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Allelopathic potential of *Senna occidentalis* (L.) Link.

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ABSTRACT

In glasshouse pot culture we determined the bioactivity of crude ethanol extract (CEE) and semi-purified fractions (SFs) obtained with hexane (HF), ethyl acetate (EAF) and aqueous-ethanol (AEF) of *Senna occidentalis* shoots on the germination and growth of lettuce (*Lactuca sativa* L.) tomato (*Lycopersicon esculentum* Mill.), onion (*Allium cepa* L) and wheat (*Triticum aestivum* L.). Both CEE and the SFs of *S. occidentalis* inhibited 50% emergence of two dicotyledon test plants. While EAF inhibited the root growth in two monocotyledon test plants and shoot growth in tomatoes. The AEF inhibited the root and shoot growth of dicots and root growth of monocots. The low concentrations of extracts and fractions stimulated the onion growth and also increased the dry biomass in all test species. Thin-layer chromatography, detected the terpenes in HF and phenolic compounds and alkaloids in EAF. The spectrophotometer analyses found the highest total phenol and flavonoid content in EAF. The bioassays showed that aerial parts of *S. occidentalis* have allelopathic potential and may be useful in weed management programmes.

Key words: Allelochemicals, dry weight, natural herbicide, pot culture, root growth inhibition, seedling emergence, *Senna occidentalis,*

INTRODUCTION

Secondary metabolism of plants provides numerous chemical structures that can be used in their natural state or modified to produce natural pesticides (4). In past 30 years, significant efforts have been done to develop the novel allelochemicals with potential application for weed control. Agrochemicals developed from the natural compounds have important advantages (different modes of action, high biodegradability and low impact on environment) over synthetic herbicides (3,15,26). In Brazil *Senna occidentalis* (L.) Link., is invasive shrub in pastures and crops in Mato Grosso do Sul state (11). From its species *Senna fistula*, *Senna torosa*, *Senna sophera* and *Senna spectalis* alkaloids (23) anthraquinones (8), flavonoids (12, 23), phenolic compounds and proanthocyanidins (12) have been isolated.

S. occidentalis extracts has purgative, hepatic, bactericidal, antipyretic, antitumor, expectorant, anti-inflammatory, diuretic, antifungal and neurotoxic effects on cattle (23), Chemical studies have isolated anthraquinones, flavonoids, polysaccharides (2, 12), piperidine alkaloids (23) and xanthones (24) from this specie. The 1,8-dihydroxi-

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anthraquinone is most active compounds isolated from *S. occidentalis* has antimalarial activity *in vitro* (23). *S. occidentalis* occurs in nearly pure colonies and hinders establishment of other species, thus changing the vegetation structure. Therefore, its biological activity diversity of its isolated compounds and its invasive impact in Cerrado (tropical savanna) led us to study its allelopathic potential, as this information is not available.

This study aimed to evaluate the allelopathic potential of crude ethanolic extract (CEE) and semi-purified fractions (SFs) of *Senna occidentalis* shoots on the emergence and growth of dicots and monocots in glasshouse bioassays.

MATERIALS AND METHODS

I. Plant collection

Fully grown *Senna occidentalis* plants were collected in Nov 2002 from a degraded pasture at Fazenda Boa Vista, Rio Brilhante, MS, Brazil (21º45'S, 54º32'W). The extracts were obtained from 2.24 kg flowering plants. One voucher specimen was deposited in the Herbarium DDMS of the Universidade Federal de Mato Grosso do Sul, MS, Brazil (Number 212). The aerial (stem, leaves and flowers) and subterranean (root) parts of the plants were cut and stored frozen in plastic bags at -7ºC until use.

II. Crude ethanol extract (CEE) and semi-purified fractions (SFs)

The aerial parts of *S. occidentalis* (1.02 kg) were cut into small pieces and extracted with ethanol (1:2 w/v ratio) for 7-days. It was further extracted three times (ethanolic extract) and the aliquots of extractions were collected and filtered. The solvent was evaporated from the filtrate in rotary evaporator under vacuum (± 40°C) and yielded 106.05 g final crude ethanolic extract (CEE).

