

1        **SHORT COMMUNICATION**

2        **Phytotoxic activity and chemical composition of *Cassia absus* seeds and aerial parts**

3        I. Zribi <sup>ab\*</sup>, H. Sbai <sup>b</sup>, N. Ghezal <sup>ab</sup>, G. Richard <sup>c</sup>, D. Trisman <sup>c</sup>, M.L. Fauconnier <sup>c</sup>, R. Haouala <sup>b</sup>

4        <sup>a</sup>.

5        <sup>b</sup> *Department of Biological Sciences and Plant Protection, Higher Agronomic Institute of Chott-*  
6        *Mariem, IRESA- 4042 Chott-Mariem, Sousse, Tunisia (UR13AGR05).*

7        <sup>c</sup> *University of Liege - Gembloux Agro-Bio Tech. General and Organic Chemistry Unit. Passage*  
8        *des Déportés, 2. B-5030 Gembloux (Belgium).*

9        Corresponding author: (I. Zribi) E-mail address: [ines\\_zraibi@yahoo.fr](mailto:ines_zraibi@yahoo.fr).

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12     **Abstract:** The present study was conducted to assess the phytotoxic potential and the  
13     phytochemical composition of *Cassia absus*. Aqueous extracts caused significant reduction of  
14     root growth of *Lactuca sativa*. Seed extract was more effective than aerial part extract.  
15     Successive extractions of this plant were performed using solvents with increasing polarities.  
16     The methanolic seed extract exerted strong phytotoxic effect on seedling growth, followed by  
17     petroleum ether extract of the aerial part. The phytochemical investigation showed that among  
18     the organic extracts, methanol extracts of seeds and aerial parts contained the highest amounts  
19     of total phenolics and proanthocyanidins. Seeds were rich in linoleic acid followed by palmitic  
20     acids. Palmitic, stearic and arachidic acids were the major fatty acids in aerial parts. HPLC-  
21     DAD analysis of the methanolic extracts revealed the presence of luteolin in *C. absus* aerial  
22     parts.

23     **Keywords:** *Cassia absus*; Phytotoxicity; WESIA; Phytochemicals; GC-FID.

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## 25 **1. Introduction**

26 The intensive use of synthetic herbicides in agriculture is of increasing concern due to contamination  
27 of environment and induction of a resistance to herbicides in weeds. Hence, for several years,  
28 scientists focus on looking for compounds issuing from plants to develop bio-herbicides as an  
29 alternative strategy for weed control (Cantrell et al. 2012). *Cassia absus* (L.), commonly known as  
30 *chaksu*, is an erect annual or biennial plant with spreading branches clothed with glandular hairs. All  
31 plant parts contain alkaloids chaksine and isochaksine (Rao et al. 1979). *C. absus* seed extracts showed  
32 antibacterial (Wath et al. 2009) and hypotensive activities (Tabassum and Ahmad 2011). Leaves are  
33 used in treatment of tumours and asthma (Dave and Ledwani 2012). Cytotoxic molecules have  
34 recently been reported in *C.italic* and *C. obtusifolia* (Mohamed, 2014; Shi et al. 2016). The objective  
35 of this study was to assess the phytotoxic activity of *C. absus* seeds and aerial parts on lettuce (*Lactuca*  
36 *sativa* L.), and to determine phytochemical content and fatty acid composition of this species.

## 37 **2. Results and discussion**

### 38 **2.1. Phytotoxic activity**

39 Our study revealed that lettuce germination was not affected by seeds and aerial part aqueous extracts  
40 of *C. absus* at all concentrations tested, 7 days after germination (data not shown). The results also  
41 showed that seeds aqueous extract reduced root growth of lettuce more than aerial parts extracts, with  
42 an average of 95 and 73% respectively at 100 g/L (Figure 1). Shoot appeared less sensitive to *C. absus*  
43 aqueous extracts than radicle. The shoot length was severely affected under high level (100g/L) of  
44 seed aqueous extract. However *C. absus* aerial part aqueous extracts affects shoot growth only at the  
45 highest concentration with 33% of inhibition (Figure 1). According to Chung et al. (2001), the higher  
46 inhibition of roots may be due to their more intimate contact with the treated filter paper.

