11-6 (11) CDCl3
@COSY_NS2_KAAU CDCl3 (D:\Umm ElQura) jaber 15

Figure S6: COSY correlations of compounds 1.

Dr.Obaid Sameer
Sample: EV-14-6H (12)
@PROTON_NSI_KAAU CDC13 {D:\Umm ElQura} jaber 12

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NAME      Obaid  EV-14-6H  04-09
EXPNO     20
PROCNO    1
Date_     20140906
Time      5.29
INSTRUM   spect
PROBHD    5 mm PATEI 1H/
PULPROG   zg30
TD         65536
SOLVENT   CDC13
NS         64
DS         2
SWH        12335.526 Hz
FIDRES     0.186225 Hz
AQ         2.6564426 sec
RG         36
CW         40.533 usec
DE         12.00 usec
TE         126.9 K
D1         1.00000000 sec
TD0        1

===== CHANNEL f1 =====
NUC1       1H
P1         7.80 usec
PL1        1.10 dB
PL12       12.96446590 W
SFO1       600.0842001 MHz
SI         32768
SF         600.0800175 MHz
WDE        32K
SSB        0
LB         0.30 Hz
GB         0
PC         2.00

```

Figure S7: ^1H NMR of compounds 2.

Dr.Obaid Sameer
Sample: EV-14-6H (12)
@C13_NS256_KAAU CDCl3 (D:\Umm ElQura) jaber 12

Figure S8: ^{13}C NMR of compounds 2.

Dr.Abeer Sameer
 Sample: EV-14-16H (12) CDCL3
 @HSQCEDsp_NS2_KAAU CDC13 {D:\Umm ElQura} jaber 3

Figure S9: HSQC correlations of compounds 2.

Dr.Abeer Sameer
 Sample: EV-14-6H (12) CDCl3
 @HMBCgplp2nd_NS4_KAAU CDC13 {D:\Umm ElQura} jaber 3

Figure S10: HMBC correlations of compounds 2.

Dr.Abeer
Sample: EV-14-6 (12) CDCL3
@COSY_NS2_KAAU CDC13 {D:\Umm ElQura} jaber 14

Figure S11: COSY correlations of compounds 2.

Figure S12. Antimicrobial activity of CHCl₃ and MeOH extracts of *Encephalartos villosus* as compared to reference antibiotics.