The semi-purified fractions (SFs) were obtained from 45.32 g of the CEE by liquid-liquid partition with hexane and ethyl acetate. The CCE was partitioned successively with hexane and ethyl acetate, after the solvents were removed under reduced pressure to afford the semi-purified fractions (SFs). The fractioning yielded 7.58 g of the hexane fraction (HF), 4.85 g of the ethyl acetate fraction (EAF) and 32.79 g of the aqueous-ethanol fraction (AEF). To determine the water content in extracts, aliquots of CEE and SFs were dried (100ºC) in oven for 10 h.

III. Plants bioassays

The dry CEE and the SFs were weighed on an analytical scale (0.001 g accuracy) and diluted in complete nutritents solution (6) to get stock solutions of 1.000 $mg.L^{-1}$ concentration. Two other solutions (500 and 250 $mg.L^{-1}$ concentration) were prepared by dilution.

The solutions were buffered with 10 mM MES (2-morpholinoethane sulfonic acid) solution and adjusted to pH 6.0 (14) with 0.1 N KOH solution. The same protocol was used to prepare a control solution without CEE or SFs.

In bioassays, the dicots were lettuce (*Lactuca sativa* L. cv. Grand Rapids) and tomato (*Lycopersicon esculentum* Mill cv. Santa Clara) and the monocots were onion

(*Allium cepa* L. cv. Baia Periforme) and wheat (*Triticum aestivum* L. cv. RRS 220), all obtained from commercial dealers.

Plastic pots (7.0 cm dia, 6.0 cm height) were filled with 160 g pre-washed sand, oven-dried (120ºC) and sieved (4.4 mm mesh). Each pot was irrigated with 40 mL of treatment solution and sown with 5-seeds of each test specie separately at ~1.0 cm depth (21). Pots were kept in glasshouse (25°C) and irrigated daily with distilled water (21). The complete nutrient solution was used once a week, instead of distilled water (1).

The number of emerged seedlings in each pot were recorded daily and the plants were harvested 28 days after sowing. Number of leaves were counted and the shoot length (cm) was measured. The harvested seedlings were dried in oven at 60ºC to reach constant dry mass for 72 h (20).

The bioassays was also done using commercial herbicides (obtained from local dealer). The herbicides used for dicots were the post-emergence [Glifosato 480 Agripec (glyphosate) and Basagran 600 (bentazon) and the mixed herbicide Atrazina Nortox 500 SC (atrazine)]. For monocots, the post-emergence herbicides used were: Glifosato 480 (glyphosate) and Poast (sethoxydim) and the pre-emergent Gesagard 500 SC (Promethrin). The herbicides were used at 10^{-2} M concentration (14). The bioassays with herbicides were done as per the protocol used for CEE and the SFs for to use as controls.

A completely random design was used to analyse data from four assays (blocks CEE, HF, EAF and AEF) with four treatments each (0, 250, 500 and 1.000 $mg.L^{-1}$) and data from three assays with herbicides with two treatments each (0 and 10^{-2} M). Eight analyses trials were done for each plot, which contained five seeds to assess emergence and five to assess growth.

The emergence index-(EI) was calculated as under:

$$EI = \Sigma(G_i/N_i),$$

Where, G_i : Number of emerged seedlings within the period $t_{i-1} \leftrightarrow t_i$, and N_i : Number of post-sowing days (16). The emerged seedlings (E%) was calculated as under:

$$E\% = (\Sigma n_i.N^{-1}).\ 100,$$

Where, n_i : Number of seedlings emerged within the period $t_{i-1} \leftrightarrow t_i$ and N : Number of seeds used in each treatment (9).

