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Phytotoxic activity of *Cleome arabica* L. and its principal discovered active compounds

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ABSTRACT

The aim of this study was to assess the phytotoxic potential of *Cleome arabica* L, as well as to isolate the main bioactive compounds. Phytotoxicity was evaluated on germination and seedling growth of *Lactuca sativa*, *Raphanus sativus*, *Peganum harmala* and *Silybum marianum*, through testing aqueous and organic extracts of different *C. arabica* organs (roots, shoots, siliquae and seeds). Results showed that siliquae methanol extract caused the greatest negative effect on lettuce germination and growth. For the bioactive subfractions (petroleum ether, ethyl acetate and methanol–water), the ethyl acetate induced highly significant reduction, showing 100% inhibition of lettuce growth at 6 g/L. The bioactive ethyl acetate subfraction was chromatographed and subjected to NMR techniques. Based on bio-guided chromatographic fractionation, five bioactive allelochemical compounds were isolated from ethyl acetate extract of siliquae of *C. arabica*. The most inhibitory compound on lettuce seedling growth was elucidated as 11-α-acetylbrachy-carpone-22(23)-ene.

1. Introduction

In recent years, a marked emphasis has been placed on the study of sustainable agriculture and major concerns have been raised about the adverse effects of extensive use of synthetic chemicals such as contamination of the environment and the resistance development of numerous plants to herbicides. Therefore scientists have focused on searching for plant compounds to develop bio-herbicides as alternative (Dayan et al., 2009; Cantrell et al., 2012). Worldwide, it is estimated that weeds are responsible for a loss of about 13.2% in eight of the most important food and cash crops, even when they are intensively controlled (Oerke et al., 1995). Therefore, considerable efforts have been dedicated to the study of allelopathic effects of different plants and their ability to control weeds in a sustainable manner (Singh et al., 2003; Macías et al., 2006). Allelochemicals that are released by some plants have been well investigated as sources of new compounds for weed control (Duke et al., 2000; Vyvyan, 2002). Chemicals with allelopathic activity belong to different categories of secondary compounds such as phenols, benzoic and cinnamic acid derivatives, flavonoids, tannins, coumarins, terpenoids, alkaloids and polyacetylenes (Duke et al., 2000). These allelopathic compounds can provide excellent inhibition of weed seed germination and seedling growth of native species in areas invaded by invasive plants (Hirtto and Callaway, 2003; Kong et al., 2004; Lorenzo et al., 2008).

Cleome L. is a large genus with 150 species in the tropical and subtropical countries both in the Old and New Worlds (Willis, 1966). *Cleome arabica* is a Capparidaceae that is widespread in North Africa. It has been used as folk medicine in the treatment of scabies and inflammation (Ahmad et al., 1990; Tsichritzis et al., 1993) as well as rheumatic pains (Bouriche and Arnhold, 2010). It also possesses antimicrobial and antifungal (Takhi et al., 2011), antioxidant (Selloum et al., 1997; Djeridane et al., 2010) and cytotoxic activities (Nagaya et al., 1997). The isolation and characterization of marker compounds from medicinal plants are one of the most important areas of research. Over the years, various types of allelochemicals have been isolated and characterized from hundreds of plants (Rice, 1984; DellaGreca et al., 2007). However, information about allelochemicals in *Cleome* species is limited. In addition, little has been reported on the flavonoids of *Cleome* species with only two species being studied so far namely, *C. viscosa* (Chauhan et al., 1979; Srivastava, 1982) and *C. droserifolia* (Seif El-Din et al., 1987). In recent studies, phytochemical investigation of *C. arabica* aerial part led to the isolation of phenolic compounds, alkaloids (Takhi et al., 2011), damarane triterpene (Khalafallah et al., 2009), cleomin (Ismail et al., 2005), new steroid derivatives (Djeridane et al., 2010), and glucosylated and rhamnosylated flavonols (Bouriche and Arnhold, 2010). Ismail et al. (2005) have also described known flavonol glycosides such as 3-*O*-glucosyl-7-*O*-rhamnopyranosides,

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3,7-di-*O*-rhamnopyranosides and 3-*O*-glucopyranosides of quercetin, kaempferol, and isorhamnetin.

Despite abundant research that has been carried out on the potential health benefits of *C. arabica*, neither the phytotoxic effect nor the constituent that is mainly responsible for its bioactivity has been known yet. Thus, we aim to assess the phytotoxic potential of *C. arabica* organs (roots, shoots, siliquae and seeds), as well as isolate and identify the main bioactive compounds. In this study, the screening was conducted under laboratory conditions on four target species: two typical acceptor plants, sensitive to most allelochemicals (lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus*)) and two common agricultural weeds (thistle (*Silybum marianum* L.) and peganum (*Peganum harmala* L.)). Then, isolation and identification of the bioactive allelochemicals from the most active plant organ were carried out through bio-guided phytotoxic tests on lettuce germination and seedling growth. Their structures were characterized by nuclear magnetic resonance of ^{1}H and ^{13}C NMR.

2. Materials and methods

2.1. Plant material

C. arabica was identified according to Tunisia flora (Pottier-Alapetite, 1979) and authenticated by Professor R. Haouala, the Department of Biological Sciences and Plant Protection. A voucher specimen (no. CC 284) was collected, dried and deposited at the herbarium of Higher Institute of Agronomy of Chott Meriem, University of Sousse Tunisia. The plant was collected during Spring 2010.

2.2. Aqueous extracts

Fresh *C. arabica* materials were rinsed with tap water and separated into roots (RT), shoots (SH), siliquae (Sl) and seeds (SD). Each of the organs was then oven-dried at 60 °C for 72 h and grinded. Fifty grams of each dried material was soaked in 1 L distilled water at room temperature for 24 h. The extracts were filtered through a filter paper several times and kept at 4 °C in the dark until use.

2.3. Organic extracts

Sequential extraction was carried out with organic solvents having increasing polarity: hexane, chloroform and methanol. Dried powder (100 g) of RT, SH, Sl and SD was immersed in the respective organic solvents for 24 h at room temperature. Organic extracts were evaporated to dryness under reduced pressure at 45 ± °C using a Rotavapor R-114 (Buchi, France). Dry fractions were stored at 4 °C until use. The extracts were tested at a concentration of 6 g/L in bioassays.

2.4. Bioassays with aqueous extracts

Aqueous extracts (RT, SH, Sl and SD) were diluted with distilled water to give 10, 20, 30, 40 and 50 g/L concentrations. They were tested on two crops (*R. sativus* L., *L. sativa* L.) and two weeds (*S. marianum*, *P. harmala* L.). Seeds were surface sterilized with 0.525 g/L sodium hypochlorite for 15 min, then rinsed four times with deionized water, imbibed in the same at 22 °C for 2 h and carefully blotted using a folded paper towel. Twenty imbibed seeds of target species were evenly placed in 9 cm plastic Petri dishes, lined with filter paper and 5 mL of respective extract was applied as per treatment. Seeds watered with distilled water were used as control. The Petri plates were then placed in a growth chamber with 400 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) at 24/22 °C for 14/10 h light and dark periods respectively. Treatments were arranged in a completely randomized design with three replications.

Cumulative germination was determined by counting the number of germinated seeds at 24 h intervals during 6 days. Shoot and root lengths of receiver species were measured after 7 days of sowing. Data were transformed to percent of control for analysis. The index of germination (GI) and total germination (GT) were determined using the following formulae (Chiapuso et al., 1997):

$$GT = N_T \times 100/N$$

where, N_T: number of germinated seeds for each treatment at the end of the assay and N: total number of seeds used in the assay. This index is the most commonly applied.

$$GI = (N_1) \times 1 + (N_2 - N_1) \times 1/2 + (N_3 - N_2) \times 1/3 + \ldots + (Nn - Nn_{-1}) \times 1/n$$

where, N_1, N_2, N_3, …., N_n: the number of germinated seeds observed afterwards 1, 2, 3,…., n − 1, n days. This index shows the delay in seed germination (Delabays et al., 1998). The inhibitory/stimulatory percentage was calculated using the equation given by Chung et al. (2001):

$$\text{Inhibition}(-)/\text{stimulation}(+)\% = [(\text{extract}-\text{control})/\text{control}] \times 100$$

where extract: parameter measured in the presence of *C. arabica* extract and control: parameter measured in the presence of distilled water.

2.5. Bioassays with organic extracts

The three extracts concentrated from hexane, chloroform and methanol were dissolved in methanol at 6 g/L, to estimate their effect on germination and early growth of target species. Two controls were considered, distilled water and methanol, to eliminate the effect of organic solvents. Filter papers placed in Petri dish were soaked with 5 mL of distilled water, methanol or the various organic extracts. Solvents were evaporated for 24 h at 24 °C, then 5 mL distilled water was added and 20 soaked seeds were placed to germinate for 7 days. Germination and shoot and root lengths of target species were estimated as before and expressed in percentage of the control. Treatments were arranged in a completely randomized design with three replications and data were transformed to percent of control for analysis.