### 47 **Figure 1**

48 Among the organic extracts of *C. absus*, the chloroform extract of aerial part decreased germination  
49 percentage to 76.7% at 6mg/ml (Supplemental Table S1). Our results showed also that the highest  
50 toxic effect on root growth was recorded in the presence of seed methanol extract at 6mg/ml. Similar  
51 result was observed for lettuce shoot growth. The variation of the phytotoxic activity between extracts  
52 was probably due to the difference of the nature of the compounds extracted with solvents with  
53 different polarity (Omezzine et al. 2014).

### 54 **Figure 2**

55 The Whole-range assessment can display a visual comparison between different biological parameters  
56 and allowed us to group and to identify the most toxic extracts (Omezzine et al. 2014). Overall, roots  
57 and shoots were more severely affected by seed aqueous extract with respective Inhibition Index (I)  
58 values of 70 and 42 % than aerial part aqueous extract (Supplemental Table S2). Among all the  
59 organic extracts of *C. absus*, the chloroform extract of aerial part exhibited the most phytotoxic effect

60 on lettuce germination. Regarding seedling growth, methanolic extract of seed was the most  
61 phytotoxic for root (I= 47.2%) and shoot growth (I= 16.3%) (Supplemental Table S3).

## 62 **2.2. Phytochemical screening**

63 In the present study, the total phenolics, flavonols and flavones and proanthocyanidins contents of  
64 organic extracts of seeds and aerial parts of *C. absus* were estimated by colorimetric methods (Table  
65 1). Among the three organic fractions, maximum amount of phenol content was found in methanol  
66 fractions of aerial parts and seeds. Proanthocyanidins content was more in methanol extracts of the  
67 seeds than in aerial parts; while total flavonols and flavones content was more in aerial parts than in  
68 seeds. Thus, the results suggest that phytotoxic activity of *C. absus* may be due to polar compounds.

### 69 **Table 1**

70 The fatty acid composition of petroleum ether fractions of *C. absus* were identified on the basis of  
71 retention time of fatty acid methyl esters by GC-FID (Table 2). The predominant fatty acid in seeds  
72 was linoleic acid followed by palmitic, oleic and stearic acids. Petroleum ether fraction of the aerial  
73 part contains 87% of saturated fatty acids. Palmitic acid was the most abundant fatty acid, followed by  
74 stearic, arachidic and behenic acids.

### 75 **Table 2**

76 In order to detect allelochemicals, preliminary HPLC analysis high-performance liquid  
77 chromatography was used to screen for the presence of 17 phenolic compounds in methanolic extracts  
78 of seeds and aerial parts (Supplemental Figure S2). Peak 17 detected in the methanolic extracts of  
79 aerial parts of *C. absus* was identified as luteolin ( $0.37 \pm 0.01$   $\mu\text{g}/\text{mg}$  DW). Overall lack of  
80 identification of other compounds in our samples could be due to the extraction method used for  
81 HPLC analysis.

## 82 **3. Conclusion**

83 It may be concluded that *C. absus* has phytotoxic potential on seed germination and seedling growth  
84 of *L. sativa*. This study suggests that seeds and aerial parts are a potential source of natural  
85 allelochemicals such as phenolic compounds, flavonoids and proanthocyanidins. However, further  
86 studies are required to test the efficacy of extracts from this plant on weed control under field  
87 conditions and to isolate the chemical constituents responsible for the phytotoxic activity.

88 **Author contribution statement** Ines Zribi carried out all the experiments, analyzed data and drafted  
89 the manuscript. Haifa Sbai and Nadia Ghezal participated in the phytotoxicity experiments. Gaëtan  
90 Richard participated in the analysis of phenolic compounds by HPLC and revised the manuscript.  
91 Danny Trisman participated in the analysis of fatty acids. Marie-Laure Fauconnier provides laboratory  
92 facilities for carrying out HPLC and GC analysis. Rabiaa Haouala directed the study and revised the  
93 manuscript.

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