IV. Statistical analyses

Data was analysed by analysis of variance (ANOVA), and Dunnett's test was used to compare the treatments means ($p < 0.05$). When parametric model predictions were violated, ANOVA was replaced by the non-parametric Kruskal-Wallis test and the Dunnett test was replaced by the Mann-Whitney U test. Alpha error was set at 5% for all the analyses. Data from herbicide bioassays were compared using Student's t test.

V. Chemical analysis

Preliminary thin layer chromatography (TLC) with silica gel $60F_{254}$ 20x20cm AL TLC chromatography plates (Merk) were used to identify the main classes of secondary compounds in CEE and in SFs. A 1.0% vanillin/ethanol H_2SO_4 solution was used as an indicator of terpenes and 1% ferric chloride as an indicator of phenolic compound.

For alkaloid detection, a CEE sample was subjected to alkaloid extraction. CEE (5.0 g) was dissolved in 10.0 mL distilled water acidified to pH 1.5 with 2.0 N HCl. After several extractions with ethyl ether, the remaining aqueous solution was alkalinized to pH 9.0 with ammonium hydroxide (NH_4OH) and extracted with either ethyl ether or with ethyl acetate. The etheric fraction and the ethyl acetate fraction were obtained after drying the solvent by rotary evaporator. The fractions were analyzed by TLC and revealed with Dragendorff's reagent (18).

The total phenolic content in CEE and SFs was determined by the Folin-Ciocalteau procedure (10,17). A standard curve plotted with gallic acid (25 to 600 µg) was used as reference. Phenolic content was determined from 5.0 g of each sample, which were dissolved in 5.0 mL distilled water. Aliquots of 1.0 mL of this solution were transferred to a 50 mL flask, and 30.0 mL distilled water and 2.0 mL of Folin-Ciocalteau's reagent were added. After 6 min, a 20% sodium carbonate solution (Na_2CO_3) was introduced and the flask was filled to volume with distilled water. The same protocol was used to prepare a blank solution without CEE or SFs samples. After 90 min at room temperature, the solutions were read with spectrophotometer at 760 nm.

To determine flavonoid content, a curve with quercetin (5.0 to 300.0 µg) was plotted as reference. Samples of 8.0 mg of CEE and SFs were dissolved in 4.0 mL pure ethanol. Thereafter, 2.0 mL aliquots of this solution were transferred to 25 mL flasks and 1.0 mL of 2.5% aluminium chloride, 1.0 mL of 10% sodium acetate and pure ethanol were added to fill the flask volume. This protocol was also used to prepare the blank solution except for the absence of CEE or SFs samples. After 40 min the solutions were read under spectrophotometer at 425 nm (10).

Data on growth and dry matter were expressed as a percentage of the control treatment, i.e., zero values are assigned to the control group, positive values indicate stimulation and negative values indicate inhibition (13).

RESULTS AND DISCUSSION

Effects of extracts and fractions

The CEE and the SFs from the aerial parts of *S. occidentalis* influenced the seedling emergence (EI and E %) of diocots (lettuce and tomato) than control (Tables 1 and 2). The effect was dose-dependent. With respect to EI, the hexane (HF) and ethanol-aqueous fractions (AEF) delayed the emergence of lettuce and tomato (65%) (Table 1). The lowest seedlings emergence (%E) was in lettuce treated with the highest concentrations of HF (82%) and AEF (54%) and in tomatoes treated with the highest concentrations of CEE (59%), AEF (59%) and HF (55%) (Table 2). These parameters were not affected in monocots.

