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## ABSTRACT

Phytotoxicity is the term used to describe the toxic effect of chemical compounds on growth and development of plants. Phytotoxic compounds vary in their chemical composition, and may be found in natural environments due to their biosynthesis and active release (allelochemicals). These compounds have the potential to be used as natural pesticides for the control of agricultural plagues. Therefore, this study aimed to evaluate the phytotoxic potential of different extracts and fractions of *Tridax procumbens*, using pre and post-emergence bioassays of *Lactuca sativa* L. seeds and mitotic index of *Allium cepa* L. root cells. Furthermore, the chemical compounds present in the extracts were phytochemically elucidated and the most bioactive fraction determined. Germination and development of tested seedlings were inhibited by the different extracts and ethyl acetate fraction of the ethanolic extract. There were notable alterations in the mitotic index of exposed *A. cepa* root cells compared to those of the negative control. Phytochemical identification was performed by HPLC–PAD, which showed that a considerable amount of flavonoid compounds were present in the ethanolic extract and its ethyl acetate fraction. According to the results obtained, it can be concluded that *T. procumbens* possesses phytotoxic compounds that are probably of flavonoid origin. The possession of these bioactive compounds may be related to its invasive nature and weed capacity.

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## 1. Introduction

Phytotoxicity is the term used to describe the toxic effects of a variety of compounds on the growth and development of plant species. These compounds can cause molecular alterations, which include modification of gene transcription and/or duplication of the DNA molecule. These alterations lead to chromosome mutation and aberration, and subsequent damage to vital processes of target plants (Inderjit et al., 1999; Gniazdowska and Bogatek, 2005). Phytotoxicity can be caused by a vast variety of compounds such as heavy metals, pesticides and allelochemical substances (Martins et al., 2007; Guimaraes et al., 2007; Carvalho et al., 2009). Compounds produced by plants can only be termed allelochemicals, if they are actively produced and released into the environment to directly or indirectly interfere with the growth and development of surrounding species (Chou and Kuo, 1986; Costa and Menk, 2000; Souza Filho and Alves, 2002).

Research related to monitoring bioactivity of extracts, fractions and isolated compounds from plants, has been frequently used to identify potentially toxic substances, especially those with allelopathic characteristics (Inderjit and Callaway, 2003; Noldin et al., 2003). Therefore,

allelopathic activity of extracts can be evaluated from their effect on germination of pre-selected sensitive species, mitotic index and chromosomal aberrations (Morel and Guillemain, 2004). Species like lettuce, tomatoes, onion, radish and cucumbers are frequently used for testing the phytotoxic effects of allelochemicals/bioactive compounds (Mazzafera, 2003; Morel and Guillemain, 2004).

Recent research has demonstrated the applicability of allelochemicals as an important means of controlling weeds, insects, algae and microorganisms, that directly and/or indirectly prejudice agricultural production and human health (Leflaive and Ten-Hage, 2007; Hajimahmoodi et al., 2010; Bártoová et al., 2011; El Marsni et al., 2011; Zak et al., 2012; Kato-Noguchi et al., 2012).

Brazil is the fifth largest consumer of pesticides worldwide. More than 150,000 tons of pesticides with herbicides representing 33% of this amount is used per year in Brazil (IBGE, 2009). Chemical control is the most common means of handling invasive plants and plagues in Brazil. However, this causes ecosystem disequilibrium, changing the physical and chemical properties of water and soil, and presenting agro-ecological management challenges (Mascarenhas, 1999; Lorenzi, 2000; Modesto Júnior and Mascarenhas, 2001; Macías et al., 2007).

*Tridax procumbens* L. (Asteraceae) popularly known as “erva-de-touro”, is an invasive perennial plant, occurring mainly in tropical and subtropical environments, and occupying pastures, meadows, fields,

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side of highways and degraded areas (Kissmann and Groth, 1999). It has therapeutic, hypotensive (Salahdeen et al., 2004), immunomodulatory, antibiotic, antioxidant, anti-inflammatory and anticancer properties (Tiware et al., 2004; Sharma and Kumar, 2009; Agrawal et al., 2009; Jachak et al., 2011). Phytochemical assays performed with this species have confirmed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins (Jude et al., 2009; Agrawal and Talele, 2011).