2.6. Isolation and identification of bioactive compound

2.6.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were run on a Varian INOVA 500 NMR spectrometer at 500 and 125 MHz, respectively, in $CDCl_3$ at 25 °C. Electronic impact mass spectra (EI-MS) were obtained with a GC–MS QP5050A (Shimadzu) equipped with a 70 eV EI detector. HPLC was performed on a Shimadzu LC-10AD by using a refractive index detector Shimadzu RID-10A. Preparative HPLC was performed using RP-18 (LiChrospher 10 μm, 250 × 10 mm i.d., Merck) columns. Flash column chromatography was performed on Merck Kieselgel 60 (230–400 mesh) at a medium pressure. Column chromatography (CC) was performed on Merck Kieselgel 60 (70–240 mesh), on Sephadex LH-20 (Pharmacia). Analytical TLC was performed on Merck Kieselgel 60 F254 or RP-18 F254 plates with 0.2 mm film thickness. Spots were visualized by UV light or by spraying with EtOH:H_2SO_4 (93:7) followed by heating for 5 min at 110 °C. Preparative TLC was performed on Merck Kieselgel 60 F254 plates, with 0.5 or 1 mm film thickness.

2.6.2. Extraction and isolation

Dried powder of siliquae of *C. arabica* was extracted twice at room temperature with MeOH during 48 h. After filtering, extracts were combined and dried at reduced pressure; the obtained residues were re-dissolved in MeOH:H_2O (1:1) and partitioned in a separator funnel with petroleum ether and ethyl acetate. The most active EtOAc extract (97 g) was separated by silica gel column chromatography and was eluted with CH_2Cl_2, EtOAc, MeOH:CH_2Cl_2 (2:1) and MeOH. Collected fractions were combined in 23 homogeneous fractions (AC_1–AC_{23}) and all of them were tested for their allelopathic activity. Bioactive

Table 1
Germination index, total germination and root and shoot lengths (expressed in % of control) of lettuce and peganum in the presence of aqueous extracts (at 50 g/L) of *C. arabica* plant parts and polyethylene glycol 4000 (PEG) solutions at the same pH and the same osmotic potential (Ψ_{Π}) (bar).

Parameter	RT extract	PEG solution	SH extract	PEG solution	Sl extract	PEG solution	SD extract	PEG solution
pH	5.88	5.88	6.43	6.43	5.89	5.89	6.63	6.63
Ψ_{Π}	0.00026	0.00022	0.00079	0.00081	0.00143	0.00131	0.000271	0.000264
Total germination								
Lettuce	0.0 ± 0.0c	101.7 ± 3.4a	0.0 ± 0.0c	96.6 ± 4.7a	0.0 ± 0.0c	103.5 ± 1.7a	0.0 ± 0.0c	80.0 ± 4.2b
Peganum	0.0 ± 0.0c	98.4 ± 6.3a	17.2 ± 2.4b	98.4 ± 6.3a	0.0 ± 0.0c	101.7 ± 2.4a	0.0 ± 0.0c	96.6 ± 3.7a
Germination index								
Lettuce	0.0 ± 0.0c	80.0 ± 1.5b	0.0 ± 0.0c	78.2 ± 1.2b	0.0 ± 0.0c	94.6 ± 1.0a	0.0 ± 0.0c	111.5 ± 1.9a
Peganum	0.0 ± 0.0c	96.3 ± 2.1a	13.0 ± 0.9b	109.0 ± 5.7a	0.0 ± 0.0c	102.1 ± 5.1a	0.0 ± 0.0c	123.9 ± 2.0a
Root length								
Lettuce	0.0 ± 0.0c	80.3 ± 6.3b	0.0 ± 0.0c	82.0 ± 3.0b	0.0 ± 0.0c	105.3 ± 2.3a	0.0 ± 0.0c	96.9 ± 4.6a
Peganum	0.0 ± 0.0c	123.6 ± 4.0a	12.4 ± 1.7b	119.4 ± 2.4a	0.0 ± 0.0c	113.9 ± 1.9a	0.0 ± 0.0c	116.6 ± 3.4a
Shoot length								
Lettuce	0.0 ± 0.0c	75.9 ± 3.7b	0.0 ± 0.0c	99.6 ± 4.6a	0.0 ± 0.0c	107.6 ± 3.8a	0.0 ± 0.0c	124.6 ± 1.0a
Peganum	0.0 ± 0.0c	117.4 ± 6.4a	38.0 ± 3.1b	109.1 ± 5.2a	0.0 ± 0.0c	108.5 ± 6.7a	0.0 ± 0.0c	117.4 ± 4.4a

RT, roots; SH, shoots; Sl, siliquae and SD, seeds. Means with the same letter in a column are not significantly different at $P < 0.05$ (LSD test). Values (N = 3 ± S.E.).

fractions (AC_3) were next fractionated by flash column chromatography on silica gel eluted with a mixture of EtOAc:hexane (1:9; 2:8; 4:6) and 100% MeOH. After TLC analysis, 16 homogeneous fractions were obtained. Allelochemicals were isolated and purified from the most active fractions and two purified compounds AC_{3-7} (36 mg) and AC_{3-16} (18 mg) were identified as compounds 1 and 5, respectively. The fraction AC_{3-10} (55 mg) was chromatographed by Sep-Pak C_{18} cartridge to obtain 3 fractions (A_1, A_2 and A_3). Fraction A_2 (9 mg) was purified by RP-18 HPLC (H_2O:CH_3CN:MeOH (2:3:5)) to obtain the compound 2. The fraction AC_{3-11} (82 mg) was re-chromatographed on TLC (EtOAc: hexane (3:7)) to obtain five fractions then the third fraction was purified by RP-18 HPLC (H_2O:CH_3CN:MeOH (2:3:5)) to give compound 3. Fraction AC_{3-15} (609 mg), eluted with MeOH, was chromatographed by Sephadex LH-20 with n-hexane:CH_2Cl_2:MeOH (7:4:0.5) as eluent, to give fractions C_1–C_{11}. Fraction C_6 was purified by RP-18 HPLC (H_2O: CH_3CN:MeOH (3:2:5)), and then by TLC (CH_2Cl_2:MeOH (1:1)) to give compound 4.

The phytotoxicity of these fractions was tested on lettuce as described before, at determined concentrations (6 g/L, 0.6 g/L and 0.06 g/L for screening extracts, 0.6 g/L for fractions and 0.06 g/L for pure fractions). Treatments were arranged in a completely randomized design with four replications.

2.7. Statistical analysis

The laboratory bioassays in a complete randomized design with three/four replications were performed to evaluate the effects of *C. arabica* extracts over the control values. ANOVA and a posthoc LSD tests were performed with PASW Statistics 18, for Windows program, to analyze treatment differences. The means were separated on the basis of least significant differences at the 0.05 probability level.

3. Results

Solutions of polyethylene glycol (PEG) having the same pH and osmotic potential of the most concentrated extracts were prepared and tested on lettuce and peganum. PEG has been widely used in experimental media at predetermined water potential values (Steuter et al., 1981). Under the same conditions, experiments with extracts of roots,

Table 2
Effect of aqueous extracts of different plant parts of *C. arabica* on germination parameters expressed in % of control of test species: lettuce, radish, peganum and thistle.