The herbicide bioassays showed that both the CEE and the SFs influenced the dicot target plants, similar to post-emergent herbicides glyphosate and bentazon. They reduced the emergence index (EI) by 47% and emergence percentage (E%) by 43%. None of the extracts influenced the monocots (onion and wheat), similar to pre-emergence herbicide Gesagard. These results suggest that both CEE and the SFs from the aerial parts of *S. occidentalis* contain chemical components that affected the physiological processes

Table 1. Emergence index (EI; mean±sd) of plants treated with shoot extracts of *S. occidentalis*

Treatment[1]	Emergence Index (EI)		
	250 mg.L^{-1}	500 mg.L^{-1}	1.000 mg.L^{-1}
Lettuce; Control: 0.37±0.06			
CEE	CEE	CEE	CEE
HF	HF	HF	HF
EAF	EAF	EAF	EAF
AEF	AEF	AEF	AEF
Tomato; Control: 0.28±0.08			
CEE	CEE	CEE	CEE
HF	HF	HF	HF
EAF	EAF	EAF	EAF
AEF	AEF	AEF	AEF
Onion; Control: 0.28±0.14			
CEE	CEE	CEE	CEE
HF	HF	HF	HF
EAF	EAF	EAF	EAF
AEF	AEF	AEF	AEF
Wheat; Control: 0.52±0.21			
CEE	CEE	CEE	CEE
HF	HF	HF	HF
EAF	EAF	EAF	EAF
AEF	AEF	AEF	AEF

*Statistically different from the control treatment (Dunnet Test, $p < 0.05$); [ns] not significantly different from the control treatment; [*2] Kruskal-Wallis Test and Mann Whitney U Test. [1] CEE = crude ethanol extract; HF = hexane fraction; EAF = ethyl acetate fraction; AEF = aqueous-ethanol fraction.

Table 2. Seedlings emergence (mean percentage±sd) of plants treated with shoots extracts of *S. occidentalis*

Treatment[1]	Emerged seedlings (%)		
	250 mg.L^{-1}	500 mg.L^{-1}	1.000 mg.L^{-1}
Lettuce; Control: 55.00±9.26			
CEE	35.00±9.26*	30.00±10.69*	28.80±9.91*
HF	50.00±10.69	25.00±9.26*	10.00±15.12*
EAF	55.00±14.14	32.50±10.35*	32.00±10.35*
AEF	32.50±10.35*	25.00±9.26*	25.00±9.26*
Tomato; Control: 67.50±21.20			
CEE	32.50±10.40*	32.50±14.90*	27.50±14.90*
HF	32.50±14.90*	32.50±14.90*	30.00±15.10*
EAF	35.00±10.40*	35.00±14.10*	32.50±14.10*
AEF	27.50±14.90*	27.50±10.40*	27.50±10.40*
Onion; Control: 57.50±24.93			
CEE	52.50±21.21	47.50±18.52	40.00±14.88
HF	57.50±12.82	37.50±12.82	37.50±12.82
EAF	67.50±21.21	57.50±19.82	42.50±19.82
AEF	52.50±23.75[ns]	42.50±19.82[ns]	35.00±17.73[ns]
Wheat; Control: 70.00± 26.20			
CEE	67.50±18.30	57.50±25.60	55.00±27.10
HF	65.00±20.70	55.00±10.70	50.00±20.70
EAF	72.50±28.20	60.00±18.30	52.50±28.30
AEF	52.50±14.90	47.50±14.90	45.00±23.30

*Statistically different from the control treatment (Dunnet Test, $p < 0.05$); [ns] not significantly different from the control treatment. [1] CEE = crude ethanol extract; HF = hexane fraction; EAF = ethyl acetate fraction; AEF = aqueous-ethanol fraction.

of emergence in dicot seedlings, delaying and reducing their establishment under glasshouse conditions. The reduction in EI may decrease the reproductive capability by compromising the uniformity of seedling establishment (22).

Dicots (Figs. 1 and 2) treated with AEF at 1.000 $mg.L^{-1}$ suffered root growth inhibition (52% in lettuce, 35% in tomato) and shoot parts (39% in lettuce, 44% in tomato). This treatment also decreased the shoot dry weight of tomato (64%). In addition, CEE and the EAF inhibited the growth of same crop (Fig. 2B). The lowest concentrations tested stimulated the shoot growth in lettuce, root growth in tomato and increased root and shoot dry weights of lettuce and tomato. When the same treatments were applied at high concentrations, growth and shoot dry weights were reduced (Figs. 1 and 2).