The present study evaluated the phytotoxic potential of different extracts and fractions of *T. procumbens*, using pre- and post-emergence bioassays of *Lactuca sativa* L. seeds and mitotic index of *Allium cepa* L. root cells. Furthermore, chemical compounds present in the most bioactive extracts and fractions were phytochemically elucidated.

## 2. Materials and methods

### 2.1. Plant material and extracts preparations

The plant parts (branches, leaves and flowers) of *T. procumbens* were collected from specimens found in the Universidade Estadual Paulista – SP (22°39'42"S e 50°24'44"W, Altitude: 546 m). The species voucher is deposited in the scientific collection of the Plant Systematics laboratory, FCL, UNESP – Assis (HASSI) sobnumber 113. For preparation of the extracts, the plant parts were pooled, washed, dried in stove (40 °C) and pulverized. The aqueous extract was obtained by mechanical agitation, in distilled water (proportion 1:10 (w:v) for 24 h at 24 °C). Right after, it was vacuum filtered, frozen and lyophilized (model: L101, Liotop, Brazil) to obtain a dry extract. The hydroethanolic extract was obtained by mechanical agitation in a ethanol:water (70:30) solution in the proportion 1:10 (w:v) for 24 h. The process was repeated 3 times with the same plant material. Then, the obtained extract was filtered and taken to a rotary evaporator (model: MA120, Marconi, Brazil) at 60 °C for the ethanol removal and later frozen and lyophilized to obtain a dry extract. The same was performed to the ethanolic extract, where only the ethanol:water (70:30) solution was substituted by absolute ethanol (IMPEX, Brazil), which had the dry extract obtained by rotary evaporator concentration followed by desiccation chamber at room temperature.

### 2.2. Extract fraction

The crude ethanol extract of *T. procumbens* parts was subjected to fractionation as it had the highest allelopathic activity in trials of pre- and post-emergence, for this reason a chromatographic column was set with about 75% silica and 25% Silica Gel 60 (Sigma-Aldrich®, USA) incorporated with 2.0 g of extract. The sequence of solvents for the elution was: n-Hexane, Dichloromethane, Ethyl Acetate, Ethyl Acetate:Methanol (70:30), Ethyl Acetate:Methanol (50:50), Ethyl Acetate:Methanol (30:70) and Methanol. Changes of solvents were performed whenever the fraction remained without evidence of separation. Filtered fractions were concentrated on a rotary evaporator at 40 ± 2 °C. Then, they were subjected to the pre- and post-emergence bioassays.

### 2.3. Bioassay for pre-emergence

The pre-emergence bioassay was performed with *L. sativa* L. cv. Grand Rapids (Lettuce) seeds by control of seeds germination of this plant in Petri dishes (60 mm × 15 mm) and germination paper, with relative humidity, temperature and luminosity controlled artificially in germination greenhouse type BOD (Biological Oxygen Demand) (model: 411/FPD, Nova Ética, Brazil). Moreover, an experiment was set with a completely randomized design (CRD), where petri dishes were separated in experimental groups and control, containing 50 lettuce seeds in each plate, with six repetitions to each experimental group, treated with 1 mL of the different extracts of *T. procumbens* (in the concentrations of 5, 10 and 20 mg mL<sup>-1</sup>), and a negative control

group (water). As the germination evaluation criteria it was used the protrusion and the geotropic curvature of the radicle, as indicated by Labouriau (1983). The seeds that presented fake germination by imbibition were not accounted in the results. The monitoring of the species germination was performed every 6 h, during 48 h.

For the results obtained in the assays, different indexes were calculated: average percentage of germination ( $[\sum n_i / A] \cdot 100$ ), average time of germination ( $T_m = [\sum n_i \cdot t_i] / \sum n_i$ ) and average germination speed ( $V_m = 1 / T_m$ ) where  $n_i$  = number of seeds germinated at each instant "ti"; A = total number of seeds put to germinate; and  $t_i$  = time between the begin of the experiment and the time i-th of observation (Santana and Ranal, 2004; Pereira et al., 2009).