Aqueous extracts/ concentration (g/L)		Lettuce		Radish		Peganum		Thistle	
		GI	GT	GI	GT	GI	GT	GI	GT
(RT)	10	19.4 ± 1.2c	40.0 ± 4.7c	97.5 ± 8.1d	96.7 ± 6.3c	69.3 ± 6.7c	80.7 ± 7.4c	46.2 ± 4.5c	54.7 ± 3.6c
	20	9.6 ± 2.0b	21.6 ± 4.0b	76.9 ± 9.0b	91.5 ± 4.6c	43.4 ±0.9b	42.4 ± 6.0b	41.7 ± 3.7c	53.1 ± 1.5c
	30	0.0 ± 0.0a	0.0 ± 0.0a	30.2 ± 1.0c	43.8 ± 6.6b	0.0 ± 0.0a	0.0 ± 0.0a	32.9 ± 4.5b	41.0 ± 2.5b
	40	0.0 ± 0.0a	0.0 ± 0.0a	9.0 ± 0.7a	11.8 ± 2.2a	0.0 ± 0.0a	0.0 ± 0.0a	19.9 ± 2.6a	27.3 ± 1.6a
	50	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	15.7 ± 3.2a	22.0 ± 1.9a
(SH)	10	66.9 ± 4.8b	91.6 ± 4.0b	92.6 ± 5.4d	95.0 ± 4.0c	85.7 ± 9.3b	94.2 ± 0.1b	85.7 ± 8.3c	77.3 ± 6.0d
	20	0.0 ± 0.0a	0.0 ± 0.0a	76.2 ± 5.9c	90.0 ± 7.0c	86.0 ± 4.8b	82.6 ± 4.8b	41.0 ± 3.2b	41.0 ± 2.5c
	30	0.0 ± 0.0a	0.0 ± 0.0a	22.3 ± 4.3b	27.1 ± 2.1b	87.5 ± 6.2b	90.3 ± 5.6b	29.8 ± 4.6ab	31.5 ± 4.2b
	40	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	75.5 ± 5.4b	86.4 ± 7.4b	18.7 ± 2.3ab	23.2 ± 5.7b
	50	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	13.0 ± 2.7a	17.2 ± 2.4a	11.1 ± 1.3a	13.6 ± 0.8a
(Sl)	10	49.6 ± 3.9b	80.0 ± 4.2b	86.8 ± 7.6d	96.6 ± 2.3c	84.9 ± 5.1c	86.4 ± 7.4c	47.9 ± 5.2d	50.0 ± 5.8c
	20	0.0 ± 0.0a	0.0 ± 0.0a	55.6 ± 3.5c	89.7 ± 7.2c	15.8 ± 2.8b	22.9 ± 4.1b	34.7 ± 2.8c	41.0 ± 8.4bc
	30	0.0 ± 0.0a	0.0 ± 0.0a	26.3 ± 2.2b	32.0 ± 2.7b	12.5 ± 1.9b	17.2 ± 4.2b	27.7 ± 3.1bc	32.1 ± 4.1ab
	40	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	18.3 ± 0.7ab	27.3 ± 1.7a
	50	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	12.5 ± 3.9a	22.6 ± 3.1a
(SD)	10	32.2 ± 0.7c	68.3 ± 4.7c	96.5 ± 7.7d	98.3 ± 2.3b	78.3 ± 4.3d	77.0 ± 7.9d	82.6 ± 3.1d	63.6 ± 5.8c
	20	14.8 ± 2.6b	35.0 ± 5.1b	81.2 ± 6.1d	96.7 ± 6.3b	57.0 ± 4.3c	71.2 ± 4.0d	44.63 ± 2.4c	40.4 ± 4.7b
	30	0.0 ± 0.0a	0.0 ± 0.0a	69.5 ± 4.9c	100.0 ± 4.1b	28.5 ± 3.7b	42.2 ± 5.1c	28.42 ± 3.6b	36.3 ± 3.8b
	40	0.0 ± 0.0a	0.0 ± 0.0a	49.3 ± 5.1b	91.4 ± 6.2b	14.2 ± 2.1ab	19.2 ± 3.0b	0.0 ± 0.0a	0.0 ± 0.0a
	50	0.0 ± 0.0a	0.0 ± 0.0a	00.0 ± 0.0a	0.0 ± 00a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a

RT, roots; SH, shoots; Sl, siliquae and SD, seeds. GI, germination index; GT, total germination. Means with the same letter in a column are not significantly different at $P < 0.05$ (LSD test). Values (N = 3 ± S.E.).

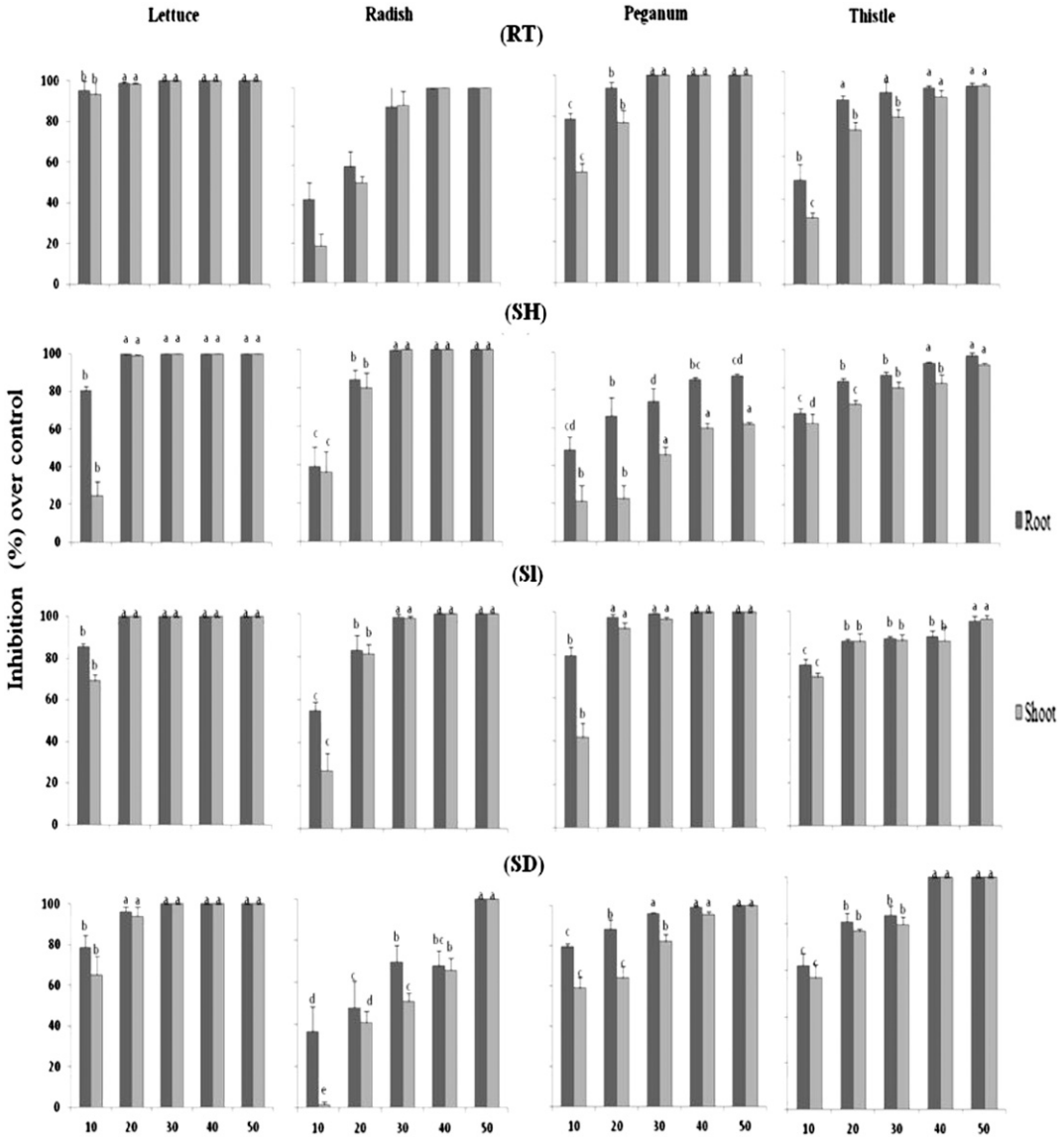


Fig. 1. Inhibition in % of control of root and shoot lengths of target species, 7 days after germination, in the presence of different concentrations of *C. arabica* root (RT), shoot (SH), siliquae (Sl) and seed (SD) aqueous extract. The bars on each column show standard error. Values (N = 3 ± S.E.). Different letters in columns indicate significant differences among treatments at $P < 0.05$ (LSD test).

shoots, seeds and siliquae of *C. arabica* and PEG experiment were concurrently conducted to distinguish between the inhibitory effects of substances and osmotic potential of the most concentrated extract. Indeed, all results were similar or improved relative to the control. None of the PEG solutions had effect neither on target plant germination nor on growth (Table 1).

3.1. Effect of C. arabica aqueous extracts

3.1.1. Germination

The *C. arabica* aqueous extracts exhibited strong inhibitory effect on the germination of the tested species and the degree of inhibition increased with increase in extract concentration which varied according to the target species and organs (Table 2). Shoot (SH) and siliquae (Sl) extracts exerted a stronger inhibitory effect on lettuce germination indicated by a total inhibition from 20 g/L for lettuce and from 40 g/L for radish. Furthermore, RT and SD extracts reduced completely the germination indices for lettuce from 30 g/L and at the highest concentration for radish. In most cases the recorded GT and GI did not exceed 85% at the lowest concentration for weeds. However, a total inhibition was recorded from 30 g/L to 40 g/L for peganum in the presence of RT and Sl, respectively. In this case, SD extract induced the same toxic effect on thistle germination (Table 2).

Table 3
Yields, in percent of dry mater (%dm), of organic extracts of *C. arabica* root (RT), siliquae (Sl), shoots (SH) and seeds (SD).