Growth was inhibited in monocots treated with EAF and AEF (66% in onion with AEF at 1.000 $mg.L^{-1}$, 40% in wheat with EAF or AEF at 1.000 $mg.L^{-1}$) (Figs. 3A and 4A). The HF treatments at any concentrations decreased the onion root dry weight by 60% (compared to control) (Fig. 3C). The lowest concentrations stimulated the root growth in the onions (Fig. 3A), increased biomass accumulation in root (Fig. 3C) and in onions shoots (Fig. 3D) and increased the biomass accumulation in shoots of wheat (Fig. 4D).

The test herbicides reduced the growth and dry weight (60%-100%) of roots and shoots in target species (onion, wheat, lettuce and tomato). These results differed from those obtained with CEE and SF treatments. The AEF inhibited the growth of both dicots and monocots and the EAF inhibited the root growth of monocots. These results suggest that these fractions have chemical compounds with allelopathic potential. A severe reduction in root growth may affect the competitive ability and productivity of plants, whereas reduction in shoot may decrease their competitiveness for light (20).

The lowest concentrations of CEE and SFs enhanced the seedling development (50%). These treatments stimulated the root growth and shoot and dry weights of target species than control. Some compounds, (phenols and terpenes), promote growth at low concentrations, but were inhibitory at high concentrations (5). In general, these compounds affect the membrane permeability and decrease the water and nutrient absorption at high concentrations, whereas, increase the absorption at low concentrations (5). This effect may change the biomass allocation (5), as observed in bioassays done with CEE and the SFs.

Biomass increase in root systems promotes better anchoring of plant body, thereby increasing root absorption area and rapid nutrient accumulation due to the close contact with soil nutrients (20).

Chemical Analysis of extracts and fractions

The TLC tests detected the terpenes in CEE and in HF, and phenolic compounds in CEE and in EAF. Alkaloids were also found in EAF. The allelopathic activity of terpenes, phenolic compounds and alkaloids is reported in other studies (5). Most of *Cassia* and *Senna* species in Brazil, such as *C. spectabilis*, *C. carnaval* and *C. Excels,* have piperidine alkaloids as the main components along with glycosidic flavones, long chain aliphatic esters, glycosidic chromone, polysaccharides (23) and anthraquinones (8). This corroborates the classes of components found in EAF in this study. The phenolic content in CEE and in SF fractions was obtained as the Folin-Ciocaulteau protocol, which is based on the chemical reduction potential compared to the equivalent reduction potential of gallic acid rather than on a quantitative determination of these compounds. According to

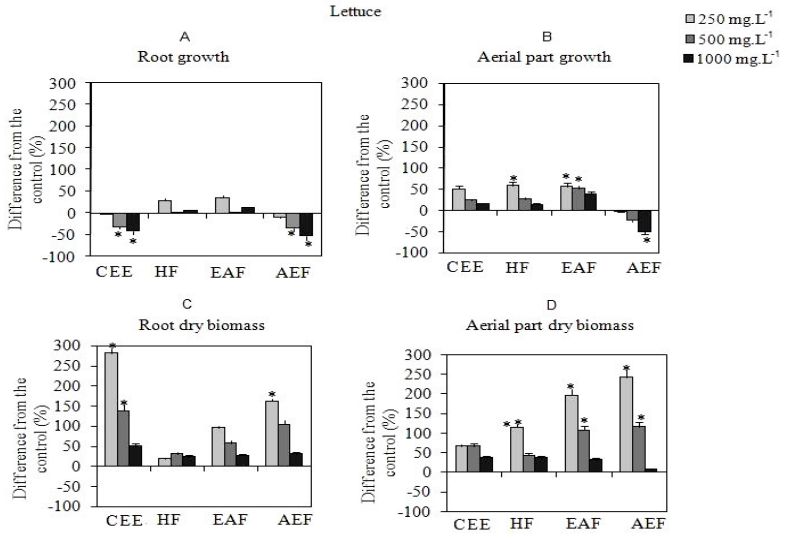

Figure 1. Effects of crude ethanol extract (CEE), hexane fraction (HF), ethyl acetate fraction (EAF) and aqueous-ethanol fraction (AEF) from shoots of *S. occidentalis* on root growth, aerial part growth, root dry biomass and aerial part dry biomass in lettuce (*Lactuca sativa*). * Means that differ statistically from control treatment (Dunnett Test).

