SUPPLEMENTARY MATERIAL

New *ent*-Kaurane Diterpenoid Dimer from *Pulicaria inuloides*.

Amal A. Galala^a, Amal Sallam^{a,*}, Osama B. Abdel-Halim^{a,b} and Sahar R. Gedara^a

^aDepartment of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura, 35516 Egypt.

^bDepartment of Pharmacognosy and Pharmaceutical Chemistry, College of Pharmacy, Taibah University, Al madinah Almonwwarah, Saudi Arabia

Abstract:

A new naturally occurring *ent*-kaurane diterpenoid dimer, 15 β , 15' β -oxybis (*ent*-kaur-16-en-19-oic acid) (**1**) along with six known compounds, 15 β -hydroxy-*ent*-kaur-16-en-19-oic acid (**2**), 15 β -hydroxy-*ent*-kaur-16-en-19-oate- β -D-glucopyranoside (**3**), 6-hydroxykaempferol-3, 7-dimethyl ether (**4**), quercetagenin 3, 7, 3'-trimethyl ether (**5**), β -sitosterol (**6**) and β -sitosterol glucoside (daucosterol) (**7**) were isolated from the aerial parts of *Pulicaria inuloides* DC. Compounds **2–5** were isolated for the first time from genus *Pulicaria*. The structures of compounds **1–7** were established on the basis of extensive 1D- and 2D-NMR spectroscopic techniques in combination with ESI-MS. The antimicrobial activity of the isolated compounds was evaluated against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Sulphorhodamine B cytotoxic assay against HepG2 (liver cancer) cell line and ABTS antioxidant assay were carried out.

Key words: *Pulicaria inuloides*; Asteraceae; *ent*-kaurane; antimicrobial; antioxidant; cytotoxicity

Figure S1. ^1H NMR spectrum of compound **1** in CD_3OD

Figure S2. APT spectrum of compound **1** in CD_3OD

Figure S3. HMQC NMR spectrum of compound **1** in CD_3OD

Figure S4. HMBC NMR spectrum of compound **1** in CD_3OD .

Figure S5. ^1H NMR spectrum of compound **1** in $\text{DMSO-}d_6$

Figure S6. APT spectrum of compound **1** in $\text{DMSO-}d_6$

Figure S7. ESI-MS^- spectrum of compound **1**

Figure S8. NOESY spectrum of compound **2** in CD_3OD .

Table S1. Results of antimicrobial activity of compounds **2-5**

Figure S1. ^1H NMR spectrum of compound **1** in CD_3OD

Figure S2. APT spectrum of compound **1** in CD₃OD

Figure S3. HMQC NMR spectrum of compound **1** in CD₃OD

Figure S4. HMBC NMR spectrum of compound **1** in CD₃OD

Figure S5. ^1H NMR spectrum of compound **1** in $\text{DMSO-}d_6$

Figure S6. APT spectrum of compound **1** in DMSO-*d*₆

Figure S7. ESI-MS⁻ spectrum of compound **1**

Figure S8. NOESY spectrum of compound **2** in CD₃OD.

Table S1: Results of antimicrobial activity of compounds **2-5**

Compound (mg/mL)	<i>E. coli</i>		<i>S. aureus</i>		<i>C. Albicans</i>	
	Diameter of inhibition zone (in mm)	% Activity index	Diameter of inhibition zone (in mm)	% Activity index	Diameter of inhibition zone (in mm)	% Activity index
2	8	34.8	NA	----	NA	----
3	6	26.1	NA	----	7	26.9
4	9	39.1	16	76.2	15	57.7
5	11	47.8	20	95.2	18	69.2
Ampicillin	23	100	21	100	NA	----
Colitrimazole	NA	----	NA	----	26	100

- NA: No Activity