	RT	Sl	SH	SD
Hexane	0.44	2.92	0.62	1.88
Chloroform	2.36	3.08	2.95	1.86
Methanol	0.14	0.48	1.33	1.71

3.1.2. Growth

The growth of target species has been significantly influenced by *C. arabica* aqueous extracts (Fig. 1). Generally, the behavior of roots and shoots was similar in the presence of all extracts except for peganum in which roots were more sensitive than shoots, in the presence of SL extract. The percentage inhibition of lettuce growth was 100%, in the presence of all extracts from 20 g/L. At 10 g/L it varied between 20% and 94% according to the organ. Furthermore, thistle growth was

Table 4
Effect of organic extracts of different plant parts of *C. arabica* (at 6 g/L) on germination parameters expressed in % of control of test species: lettuce, radish, peganum and thistle.

Organic extracts		Lettuce		Radish		Peganum		Thistle	
		GI	GT	GI	GT	GI	GT	GI	GT
(RT)	Hexane	30.2 ± 1.8a	71.1 ± 2.8ab	96.6 ± 6.8a	98.3 ± 2.3a	68.7 ± 6.0b	83.3 ± 4.7b	50.2 ± 4.1c	54.1 ± 7.2c
	Chloroform	36.5 ± 0.4a	67.8 ± 8.2a	95.7 ± 3.9a	98.3 ± 2.3a	41.9 ± 3.9a	71.2 ± 1.7ab	0.0 ± 0.0a	0.0 ± 0.0a
	Methanol	39.9 ± 2.6a	88.3 ± 7.4b	92.4 ± 7.6a	96.6 ± 4.7a	39.0 ± 3.0a	55.7 ± 2.6a	36.7 ± 2.2b	36.3 ± 2.5b
(SH)	Hexane	44.5 ± 2.5a	55.7 ± 7.2a	97.3 ± 5.4a	100.0 ± 0.0a	50.4 ± 5.7b	69.4 ± 0.7a	0.0 ± 0.0a	0.0 ± 0.0a
	Chloroform	66.7 ± 4.3b	49.0 ± 1.5a	95.5 ± 3.0a	96.6 ± 2.3a	41.6 ± 3.2ab	64.5 ± 6.4a	0.0 ± 0.0a	0.0 ± 0.0a
	Methanol	44.2 ± 0.3a	47.4 ± 2.0a	94.0 ± 2.7a	100.0 ± 0.0a	38.4 ± 2.2a	62.8 ± 5.6a	0.0 ± 0.0a	0.0 ± 0.0a
(Sl)	Hexane	55.3 ± 1.0c	69.6 ± 1.4c	94.1 ± 3.4a	96.6 ± 4.7a	52.3 ± 5.0b	69.4 ± 0.9b	0.0 ± 0.0a	0.0 ± 0.0a
	Chloroform	35.6 ± 3.2b	44.0 ± 1.3b	95.8 ± 7.3a	98.3 ± 2.3a	48.6 ± 5.8b	74.5 ± 8.1b	0.0 ± 0.0a	0.0 ± 0.0a
	Methanol	12.0 ± 1.9a	22.0 ± 2.1a	94.9 ± 4.2a	98.3 ± 2.3a	31.4 ± 2.7a	38.8 ± 1.1a	0.0 ± 0.0a	0.0 ± 0.0a
(SD)	Hexane	49.6 ± 4.3c	64.4 ± 3.4c	95.0 ± 6.2a	100.0 ± 0.0a	39.3 ± 4.3a	52.2 ± 4.2a	0.0 ± 0.0a	0.0 ± 0.0a
	Chloroform	14.2 ± 1.3b	20.3 ± 0.4a	93.5 ± 3.2a	95.0 ± 4.0a	36.6 ± 4.9a	55.8 ± 3.0a	0.0 ± 0.0a	0.0 ± 0.0a
	Methanol	33.5 ± 2.3a	45.6 ± 1.2b	91.8 ± 4.0a	96.6 ± 2.3a	31.4 ± 3.4a	52.5 ± 2.0a	0.0 ± 0.0a	0.0 ± 0.0a

RT, roots; SH, shoots; Sl, siliquae and SD, seeds. GI, germination index; GT, total germination. Means with the same letter in a column are not significantly different at $P < 0.05$ (LSD test). Values ($N = 3 \pm$ S.E.).

inhibited by percentages ranging between 50% and 100% in all cases. For radish and peganum, their greater sensitivity was registered with RT and Sl extracts, where a total inhibition was recorded from 40 g/L, below this concentration average percentage inhibition was 64%. SH extracts were more toxic for radish compared to peganum, hence it induced an inhibition varying from 36% to 100% for the first and from 21% to 87% for the second. Contrarily, S extract was more toxic for peganum provoking an average reduction of 86% for all

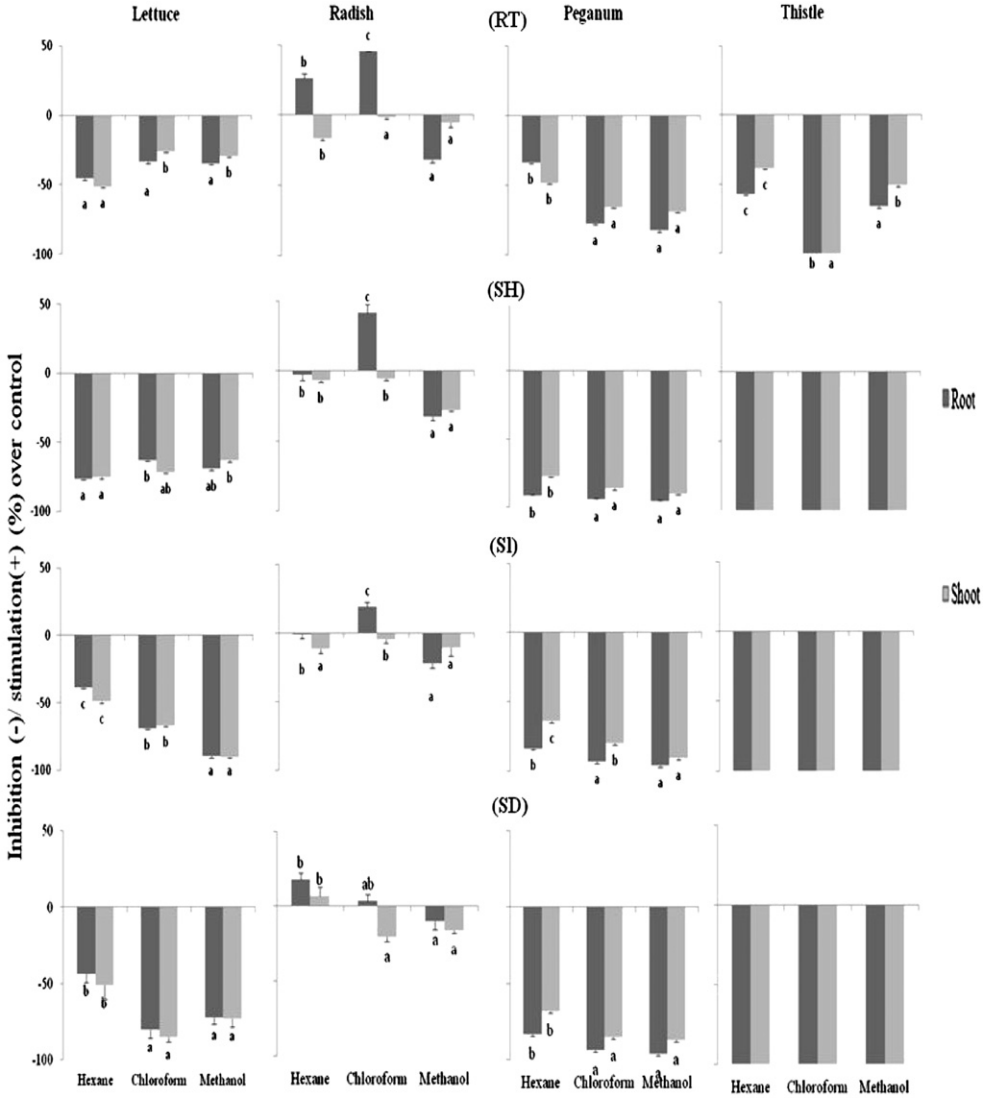


Fig. 2. Inhibition in % of control of root and shoot lengths of target species, 7 days after germination, in the presence of three organic extracts of *C. arabica* root (RT), shoots (SH), siliquae (Sl) and seeds (SD), at 6 g/L. The bars on each column show standard error. Values ($N = 3 \pm$ S.E.). Different letters in columns indicate significant differences among treatments at $P < 0.05$ (LSD test).