### 2.4. Bioassay for post-emergence

The bioassay was performed according to the methodology suggested by Soares and Vieira (2000) and Alves et al. (2004) and adapted to our laboratory conditions. The lettuce seeds were previously germinated in Petri dishes, covered with germination paper and dampened with distilled water. After 24 h in BOD greenhouse conditions, the seedlings that presented an average length of 2 mm were used in the bioassay, which was set with a completely randomized design (CRD) with Petri dishes containing as substrate germination paper, dampened with 1 mL of different *T. procumbens* extracts solution. These were separated in experimental and control groups, containing 25 seedlings in each plate, with four replicates for each treatment and control (water).

The evolution process of the treatments was performed by observation and measurement of seedlings radicles and hypocotyl, using a digital caliper rule (model: IP65, DIGIMESS®, Brazil), at each 24 h until it completed 48 h of exposition (MIRÓ et al., 1998; PROCOPPIO et al., 2005).

### 2.5. Statistical analysis for pre- and post-emergence testing

For the statistical analysis of pre- and post-emergence tests were performed the normality tests and homogeneity. The transformed data did not present normality and its variances were not homogeneous, therefore the results were analyzed by Kruskal–Wallis and Dunn ( $\alpha = 0.05$ ) with the support of the BioEstat 5.3 software, according to Santana and Ranal (2004).

### 2.6. Determination of osmotic potential, pH and electrical conductivity

The osmotic potential determination was performed according to the technique described by Villela et al. (1991). The treatment was evaluated by osmotic solutions obtained using Polyethylene Glycol 6000 (PEG 6000). The values obtained in the PEG 6000 osmotic potential solutions were compared to the values found in the *T. procumbens* extracts.

The pH of the different *T. procumbens* extracts was determined using a pH meter (Tecnopon® model MPA210). Similarly, the electrical conductivity was measured with a conductivity meter (Conductivity Meter Instrutherm®, model CD860).

### 2.7. A. cepa root cell mitotic index

*A. cepa* (onion) seeds were germinated in Petri dishes, when the roots of the seedlings reached 1 cm in length they were exposed to extracts at concentrations that presented highest activity in pre- and post-emergence experiments, for a period of 48 h. After this period the seedlings were replaced in Petri dishes containing distilled water until they reached 5 cm in length average (recovery period). The entire experiment was conducted in a BOD germination greenhouse conditions. The roots were fixed in Carnoy (absolute ethyl alcohol and glacial acetic acid, 3:1). Following for setting and analysis of the roots, they were hydrolyzed in hydrochloric acid (HCl) 1 N at 60 °C for 8 min, and right after, were stained with Schiff Reactive for 2 h in the dark.

**Table 1**Effects of different aqueous, hydroethanolic and ethanolic extracts of *Tridax procumbens* on seed germination and seedling growth of *Lactuca sativa* (lettuce).

Treatment	Extract (mg mL <sup>-1</sup> )	G ± SD (%)	Tm ± SD (hours)	Vm ± SD (seeds/hs)	Radicle (mm)	Hypocotyl (mm)
Water	–	99.00 ± 1.09a	14.43 ± 1.01a	0.069 ± 0.0046a	21.113 ± 6.468a	3.502 ± 0.773a
Aqueous	5	98.33 ± 1.50a	22.70 ± 1.24b	0.044 ± 0.0024b	07.168 ± 1.520b	3.605 ± 0.863a
	10	84.00 ± 6.81b	36.13 ± 1.95b	0.027 ± 0.0015c	04.172 ± 1.116c	2.924 ± 0.732b
	20	11.00 ± 7.56c	45.58 ± 1.89b	0.022 ± 0.0009c	02.638 ± 0.623d	1.831 ± 0.817c
Hydroethanolic	5	92.33 ± 3.44b	29.22 ± 3.57b	0.034 ± 0.0043b	06.187 ± 1.450b	3.193 ± 0.754b
	10	39.33 ± 17.04c	38.88 ± 3.54c	0.025 ± 0.0024b	05.612 ± 1.476c	2.808 ± 0.542c
	20	01.00 ± 1.67d	48.00 ± 0.00d	0.020 ± 0.0000c	03.853 ± 0.914d	2.162 ± 0.457d
Ethanolic	5	42.66 ± 18.27b	25.24 ± 2.46b	0.039 ± 0.0042b	08.662 ± 2.311b	3.190 ± 0.824b
	10	17.66 ± 6.97c	30.40 ± 5.29b	0.033 ± 0.0059b	06.799 ± 1.844c	2.909 ± 0.560b
	20	00.00 ± 0.00d	00.00 ± 0.00c	0.000 ± 0.0000c	06.831 ± 1.552c	3.151 ± 0.796b

Data are presented as mean ± standard deviation. Means with the same letter in the column do not differ by Dunn's test ( $\alpha = 0.05$ ). Legend: G% = germination mean percentage, Tm = germination mean time and Vm = germination average speed.

