## Systematic phytochemical screening of different organs of *Calotropis procera* and the ovicidal effect of their extracts to the foodstuff pest *Cadra cautella*.

Ammar Bader<sup>1</sup>, Ziad Omran<sup>1,5</sup>, Ahmed I. Al-Asmari<sup>2,</sup> \*, Valentina Santoro<sup>3</sup>, Nunziatina De Tommasi<sup>3</sup>, Massimiliano D'Ambola<sup>3</sup>, Fabrizio Dal Piaz<sup>3</sup>, Barbara Conti<sup>4,</sup> \*, Stefano Bedini<sup>4</sup>and Majed Halwani<sup>5,</sup> \*

- <sup>1</sup> Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, Makkah, 21955, Saudi Arabia; <u>ambader@uqu.edu.sa</u>; <u>zhomran@uqu.edu.sa</u>
- <sup>2</sup> King Abdulaziz Hospital, Laboratory Department, Jeddah, Saudi Arabia; <u>ahmadalasmari@yahoo.com</u>
- <sup>3</sup> Dipartimento di Farmacia, Università di Salerno, Fisciano (SA), Italy; <u>vsantoro@unisa.it; detommasi@unisa.it;</u> <u>mdambola@unisa.it; fdalpiaz@unisa.it</u>
- <sup>4</sup> Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, Pisa, Italy; <u>barbara.conti@unipi.it</u>; <u>stefano.bedini@unipi.it</u>
- <sup>5</sup> Nanomedicine Department, King Abdullah International Medical Research Center, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia; <u>halawanima@ngha.med.sa</u>
- \* Correspondence: <u>ahmadalasmari@yahoo.com</u>, Tel.: +9665599155725 (A.I.A.); <u>barbara.conti@unipi.it</u>, Tel.: +390502216125 (B.C.) and <u>halawanima@ngha.med.sa</u>, Tel.: +966114294433 (MH).

## **Content:**

## Page No.

2	Extraction and Isolation method
3	Figure S1. <sup>1</sup> H NMR spectrum of MeOH Extract of leaves
4	Figure S2. <sup>1</sup> H NMR spectrum of MeOH Extract of flowers
5	Figure S3. <sup>1</sup> H NMR spectrum of MeOH Extract of stems
6	Figure S4. <sup>1</sup> H NMR spectrum of MeOH Extract of roots
7	Figure S5. <sup>1</sup> H NMR spectrum of Latex Extract

**Extraction and Isolation:** Dried and powdered leaves of *C. procera* (500 g) were extracted with MeOH-H<sub>2</sub>O to give 33.0 g of crude methanolic extract. The *C. procera* obtained crude extract was dissolved in water and successively partitioned with *n*-hexane, and *n*-butanol, to yield 6.0 g and 11.5 g, respectively. The *n*-butanol fraction was subjected to flash silica gel CC using on Isolera Biotage (SNAP 340 g column, flow rate 90 mL/min), eluting with CHCl<sub>3</sub> followed by increasing concentrations of MeOH in CHCl<sub>3</sub> (between 1 and 100%) collecting 27 mL of fractions, that were grouped by TLC into 10 major fractions (A-J). Fractions C (95.6.0 mg) and D (205.6 mg) were subjected to RP-HPLC with MeOH-H<sub>2</sub>O (5:5) to yield calotropin (1.1 mg,  $t_R$  31 min) and calactin (3.8 mg,  $t_R$  18 min) from fraction C, and uscharin (0.9 mg,  $t_R$  31.5 min) from fraction D.

Fraction F (239.7 mg) was purified by RP-HPLC with MeOH-H<sub>2</sub>O (1:1) to afford compound 16 $\beta$ -hydroxy-calactin (1.2 mg,  $t_R$  15 min) and isorhamnetin (1.2 mg,  $t_R$  26 min).

Fractions G (56.0 mg), and H (181.0 mg), were separately subjected to RP-HPLC with MeOH-H<sub>2</sub>O (3:2) to yield 15 $\beta$ -hydroxy-calactin (2.8 mg,  $t_R$  15 min), and 12 $\beta$ -hydroxy-calactin (1.2 mg,  $t_R$  18 min) from fraction G, 15 $\beta$ -hydroxy-calactin (1.7 mg,  $t_R$  15 min) and calactin (2.5 mg,  $t_R$  25 min) from fraction H.

Fractions I (93.0 mg) and J (201.0 mg) were individually chromatographed by RP-HPLC with MeOH-H<sub>2</sub>O (3.5:6.5) to give rutin (2.9 mg,  $t_R$  12 min) and kampferol (1.0 mg,  $t_R$  15 min) from fraction I and rutin (4.2 mg,  $t_R$  12 min), and isorhamnetin (3.7 mg,  $t_R$  21 min) from fraction J.



Figure S1. <sup>1</sup>H NMR spectrum of MeOH Extract of leaves (CD<sub>3</sub>OD, 500 MHz)



Figure S2. <sup>1</sup>H NMR spectrum of MeOH Extract of flowers (CD<sub>3</sub>OD, 500 MHz)

![](_page_4_Figure_0.jpeg)

Figure S3. <sup>1</sup>H NMR spectrum of MeOH Extract of stems (CD<sub>3</sub>OD, 500 MHz)

![](_page_5_Figure_0.jpeg)

Figure S4. <sup>1</sup>H NMR spectrum of MeOH Extract of roots (CD<sub>3</sub>OD, 500 MHz

![](_page_6_Figure_0.jpeg)

Figure S5. <sup>1</sup>H NMR spectrum of Latex Extract (CD<sub>3</sub>OD, 500 MHz