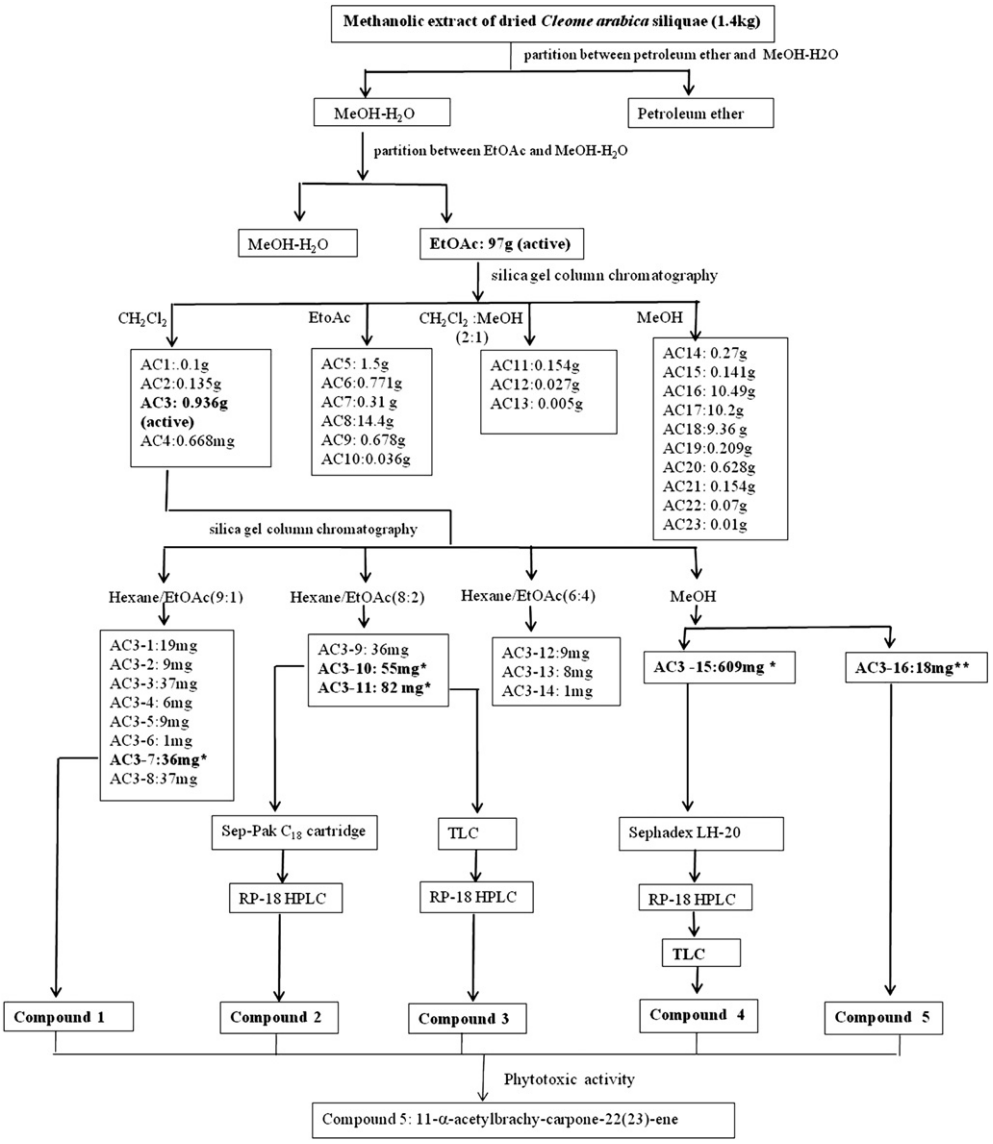


Fig. 3. Chromatographic steps led to the isolation of active compounds.

concentrations however; this reduction was about 47% until 40 g/L and 100% at 50 g/L for radish (Fig. 1).

3.2. Effect of C. arabica organic extracts on germination and growth

3.2.1. Yield of organic extracts

Siliquae and seeds possess the highest yields with chloroform solvent (3.08% and 2.95%, respectively). However, root methanol extract gave the lowest yield (0.14%). For seed extract, hexane solvent gave better yield (1.88%) than chloroform and methanol (1.86% and 1.71%, respectively) (Table 3).

3.2.2. Germination

Methanol, where residues were dissolved, had no effect on germination; hence effects could be attributed to allelochemicals present in organic extracts. Thistle germination was the most sensitive compared to the other target species, indeed a total inhibition was recorded in the presence of all organic extracts, except RT hexane and methanol extracts which gave respective GT of 36% and 54% (Table 4). However, radish germination was the most resistant which gave similar values of GT than control in all cases. Moreover, peganum germination was significantly delayed by all methanol extracts with an average GI of 35% while in other cases this index varied between 36% and 68%. The lowest GT (38%) was observed in the presence of Sl methanol extract. For

Table 5
Effect of *C. arabica* siliquae extracts on germination parameters expressed in % of control of lettuce.

Siliquae extracts		Petroleum ether		Ethyl acetate		Methanol–water	
		GI	GT	GI	GT	GI	GT
Concentrations (g/L)	0.06	84.0 ± 5.5a	100.0 ± 5.3a	65.7 ± 8.8c	68.8 ± 6.4c	67.9 ± 6.3b	76.5 ± 5.2b
	0.6	87.0 ± 7.3a	91.3 ± 6.4a	26.7 ± 3.6b	34.7 ± 3.4b	58.3 ± 7.6b	68.1 ± 4.7b
	6	67.2 ± 4.2b	91.3 ± 6.4a	0.0 ± 0.0a	0.0 ± 0.0a	33.7 ± 1.6	30.4 ± 2.8a

GI, germination index; GT, total germination. Means with the same letter in a column are not significantly different at $P < 0.05$ (LSD test).Values ($N = 4 \pm S$).

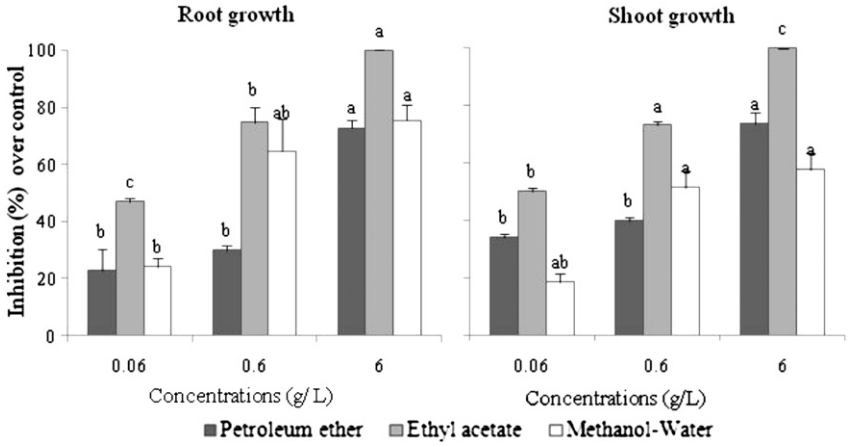


Fig. 4. Inhibition in % of control of root and shoot lengths of target species, 7 days after germination, in the presence of *C. arabica* siliquae petroleum ether, ethyl acetate and methanol–Water extracts (at 0.06, 0.6 and 6 g/L). The bars on each column show standard error. Values (N = 4 ± S.E.). Different letters in columns indicate significant differences among treatments at $P < 0.05$ (LSD test).

lettuce, which was the most sensitive species, Sl methanol extract and SD chloroform extract were the most toxic; they gave respective GI of 12% and 14.2% and an average GT of 21% (Table 4).

3.2.3. Growth

For seedling growth, weeds were more sensitive than crops with RT and SH growth showing similar behavior except for radish where roots were less sensitive in the presence of all chloroform fractions and in the presence of RT and SD hexane extracts. Indeed, Sl and SH chloroform extracts provoked a respective stimulation of 19% and 41% for radish root length while its shoot length was reduced by 5% compared to the control (Fig. 2). With the exception of RT hexane and methanol extracts which induced a reduction to the half for thistle growth, all the other organic extracts provoked its total inhibition. Similarly, all organic extracts induced a significant inhibition of peganum growth by more than 70%, excluding RT hexane extract which reduced root and shoot lengths by an average of 40%. Lettuce growth was sensitive especially to Sl methanol and SH hexane extracts which induced respectively an average inhibition of 89% and 75%. Both SD chloroform and methanol extracts induced a significant inhibition of seedling lettuce by 78%. Organic extracts of RT were less toxic for lettuce inducing an inhibition less than 50% (Fig. 2).

Table 6
Germination index (GI) and total germination (GT) expressed in % of control of lettuce in the presence of *C. arabica* siliquae ethyl acetate fractions at 0.6 g/L.