The roots were placed on slides, added a drop of 2% acetic carmine and covered with coverslips. After, they were crushed and fixed. Analyses were performed using an optical microscope (100×) and 5000 cells/treatment were observed. Phytotoxic effects of the extracts were determined by mitotic index analysis (total n° of dividing cells / total n° of analyzed cells × 100).

For the *A. cepa* test statistical analysis, results were submitted to non-parametric Kruskal–Wallis and Mann–Whitney tests (analysis significance level of 5% and 1%) according to Leme and Marin-Morales (2009).

### 2.8. High performance liquid chromatography (HPLC-PDA)

Chromatographic separations were performed on high performance liquid chromatography (analytical, quaternary gradient) model PU-2089S Plus (Jasco®), coupled to a diode array detector with photo scan range 200–900 nm, MD-2015 model Plus (Jasco®), automatic injector model AS-2055 (Jasco®) with 50 µL loop and column oven model CO-2060 Plus. The Jasco ChromPass (version 1.8.1.6) was used during the acquisition and processing of chromatographic data. Reverse phase column immobilized with octadecylsilane was used, model Luna C<sub>18</sub> (2) 100A (Phenomenex®) of 250 × 4.6 mm i.d., with an average particle size of 5 µm with guard column (Phenomenex®) of 4 × 3 mm i.d.. An aliquot of 10 mg of the ethanol extract and ethyl acetate fraction was dissolved in 1 mL of acetonitrile (ACN) 100% and filtered with syringe filter with pore size of 0.45 µm. The samples were monitored by PDA detector in the range of 200–600 nm. The chromatogram was obtained at 334 nm. Mobile phase: Acetonitrile 99.9% + 0.1% Formic Acid (A) and Water 99.9% + 0.1% Formic Acid (B). Gradient: 10–35% of A in B during 60 min.

## 3. Results

### 3.1. Pre-emergence assay with crude extracts and ethanolic extract fractions

The pre-emergence test results of seeds exposed to different concentrations of *T. procumbens* aqueous extract are shown in Table 1. Seeds

treated with the 5 mg mL<sup>-1</sup> concentration presented no significant difference in germination compared to the control, but differed when compared to the 10 and 20 mg mL<sup>-1</sup> treatments that decreased by 16.0% and 89.0%, respectively. These treatments differed from each other and the control (99.33% germination). Germination time did not significantly vary between the different extract concentrations, but were significantly different from the control. For average germination rate, the 5 mg mL<sup>-1</sup> treatment was significantly different from the control, 10 and 20 mg mL<sup>-1</sup> treatments. Furthermore, germination rates were not significantly different between the 10 and 20 mg mL<sup>-1</sup> treatments, but those of the two concentrations were different from the control.

Treatments having 5, 10 and 20 mg mL<sup>-1</sup> hydroethanolic extract caused 7.67, 60.67 and 99.00% germination rate reduction, respectively. These germination rates were statistically different between the treatments and the control. In addition, average germination times varied significantly between the different hydroethanolic extract treatments and the control group (Table 1).

Results of the different ethanolic extract treatments (5, 10, and 20 mg L<sup>-1</sup>) demonstrated a concentration dependent reduction (57.34, 82.34 and 100%, respectively) of germination rates. The average germination time and germination rate of seeds treated with 20 mg mL<sup>-1</sup> were significantly distinct from those of the 5 and 10 mg mL<sup>-1</sup> treatments and the control (Table 1).

Due to the high biological activity observed with the ethanolic extract, it was fractionated, and 4.5 mg mL<sup>-1</sup> of the fractions subjected to pre- and post-emergence tests, and the results are given in Table 2. Only the dichloromethane (14.67%) and ethyl acetate (97.34%) fractions caused a reduction in germination during the pre-emergence assay. Germination time and rate were only influenced by ethyl acetate:methanol (70:30) and methanolic fractions.