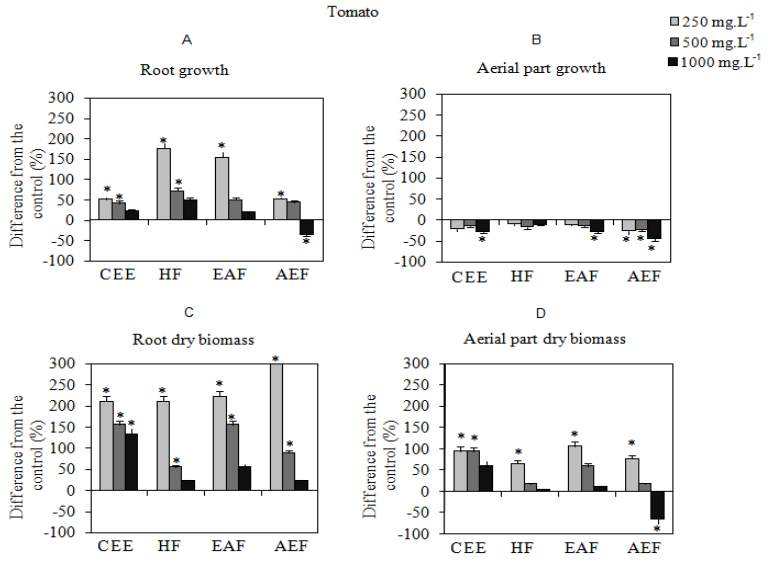

Figure 2. Effects of crude ethanol extract (CEE), hexane fraction (HF), ethyl acetate fraction (EAF) and aqueous-ethanol fraction (AEF) of *S. occidentalis* shoots on root growth, aerial part growth, root dry biomass and aerial part dry biomass in tomato (*Lycopersicon esculentum*). * means that differ statistically from the control treatment (Dunnett Test).

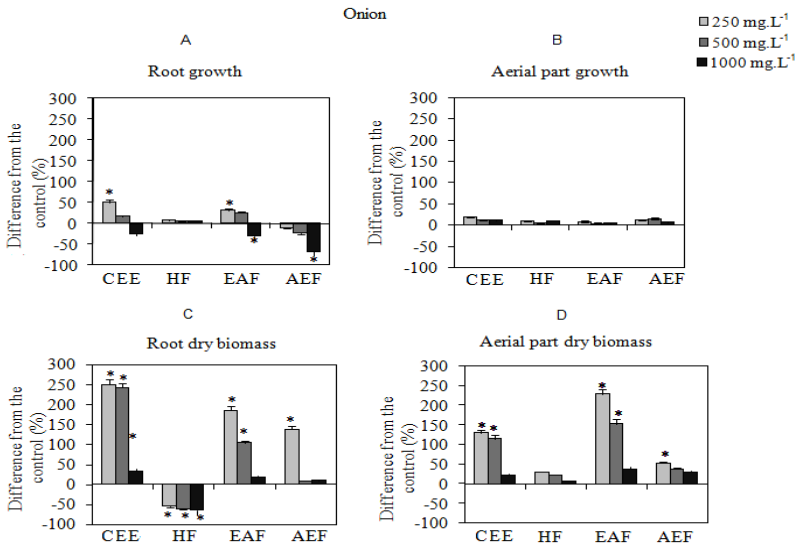

Figure 3. Effects of crude ethanol extract (CEE), hexane fraction (HF), ethyl acetate fraction (EAF) and aqueous-ethanol fraction (AEF) of *S. occidentalis* shoots on root growth, aerial part growth, root dry biomass and aerial part dry biomass in onion (*Allium cepa*). *means that differ statistically from the control treatment (Dunnet Test).