Fractions	GI	GT
AC_1	86.5 ± 1.5defg	82.8 ± 6.8d
AC_2	57.2 ± 2.9bc	52.6 ± 3.7c
AC_3	5.0 ± 0.8a	5.0 ± 0.1a
AC_4	20.0 ± 3.2ab	15.3 ± 4.5a
AC_5	46.9 ± 4.1b	35.6 ± 4.1b
AC_6	73.6 ± 8.7cd	67.7 ± 3.2cd
AC_7	94.7 ± 4.1defgh	81.5 ± 2.4d
AC_8	81.5 ± 8.1defg	69.6 ± 7.7cd
AC_9	79.2 ± 2.8cd	71.2 ± 1.7cd
AC_{10}	90.8 ± 1.0defgh	77.9 ± 2.1d
AC_{11}	91.9 ± 0.1defgh	86.3 ± 6.6d
AC_{12}	101.7 ± 6.0fghi	82.8 ± 8.9d
AC_{13}	97.1 ± 4.0defgh	83.0 ± 6.1d
AC_{14}	86.0 ± 1.6defg	73.0 ± 7.9cd
AC_{15}	93.9 ± 5.9defgh	81.3 ± 2.6d
AC_{16}	87.4 ± 1.6defg	77.8 ± 5.0d
AC_{17}	100.7 ± 1.2fghi	79.8 ± 1.6d
AC_{18}	111.5 ± 5.8hi	81.4 ± 4.3d
AC_{19}	83.6 ± 1.2defg	67.8 ± 2.0cd
AC_{20}	117.0 ± 1.7hi	83.2 ± 3.3d
AC_{21}	75.6 ± 5.9cd	71.4 ± 2.1cd
AC_{22}	76.1 ± 2.3cd	77.9 ± 6.1d
AC_{23}	79.4 ± 1.5cd	68.0 ± 3.3cd

Means with the same letter in a column are not significantly different at $P < 0.05$ (LSD test). Values (N = 4 ± S.E.).

3.3. Isolation and identification of bioactive compounds from siliquae of *C. arabica*

After testing *C. arabica* aqueous and organic extracts, results revealed that siliquae methanol extract was the most active one. For this reason, it was selected for further chemical study and the extraction series diagram from this extract is shown in (Fig. 3). Lettuce was used as target species as it was most sensitive to chemicals at low concentrations (Olofsdotter, 2001).

3.3.1. Biological activity of petroleum ether, ethyl acetate and methanol–water extracts

Methanolic extract of siliquae (Sl) was separated into three fractions: petroleum ether, ethyl acetate and methanol–water extracts, their bioassays were tested at 0.06, 0.6 and 6 g/L on lettuce germination and seedling growth (Table 5 and Fig. 4). Results exhibited a significant inhibition in which magnitude increased with the increasing concentration. At the highest concentration ethyl acetate was the most toxic, it induced a total inhibition of germination (Table 5). Petroleum ether fraction was less active than MeOH–H_2O yet they induced a disparity inhibition by 8.7% and 69.6% with a significant delayed germination of 67.2% and 33.7% at the highest concentration, respectively.

In the same trend, ethyl acetate showed a highly significant phytotoxic effect on lettuce seedling growth which was completely inhibited at the highest concentration (Fig. 4). However, petroleum ether and MeOH–H_2O fractions induced a slight phytotoxic effect with an average of 24% at 0.06 g/L and 34.9% and 58% at 0.6 g/L, respectively.

The data generated led us to select ethyl acetate extract for further bio-guided phytotoxic assays.

3.3.2. Biological activity of ethyl acetate fractions

The ethyl acetate extract was chromatographed over flash chromatography on silica gel and the fractions obtained were tested on lettuce germination and seedling growth at 0.6 g/L.

The ethyl acetate fractions induced a significant effect on lettuce germination that depended on fraction polarity, since the inhibition decreased with polar fractions (Table 6). Thus, the greatest inhibition was registered with fractions eluted by CH_2Cl_2 and the most toxic one was AC_3 which induced an inhibition of 95%. The inhibition of GT varied from 18.5% to 64.4% and the GI varied between 5% and 53% when

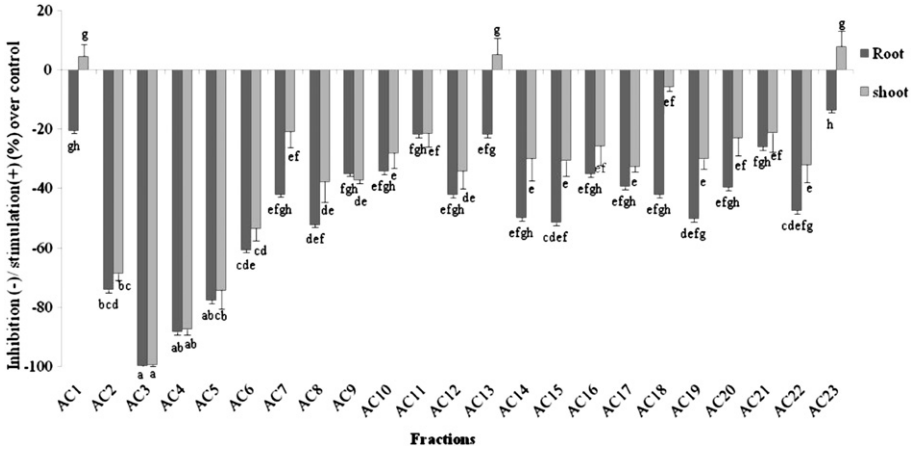


Fig. 5. Inhibition in % of control of *L. sativa* root and shoot lengths, 7 days after germination, in the presence of *C. arabica* siliquae ethyl acetate fractions at 0.6 g/L. Values (N = 4 ± S.E.). Different letters in columns indicate significant differences among ethyl acetate fractions at $P < 0.05$ (LSD test).

fractions were eluted by EtOAc. The polar fractions induced a slight inhibition which was varied between 2 and 24% (Table 6).

The same result was obtained on seedling growth with the root more sensitive (Fig. 5). AC_3 was the most active fraction; it was eluted by CH_2Cl_2 and induced 99% inhibition. Moreover, the other fractions also induced a significant inhibition with varied disparity. The inhibition of fractions eluted by EtOAc ranged between 30% and 75% and was less than 40% for the other fractions (Fig. 5).

3.3.3. Biological activity of the fractions from flash silica gel column chromatography

The active fraction AC_3 was loaded on flash column chromatography silica gel. A total of 16 fractions were obtained and screened for their allelopathic effect on lettuce at 0.06 g/L. Lettuce total germination was not affected as it gave similar values to control in all subfractions. In this case, germination index was more or less inhibited by all subfractions, and percentage inhibition varied from 1% to 12%, except AC_{3-4} and AC_{3-16} which gave similar values than control (Table 7). However, seedling growth was significantly affected by all subfractions and root was more sensitive compared to shoot (Fig. 6). The most active subfraction was AC_{3-16}, it was eluted by MeOH and induced 73% inhibition for root length and 57% for shoot length. The subfractions AC_{3-7}, AC_{3-10}, AC_{3-11} and AC_{3-15} revealed a significant inhibition which exceeded 50%, while the other fractions did not exhibit strong phytotoxicity to lettuce seedling growth (Fig. 6).

Table 7
Germination index (GI) and total germination (GT) expressed in % of control of lettuce in the presence of siliquae fractions from flash silica gel column chromatography at 0.06 g/L.

Fractions	GI	GT
AC_{3-1}	96.4 ± 3.6abc	100.0 ± 0.0a
AC_{3-2}	93.2 ± 4.2abc	100.0 ± 0.0a
AC_{3-3}	98.0 ± 2.8c	100.0 ± 0.0a
AC_{3-4}	100.9 ± 1.9c	100.0 ± 0.0a
AC_{3-5}	92.8 ± 4.1abc	100.0 ± 0.0a
AC_{3-6}	96.1 ± 3.5abc	100.0 ± 0.0a
AC_{3-7}	89.8 ± 5.0a	100.0 ± 0.0a
AC_{3-8}	94.0 ± 6.8ab	98.3 ± 2.3a
AC_{3-9}	90.0 ± 5.4a	98.3 ± 2.3a
AC_{3-10}	87.7 ± 3.0a	98.3 ± 2.3a
AC_{3-11}	91.7 ± 5.1abc	100.0 ± 0.0a
AC_{3-12}	92.1 ± 4.4abc	100.0 ± 0.0a
AC_{3-13}	96.1 ± 4.3bc	100.0 ± 0.0a
AC_{3-14}	97.8 ± 2.2bc	100.0 ± 0.0a
AC_{3-15}	99.7 ± 4.6c	100.0 ± 0.0a
AC_{3-16}	100.2 ± 2.3c	96.6 ± 2.3a

Means with the same letter in a column are not significantly different at $P < 0.05$ (LSD test). Values (N = 4 ± S.E.).