### 3.2. Post-emergence bioassay with crude extracts and fractions of ethanolic extract

In post-emergence bioassays, seedlings were exposed to 5, 10 and 20 mg mL<sup>-1</sup> of the aqueous, hydroethanolic and ethanolic extracts.

**Table 2**Effects of different ethanolic extract fractions (Dichloromethane, Ethyl Acetate, Ethyl Acetate/Methanol 70%/30%, Ethyl Acetate/Methanol 50%/50%, Ethyl Acetate/Methanol 30%/70% and Methanol) (4.5 mg mL<sup>-1</sup>) on germination and seedling growth of *Lactuca sativa* (lettuce).

Treatment	G ± SD (%)	Tm ± SD (hours)	Vm ± SD (seeds/hs)	Radicle (mm)	Hypocotyl (mm)
Water	96.66 ± 3.05a	18.20 ± 0.36a	0.055 ± 0.0011a	17.19 ± 5.29a	3.95 ± 0.92a
Dichloromethane	85.33 ± 5.03b	26.36 ± 1.63b	0.038 ± 0.0023c	08.62 ± 1.94c	2.65 ± 0.66c
Ethyl acetate	02.66 ± 3.05c	41.00 ± 9.89c	0.025 ± 0.0061d	10.42 ± 2.41b	3.26 ± 0.68b
70%/30%	95.33 ± 2.30a	24.35 ± 1.38b	0.041 ± 0.0024bc	11.73 ± 2.02b	3.00 ± 0.44bd
50%/50%	97.33 ± 2.30a	21.94 ± 1.84b	0.045 ± 0.0038b	06.57 ± 0.95d	2.94 ± 0.41 cd
30%/70%	99.33 ± 1.15a	19.49 ± 0.40a	0.051 ± 0.0011a	06.91 ± 1.11d	3.19 ± 0.49bd
Methanol	98.00 ± 2.00a	19.17 ± 0.58a	0.052 ± 0.0016a	06.73 ± 1.30d	2.84 ± 0.63 cd

Means with the same letter in the column do not differ by Dunn's test ( $\alpha = 0.05$ ).

**Table 3**  
pH, osmotic potential and electrical conductivity of organic extracts of *T. procumbens*.

Tratament	Extract (mg mL <sup>-1</sup> )	pH	Osmotic Potential (MPa)	Electric Conductivity (mS cm <sup>-1</sup> )
Water		6.06	0.0	0.014
Aqueous	5	6.90	−0.00392	2.75
	10	6.39	−0.00814	4.98
	20	6.90	−0.01444	9.07
Hydroethanolic	5	6.46	−0.00180	2.32
	10	6.67	−0.00884	4.56
	20	6.31	−0.01588	7.64
Ethanollic	5	5.40	−0.00251	1.11
	10	5.30	−0.00673	1.60
	20	5.12	−0.01518	3.43

Compared to the control, the extracts significantly altered radicle and hypocotyl length after 48 h exposure (Table 1).

The effect of ethanolic extract fractions on the initial phase of *L. sativa* seedling development, showed that the dichloromethane fraction differed from the other extracts. On the other hand, the results obtained from the ethyl acetate and ethyl acetate:methanol (70:30) fractions were different from those of the following fractions: ethyl acetate:methanol (50:50), ethyl acetate:methanol (30:70) and methanol. Average hypocotyl length was affected by all the different fractions in relation to the control (Table 2).

### 3.3. pH, osmotic potential and electrical conductivity

The physicochemical results of *T. procumbens* extracts are shown in Table 3. The extracts had a pH range of 5.12 to 6.90, while the control had 6.06. Osmotic potential of the extracts ranged from −0.00180 to −0.01588 MPa, and their electrical conductivity from 1.11 to 9.07 mS cm<sup>-1</sup>.

### 3.4. Mitotic index

At 20 mg mL<sup>-1</sup>, *A. cepa* root meristem cells exposed to aqueous, hydroethanolic and ethanolic extracts had 10.84, 8.90 and 11.30 mitotic index values, respectively. Mean mitotic index values between the three extracts did not differ statistically between themselves and the positive control MMS (9.74). Among the extracts, the hydroethanolic extract caused a significant variation in mitotic index compared to the negative control (14.52). Prophase frequency declined in root cells exposed to aqueous extract, while metaphase, anaphase and telophase frequencies were only reduced by the hydroethanolic extract (Table 4).