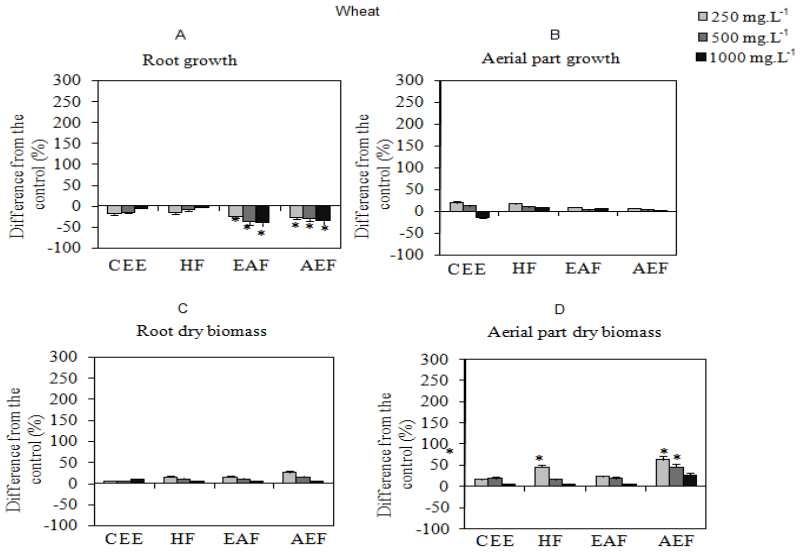

Figure 4. Effects of crude ethanol extract (CEE), hexane fraction (HF), ethyl acetate fraction (EAF) and aqueous-ethanol fraction (AEF) of *S. occidentalis* shoots on root growth, aerial part growth, root dry biomass and aerial part dry biomass in wheat (*Triticum aestivum*). * means that differ statistically from the control treatment (Dunnet Test).

Katalinic *et al.* (7), many phenolic compounds show different responses in this type of assay. The spectrophotometric determination of flavonoid content considered the formation of aluminium-flavonoid complexes and used quercetin as reference. The EAF activity was differentiated from CEE, HF and AEF, owing to its higher total phenolic and flavonoid content (Fig. 3).

Table 3. Total phenolic and flavonoid content (mean±sd) in extracts from shoots of *S. occidentalis*

Extract*	Phenols (µg EAG mg^{-1})	Flavonoids (µg EQ mg^{-1})
CEE	63.040±0.007	37.500±0.009
HF	54.050±0.012	37.500±0.008
EAF	101.860±0.037	50.500±0.060
AEF	50.037±0.016	7.180±0.016

* CEE = crude ethanol extract; HF = hexane fraction; EAF = ethyl acetate fraction; AEF = aqueous-ethanol fraction.

Other studies have described more than 350 secondary metabolites in *Senna* species from tropical and subtropical areas worldwide. Chemical studies indicate that these species contain different classes of components, but anthraquinones and flavonoids are most frequent groups these species (8,12,19). Many alkaloids, terpenes and phenolic compounds have already been identified as allelochemicals in other plant species (5). These compounds can act individually or in synergism, and may interfere with physiological processes during the emergence and growth of target dicots and monocot plants investigated in this study (5,25).

Our results showed inhibitory effect of CEE and SFs on the emergence and growth of lettuce and tomato. The AEF inhibited the growth in both dicots and monocots, while EAF inhibited the growth of tomato, onion and wheat. CEE and SF treatments influenced the emergence, growth and biomass accumulation of target species. After the bioassays, the isolation and identification studies of these chemical compounds are required to show their potential in weed control programmes.

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