3.3.4. Compound characterization

The most active allelopathic substance was isolated as a yellow amorphous powder (18 mg). The molecular formula of this substance was determined to be $C_{31}H_{44}O_8$ based on its mass spectrum (*m/z*: 544.5 for $[M]^+$). $[\alpha]_D = +18°$ (CH_2Cl_2); IR ν_{max} (CH_2Cl_2, c 0.03) cm^{-1}: 3050, 2980, 1734, 1722, 1580, 1421, 1270, and 895. ^1H NMR (500 MHz, $CDCl_3$) δ 7.34 (1H, d, J = 5.7 Hz, H-22), 6.05 (1H, d, J = 5.7 Hz, H-23), 5.19 (1H, br d, J = 5.7 Hz, H-1), 5.17 (1H, m, H-11), 3.18 (1H, dd, J = 15.5, 5.5 Hz, H-2a), 2.91 (1H, br d, J = 15.4 Hz, H-2b), 2.00 (3H, s, CH_3CO), 1.94 (3H, s, CH_3CO), 1.47 (3H, s, Me-28), 1.43 (3H, s, Me-30), 1.41 (3H, s, Me-29), 1.21 (3H, s, Me-21), 1.11 (3H, s, Me-19), and 0.92 (3H, s, Me-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2 (C-24), 172.1 (C-3), 170.6 ($\underline{C}OCH_3$), 170.4 ($\underline{C}OCH_3$), 159.4 (C-22), 121.1 (C-23), 91.1 (C-20), 84.5 (C-4), 78.8 (C-11), 72.7 (C-1), 53.6 (C-17), 51.1 (C-13), 50.0 (C-14), 46.5 (C-9), 43.9 (C-10), 41.9 (C-5), 41.5 (C-8), 36.9 (C-2), 34.8 (C-15), 34.4 (C-12), 30.5 (C-7), 30.4 (C-28), 28.9 (C-16), 26.6 (C-29), 23.5 (C-6), 23.1 (C-30), 21.7 ($CO\underline{C}H_3$), 21.6 ($CO\underline{C}H_3$), 17.1 (C-21), 15.7 (C-19), and 14.4 (C-18). From a comparison of these data with those reported in the literature (Ahmed et al., 1997), the substance was identified as 11-α-acetylbrachy-carpone-22(23)-ene (compound 5) (Fig. 7).

Compound 1 was identified as β-sitosterol: IR ν_{max} (CH_2Cl_2, c 0.03) cm^{-1}: 3040 and 2935. ^1H NMR (500 MHz, $CDCl_3$) δ 5.35 (1H, m, H-6), 3.53 (1H, m, H-3), 1.00 (3H, s, H-19), 0.91 (3H, d, J = 6.2 Hz, H-21), 0.85 (3H, t, J = 5.9 Hz, H-29), 0.83 (3H, d, J = 6.0 Hz, H-26), 0.81 (3H, d, J = 6.0 Hz, H-27), and 0.67 (3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.7 (C-5), 121.6 (C-6), 71.7 (C-3), 56.7 (C-14), 56.0 (C-17), 51.1 (C-9), 45.8 (C-24), 44.0 (C-13), 42.2 (C-4), 39.8 (C-12), 37.3 (C-1), 36.4 (C-10), 36.1 (C-20), 33.9 (C-22), 31.9 (C-7), 31.8 (C-8), 31.6 (C-2), 29.1 (C-25), 28.2 (C-16), 26.1 (C-23), 24.1 (C-15), 23.1 (C-28), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 19.2 (C-27), 18.7 (C-21), 11.8 (C-18), and 11.0 (C-29) (Fig. 7).

Compound 2 was identified as 17-α-hydroxycabraleactone: $[\alpha]_D = +18$ (CH_2Cl_2); IR ν_{max} (CH_2Cl_2, c 0.03) cm^{-1}: 3520, 3058, 2988, 1734, 1421,1270, and 895. ^1H NMR (500 MHz, $CDCl_3$) δ 2.70, 2.68, 2.66, 2.63, 2.61, 2.60, 2.51, 2.49, 2.47, 2.46, 2.45, 2.44, 1.45 (Me-21), 1.17 (Me-28), 1.09 (Me-19), 1.04 (Me-29), 1.00 (Me-18), and 0.95 (Me-30) (Fig. 7).

Compound 3 was identified as amblyone: $[\alpha]_D = +18$ (CH_2Cl_2); IR $_{max}$ (CH_2Cl_2, c 0.02) cm^{-1}: 3520, 3058, 2988, 1734, 1421,1270, and 895. ^1H NMR (500 MHz, $CDCl_3$) δ 4.24 (1H, dd, J = 8.9, 1.4 Hz, H-19), 3.73 (1H, dd, J = 8.9, 1.0 Hz, H-19′), 2.64 (1H, m, H-23), 2.54 (3H, m, H-22

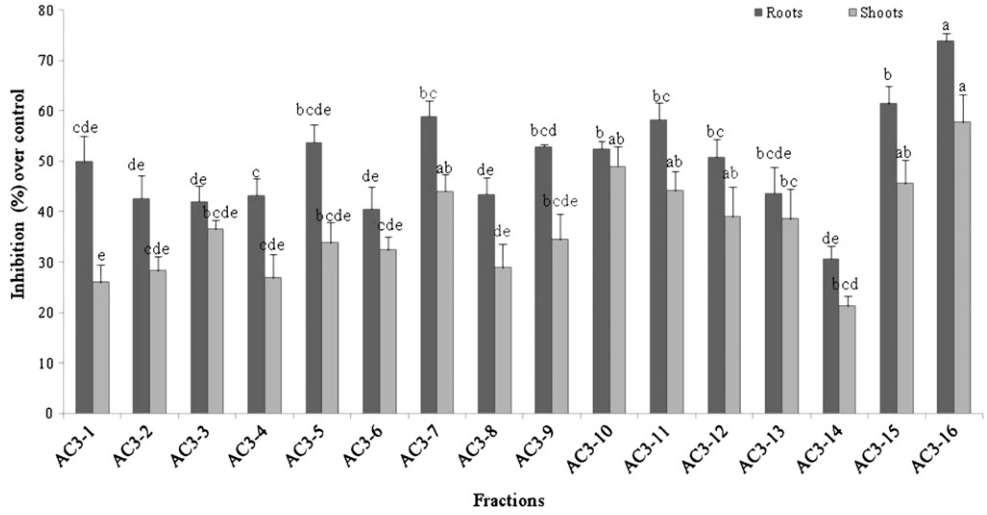
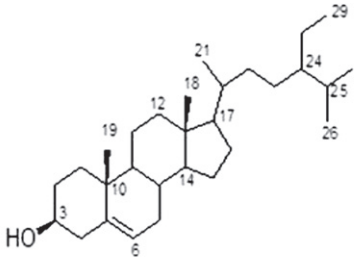


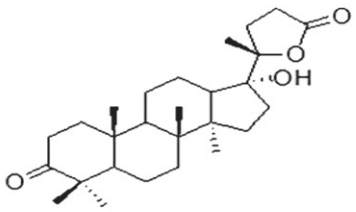
Fig. 6. Inhibition in % of control of *L. sativa* root and shoot lengths, 7 days after germination, in the presence of siliquae fractions from flash silica gel column chromatography at 0.06 g/L. Values (N = 4 ± S.E.). Different letters in columns indicate significant differences among treatments at P < 0.05 (LSD test).

and H-23′), 1.36 (3H, s, Me-21), 1.03 (3H, s, Me-28), 0.98 (3H, s, Me-29), 0.89 (3H, s, Me-18), and 0.86 (3H, s, Me-30) (Fig. 7).

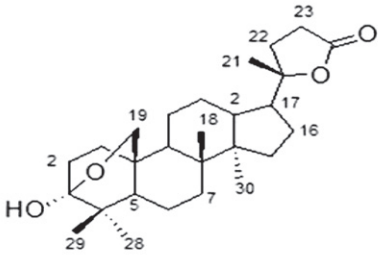
Compound 4 was identified as calycopterin: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (2H, d, J = 8.1, H-2′ and H-6′), 6.98 (2H, d, J = 8.1, H-3′ and H-5′), 4.11 (3H, s, 7-OMe), 3.95 (6H, s, 6-OMe and 8-OMe), and 3.87 (3H, s, 3-OMe). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.6 (C-2), 138.8 (C-3), 178.6 (C-4), 148.4 (C-5), 135.9 (C-6), 153.1 (C-7), 133.5 (C-8), 145.0 (C-9), 107.0 (C-10), 123.3 (C-1′), 130.8 (C-2′ and C-6′), 116.4 (C-3′ and C-5′), 158.6 (C-4′), 62.7 (7-OMe), 61.2 (8-OMe), 61.0 (6-OMe), and 60.1 (3-OMe) (Fig. 7).