### 3.5. High performance liquid chromatography (HPLC-PAD) analysis of *T. procumbens* extracts

The HPLC-PAD screening of the ethanol extract of *T. procumbens* and its ethyl acetate fraction gave very similar chromatographic profiles (Fig. 1A and B) indicating that the extractor ethyl acetate had higher affinity due to its polarity with most of the ethanolic extract compounds.

**Table 4**

Mitotic index of *Allium cepa* root meristem treated with *T. procumbens* (ethanolic, hydroethanolic and Aqueous) extracts at 20 mg mL<sup>-1</sup>. The negative control (NC) were treated with water and positive control with 0.0077 µL mL<sup>-1</sup> of methyl methanesulfonate (MMS).

Tratament	Cell division					Mitotic index <sup>a</sup>
	Interphase	Prophase	Metaphase	Anaphase	Telophase	
NC	3387	386	70	39	86	14.52 ± 02.17a
Ethanolic	4394	305	96	52	112	11.30 ± 02.63ab
Hydroethanolic	4473	274	64	25	82	08.90 ± 00.79b
Aqueous	4383	290	104	46	102	10.84 ± 01.86ab
MMS	4353	322	65	23	77	09.74 ± 02.50b

<sup>a</sup> Mitotic index = (total number of dividing cells / total number of analyzed cells × 100). Same letters in columns do not differ statistically averages evaluated with the Kruskal–Wallis test ( $p < 0.05$ ).

Peaks with absorption bands typical of flavonoids (Fig. 2A), which are recognized by the characteristic Band II, with maximum wavelength in the spectral range of 240–290 nm, assigned to the A-ring, and the Band I, with maximum length in the spectral range of 300–390 nm, assigned to the B-ring, presenting higher incidence of molecules from the flavones and flavonols group (Fig. 2B and C).

## 4. Discussion

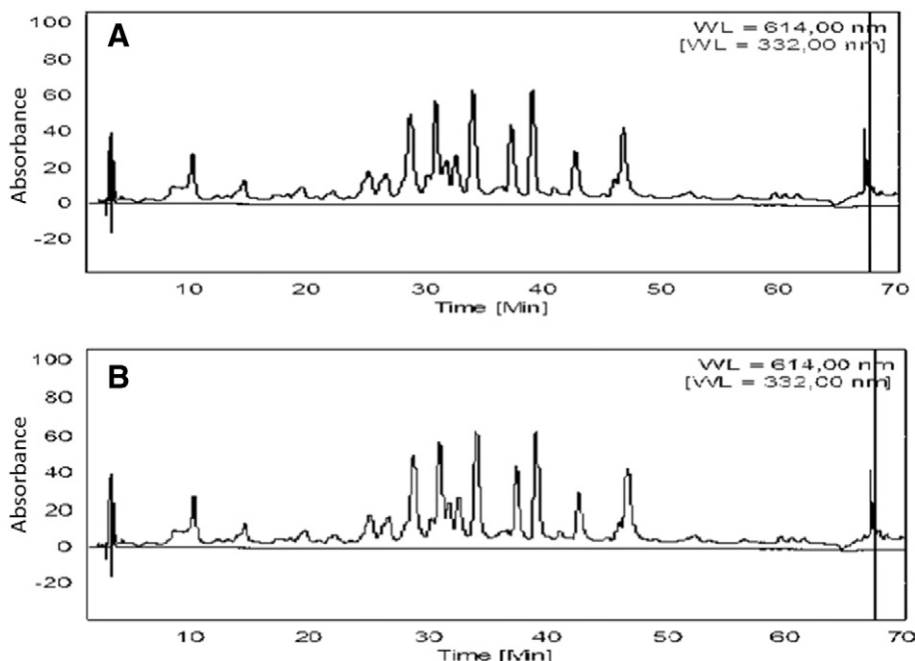
According to Lorenzi (2000) and Kissmann and Groth (1999), *T. procumbens* is an important invasive plant and weed that infests pastures, roadsides, vacant lands and urban areas, mainly in the Southeastern and Midwestern regions of Brazil. A study carried out by Vivian et al. (2013) demonstrated that this species spreads rapidly in annual agricultural crop cerrado lands of Brazil's Central-West region. Furthermore, recent studies by Jachak et al. (2011), Christudas et al. (2012), Algariri et al. (2013) and Policegoudra et al. (2014) presented different applications of this species based on its biological and pharmacological activities.

Silva et al. (2014) are of the opinion that for the detection of the phytotoxic potential of a given plant extract, pre- and post-emergence bioassays are valid and reliable. In agreement with Inderjit and Nilsen (2003) and Silva et al. (2014), pre-emergence bioassays demonstrated that different concentrations of the ethanolic extract and its ethyl acetate fraction significantly altered all indexes analyzed for the target plant. The researchers showed that allelochemicals interfere with essential metabolic activities, thus causing changes in the germination process. Significant radical and hypocotyl growth inhibition was observed during the growth assays. The extracts and fractions (or allelochemicals) responsible for growth inhibition in our study have been previously reported to have phytotoxic effects on plant growth (Silva et al., 2013, 2014; Poonpaiboonpipat et al., 2013). During both trials in our study, increased biological activity was recorded in parallel with increasing of exposure concentrations. This dose dependent bioactivity has been demonstrated in research performed by Jinhu et al. (2012) and Tigre et al. (2012). Also, preliminary research conducted by Krautmann et al. (2001) and Femina et al. (2012) indicated possible biological activity of *T. procumbens* on plant development, which agrees with the results of the present study.

Phytotoxic assays performed with plant extracts show that aqueous extracts can alter growth medium physicochemical characteristics such as pH, osmotic potential and electrical conductivity, thereby directly affecting the germination capacity and development of seedlings. This highlights the importance of physicochemical characterization of extracts, in order to avoid their erroneous classification. Gatti et al. (2004) recommend that the osmotic potential of the extracts involved in germination tests does not exceed −0.2 MPa (Table 3).

The different extracts of *T. procumbens* presented pH variation from 5.12 to 6.90 (Table 3), and these are within the range of values that do not influence the germination process, as demonstrated by Baskin and Baskin (1998) and Carmo et al. (2007), which evaluated the germination of lettuce on a wide range of pH values, from 3.0 to 7.0. Electrical conductivity of extracts and their different concentrations are within





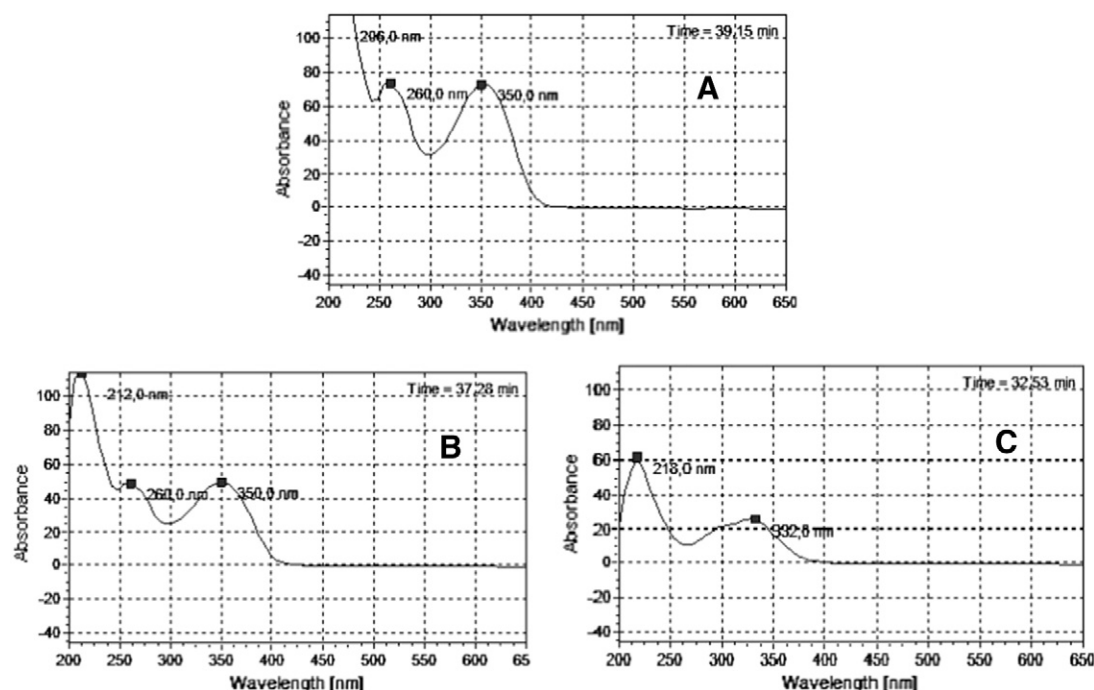
**Fig. 1.** (A) Chromatographic profile of ethanolic extract obtained by HPLC-PAD. (B) Chromatographic profile of the ethyl acetate fraction of ethanolic extract obtained by HPLC-PAD. Eluting system: A (Acetonitrile + 0.1% Formic Acid) and B (Water + 0.1% Formic Acid) Gradient: 10–35% A in B in 60 min. Phenomenex® Luna C18 column (250 × 4.6 mm id. 5  $\mu$ m). HPLC (Jasco®), flow 1.0 mL min<sup>-1</sup>,  $\lambda$  = 334 nm. Injection volume: 20  $\mu$ L. Column oven: 40 °C.