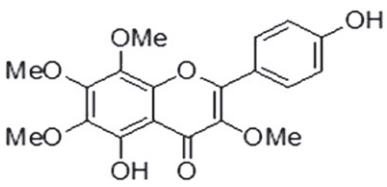
Compound 1:β-sitosterol



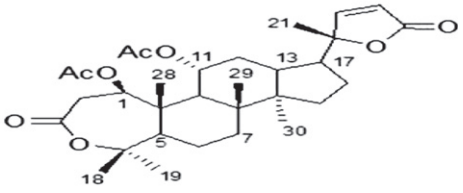
Compound 2: 17- α -hydroxycabraleactone



Compound 3: Amblyone



Compound 4: Calycopterin



Compound 5 : 11-α-acetylbrachy-carpone-22(23)-ene

Fig. 7. Chemical structure of *C. arabica* siliquae active allelochemicals.

3.4. Biological activity of purified compounds

Isolated compounds were bio-assayed for their inhibitory effect on lettuce germination and growth at 0.06 g/L (Table 8). Results revealed that the identified compounds differed markedly in their phytotoxic effects on lettuce seedling growth. Root growth was the most sensitive variable, followed by shoot growth then seed germination. Compound 5 was the most toxic on GT and GI with a respective inhibition of 21% and 42%. Furthermore, the other compounds induced a slight inhibition on lettuce germination that did not exceed 32.5% Similarly, lettuce growth was significantly affected by compound 5; showing 73% and 67% inhibitory effect towards the growth of root and shoot respectively. Indeed, root length was affected by an average inhibition of 62% in the presence of the other compounds while shoot length was affected by an average inhibition of 61% by compounds 2 and 3 and a less toxic effect was recorded for compounds 1 and 4 (33% of inhibition) (Table 8).

4. Discussion

The present study revealed a potent phytotoxic effect for different parts of *C. arabica* on germination and growth of target species. All aqueous extracts at all concentrations markedly reduced germination and growth of target species with concentration-dependent mechanism. A number of previous studies reported that the degree of inhibition increased with increasing extract concentrations (Chung and Miller, 1995; Laosinwattana et al., 2009). This result suggests that *C. arabica* aqueous extracts contain water-soluble substances which inhibited germination and growth of target species. Root (RT) aqueous extract was the most toxic for lettuce seedling growth, which showed a significant inhibition of 94%, followed by siliquae (Sl) and seed (SD) extracts inducing 74% inhibition, then shoot (SH) extract with 53% inhibition at the lowest concentration. In fact, Chon et al. (2004) concluded from studies using aqueous alfalfa leaf extract that delayed seed germination and especially reduced root elongation were due mainly to toxic factors of the leaf extract. According to Alam and Islam (2002), inhibition of seed germination of crop plants is due to disturbance of the peroxidase activities, alpha-amylase and acid phosphates. These results were confirmatory to other findings where reduction in root length, and germination by aqueous extracts has been reported (Kil et al., 2002; Siddiqui, 2007).

To determine the chemical group to which bioactive molecules of *C. arabica* belong, we conducted a fractional extraction in three organic solvents with increasing polarity. A contrast between solvents indicates that the hexane extract was significantly less potent than chloroform and methanol extracts. The different organic extracts influence germination and seedling growth for all target species. The lettuce seeds are highly sensitive to inhibitory and stimulatory chemical compounds (Fujii et al., 2003). Besides this, root growth is substantially sensitive compared to shoots, which was suggested as the best indicator of phytotoxic effect of allelochemicals (Chon et al., 2000; Haouala et al., 2008; Omezzine et al., 2011). Furthermore, the root tissue permeability to allelochemicals was reported to be greater than that of shoot tissue (Nishida et al., 2005). The inhibitory effect might have occurred through a variety of mechanisms like reduced mitotic activity in roots and shoots, reduced rate of ion uptake, inhibition of photosynthetic respiration and enzyme action (Rice, 1974). Such an outcome might be expected because it is likely that roots are first to absorb allelochemicals from the environment (Turk and Tawaha, 2002) which in turn might inhibit cell division (Rietjens and Alink, 2003) and is highly active at meristimatic tissue of the growing root tip.

Table 8
Effects of identified compounds from siliquae *C. arabica* on germination index (GI), total germination (GT), root length (R^L) and shoot length (S^L) of *L. sativa* at 0.06 g/L.

Compounds	(GI)	(GT)	(R^L)	(S^L)
1) β-Sitosterol	70.2 ± 3.8b	98.2 ± 3.5a	49.7 ± 2.0a	64.4 ± 2.3a
2) 17-α-Hydroxycabraleactone	80.1 ± 1.7a	96.6 ± 2.3a	31.9 ± 1.6b	35.0 ± 1.8c
3) Amblyone	83.8 ± 2.4a	88.3 ± 2.8b	36.4 ± 2.7b	42.2 ± 1.7b
4) Calycopterin	67.5 ± 1.1b	93.2 ± 5.3a	34.2 ± 0.4b	68.9 ± 2.2a
5) 11-α-Acetylbrachy-carpone-22(23)-ene	57.4 ± 0.2c	78.6 ± 1.9b	26.6 ± 1.0b	32.7 ± 0.5c

Means with the same letter in a column are not significantly different at $P < 0.05$ (LSD test). Values (N = 4 ± S.E.).

The isolation and characterization of marker compounds are one of the most important areas of research on plants rich in molecules of interest. The isolation of markers allows structural determination of bioactive compounds that may enable production of synthetic material, incorporation of structural modification and rationalization of mechanism of action (Khan et al., 2006). Hence, the present study aimed to isolate different chemical constituents from *C. arabica* methanol extract. The relatively high inhibitory property of Sl methanol extract subfractions suggested the presence of many active chemical constituents possibly acting together. These results indicated that extraction of natural compounds by appropriate solvent systems would be important to obtain fractions with high allelopathic potential. This finding is supported by Li et al. (2006) and Luthria et al. (2007). These authors reported that different solvent systems used for the extraction of secondary metabolites from plant materials resulted to extraction efficacy. The acetate ethyl fraction exhibited a total inhibition on lettuce germination and seedling growth at the highest concentration. The phytotoxic effect might be related to specific allelopathic compounds being produced in larger quantities in certain fraction, imparting a higher level of allelopathy. A compound responsible for this toxicity is identified as damarane type triterpene in particular 11-α-acetylbrachy-carpone-22(23)-ene, 17-α-hydroxycabraleactone and amblyone which were previously isolated from *Cleome amblyocarpa* (Ahmed et al., 1997). Indeed, Khan et al. (2008) reported that amblyone have induced several effects such as antibacterial, antifungal and cytotoxic activities. However, calycopterin and β-sitosterol showed less phytotoxic effect on lettuce germination and seedling growth compared to the other separated molecules. β-Sitosterol is a known plant sterol which has been reported to be abundant in wheat germ oil, cotton seed oil, corn oil, and soybean oil (Chen, 1991). This phytosterol was reported to possess analgesic, anthelminthic, and antimutagenic activities (Villaseñor et al., 2002), anti-proliferative and apoptotic potential in several cancer models (Awad and Fink, 2000). It was also considered as a chemopreventive agent (Rao and Koratkar, 1997; Madhavi et al., 1998). Although the mechanism of action is not exactly known. It can also be the factor used to form the lympho and natural killer cells (NK cells) in the immunity process circulation (Bouic et al., 1996). Calycopterin, the major flavonol of *Digitalis thapsi* L. (Scrophulariaceae) and *Calycopteris floribunda* Lamk (Combretaceae) was isolated by Ratnagiriswaran et al. (1934). This compound inhibited lymphocyte proliferation in a dose-dependent manner with an IC_{50} value of 1.7 μg/mL (Faham et al., 2008). Despite the medicinal values of these compounds there is no available information on their role in allelopathy.

There are increasing evidences showing that allelochemicals have significant effects on cell division, cell differentiation, ion and water uptake, water status, phytohormone metabolism, respiration, photosynthesis, enzyme function, and signal transduction as well as gene expression (Singh and Thapar, 2003; Belz and Hurle, 2004). It is quite possible that allelochemicals may produce more than one effect on the cellular processes responsible for reduced plant growth. However, the details of the biochemical mechanism through which a particular compound exerts a toxic effect on the growth of plants are not well known.

5. Conclusion

The present study reported the phytotoxic activity of *C. arabica* extracts of which the siliquae fractions and the most active were used

to isolate the active compound elucidated as 11-α-acetylbrachy-carpone-22(23)-ene. This compound can be employed in developing new types of herbicides as well as for biorational management tools for controlling weeds on crops. However, their effects on natural enemies, crops, or the environment have not been fully investigated.

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