the established by Souza et al. (2003), who found values below 20 mS cm<sup>-1</sup> are not harmful to the germination of seeds.

The mitotic index of meristematic root cells of *A. cepa* provides an additional means to evaluate the phytotoxic potential of the different extracts and their fractions, as was previously also demonstrated by Batish et al. (2006) and Pinho et al. (2010). Furthermore, according to Salehzadeh et al. (2003), the reduction in the rates of cell division can be directly related to the fact that many cells are retained at the interphase stage. They showed that DNA synthesis (S) and GAP 2 (G2)

phases are easily altered by chemicals due to the over production of reactive oxygen species, which can damage DNA and protein synthesis.

Analysis of the possible compounds involved in the phytotoxicity recorded in the present study was performed by HPLC-PAD in both the ethanolic extract and its ethyl acetate fraction (Fig. 1). This analysis showed very similar chromatographic profile between the extract and its ethyl acetate fraction. According to assays performed by Mabry et al. (1970) and Merken and Beecher (2000), the spectral bands reported in our study correspond to the presence of flavones and flavonol



**Fig. 2.** (A) Maximum absorption bands in the UV region illustrated for flavonoids. (B) UV spectrum typical of a flavonol and (C) UV spectrum typical of a flavone.

molecules. This is because their peaks for Band II can be found around 240–280 nm and Band I around 300–380 nm. In addition, glycosylated flavonoids derivative of quercetin can also be identified within this spectral region.

The phytotoxic potential of many compounds, including flavonoids, are recognized because they may affect cell membrane permeability (Cordeiro-Araújo et al., 2015), promote damage to DNA and proteins, and cause lipid peroxidation (Yu et al., 2003), and may subsequently lead to cell death. Similarly, Sanevas et al. (2007) found that seaweed extract induced oxidative stress in onion and wheat seedlings. Another example, and one of the best characterized, is the capacity of sorgoleone to inhibit photosynthesis by blocking the electron transport chain of photosystem II due to oxidative stress (Czarnota et al., 2001).

Our results corroborate those of previous studies by Saraf et al. (1991) and Jude et al. (2009), who showed the presence of flavonoids in extracts of *T. procumbens*. Jachak et al. (2011) demonstrated the presence of the flavonoid compounds centaurein and bergenin, which have anti-inflammatory properties. Agrawal et al. (2009) and Sailaja et al. (2011) observed the antioxidant properties of different extracts of this plant.

Considering the results obtained in the different assays, as well as the phytochemical characterization of the extracts and their fractions, it is possible to conclude that this species possesses compounds with phytotoxic potential. This is due to the interference with germination, seedling development and stabilization of lettuce. Furthermore, phytochemical characterization revealed the presence of flavonoid compounds, and the ethanolic extract and its ethyl acetate fraction had the highest biological activity. Although these results suggest that *T. procumbens* have active components with phytotoxic potential other assessments are needed before it can be used in weed control and on plants of agronomic interest.

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