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Research Article

Phytotoxicity and allelopathic potential of extracts from rhizomes and leaves of *Arundo donax*, an invasive grass in neotropical savannas

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Abstract

The perennial rhizomatous grass *Arundo donax* L. (Poaceae), the giant reed, is an exotic invasive species in several countries of Europe that is rapidly spreading in the savannas of Central Brazil, locally known as Cerrado. Allelopathy could facilitate the successful invasion of this species by hampering or suppressing the regeneration of the native vegetation. However, information on the phytotoxicity of *A. donax* extracts is limited. We investigated the allelopathic potential of *A. donax* leaf and rhizome extracts, screened them for phytochemicals by thin-layer chromatography (TLC) and nuclear magnetic resonance (^{1}H-NMR), and tested the extracts for antioxidant activity, antimicrobial activity, and cytotoxicity against *Artemia salina*. Aqueous and methanolic extracts were initially tested in germination and seedling growth bioassays using *Lactuca sativa* L. (Asteraceae). The aqueous extracts were then tested on five Cerrado tree species and on *Megathyrsus maximus*, an invasive, alien grass in the Cerrado. Extracts negatively affected germination and seedling growth of the target species. Leaf extracts were more inhibitory. Extracts did not show antioxidant and cytotoxic activity and had very low antimicrobial activity. Flavonoids, and other phenolics were detected mostly in leaves. Terpenes, which were also present in the leaves, were the main secondary metabolites in rhizomes. Alkaloids were detected by TLC in leaf methanolic extracts. However, ^{1}H-NMR revealed the presence of indole alkaloids in methanolic extracts from rhizomes and leaves. We confirmed the allelopathic potential of this species and caution against weed control methods relying on cutting the plant back to soil level for favouring release of allelochemicals.

Keywords: biological invasion; Cerrado; giant reed; phytochemistry

Introduction

Arundo donax L. (Poaceae), known as giant reed, is a perennial rhizomatous grass, native to Asia and the Mediterranean (Mariani *et al.*, 2010) that is an exotic invasive species in several countries of Europe and the Americas (Boose and Holt, 1999; Favaretto *et al.*, 2018). Although it thrives in the most different types of

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soil and environmental conditions, *A. donax* is considered a hydrophyte and has its growth favoured in wetlands, where it can quickly expand and form large stands, especially along river channels (Bell, 1997; Corno *et al.*, 2014). The plant spreads vegetatively from the stem and the rhizome (Mariani *et al.*, 2010). Its establishment may result in losses of composition, structure, and functioning of native plant and animal communities and of ecosystem processes, which can often not be recovered (Bell, 1997; Coffman *et al.*, 2010; Maceda-Veiga *et al.*, 2016).

This is especially critical in the Cerrado of Central Brazil, a complex of seasonal savannas and a hotspot of highly threatened biodiversity (Gomes *et al.*, 2020). The propagation of *A. donax* in the Cerrado is favoured by the characteristics of its phytophysiognomies, with the dominance of open, herbaceous vegetation with scattered trees and shrubs, which is undergoing fast rates of degradation by human activities and increasingly frequent fires (Myers *et al.*, 2000; Gomes *et al.*, 2020). Colonization by this species increases fuel loads as well as fire frequency and intensity. In addition to increasing the risk of fires, *A. donax* has a rapid recovery after fire, therefore, imposing a strong negative effect on the regeneration of the native vegetation (Coffman *et al.*, 2010). *Arundo donax* has been rapidly spreading throughout anthropic areas in Central Brazil, and, more recently, invaded wetlands and preserved vegetation areas (Simões *et al.*, 2013; IABIN, 2019).

Among the mechanisms responsible for the success in the invasion of some exotic plant species is the release of chemical compounds, products from secondary metabolism, which have harmful or positive effects on community members living in the environment, a process known as Allelopathy (Fujii, 2003; Favaretto *et al.*, 2018; Możdżeń *et al.*, 2020). Understanding whether the interaction of *A. donax* with other plant species involves allelochemicals is important to elucidate the mechanisms that favour the invasion of this species into a new area (Hierro and Callaway, 2003).

Indole alkaloids and other nitrogenous aromatic substances were isolated from the leaves (Hong *et al.*, 2010), rhizomes, and roots (Al-Snafi, 2015) of *A. donax*. However, information on the allelopathic potential of *A. donax* is limited. The crude aqueous extract of *A. donax* leaves inhibited the germination and growth of lentil seedlings (*Lens culinaris* Medik) (Abu-Romman and Ammari, 2015). The methanolic extract of *A. donax* leaves had an inhibitory effect on the proliferation of *Microcystis aeruginosa*, cyanobacteria responsible for water contamination in reservoirs (Hong *et al.*, 2010). As far as we know, there are no phytotoxic studies performed with extracts from *A. donax* rhizomes. The allelopathic potential of this structure was shown for other grasses with invasive behaviour, such as *Cynodon dactylon*, whose aqueous rhizome extract inhibited almost entirely the germination and growth of several other plant species (Mahmoodzadeh and Mahmoodzadeh, 2014).

In the present study, we evaluated the phytotoxic potential of extracts from leaves and rhizomes of *A. donax*. Because this species forms dense stands and is invasive of wetlands in the Cerrado, we expected that aqueous extracts of leaves and rhizomes would affect the germination and growth of other plants, show antimicrobial activity, and affect water quality. We also screened the crude extracts for the main secondary metabolites.

Materials and Methods

Plant material

Leaves and rhizomes of *A. donax* were collected from ten adult plants, with no inflorescences, in an area of degraded Cerrado in the Federal District, Brazil (15°46'07.91"S; 47°51'51.19"W). The material was immediately transported to the University of Brasilia, washed in running water, and dried in a drying oven at 60 °C for 72 hours.

Plant extracts

Aqueous extracts: crude 10% (w/v) water extracts were obtained from leaves and rhizomes. The leaves were shredded in a cutting mill (Tecnal R-TE-650/1) and placed in an ultrasonic bath for 45 minutes. The fibrous rhizome was cut into pieces (≤ 1 cm^3), placed in suspension in deionized water for 48 hours, and extracted for 4 hours in an ultrasonic bath. Both extracts were filtered twice on filter paper with the aid of a vacuum pump and lyophilized.

Methanolic extracts: the procedure was the same described for the aqueous extracts, replacing the water with 99.9% methanol. After obtaining the extracts, the solvent was evaporated in a rotary evaporator until the complete drying of the material. Dry fractions were weighed, dissolved in distilled water, and added dimethylsulfoxide (DMSO) 0.05% (v/v).

Dilutions of 10, 5, 2.5, and 1.25% were prepared from the aqueous and methanolic crude extracts.

Germination and seedling growth bioassay

The allelopathic potential of aqueous and methanolic extracts from leaves and rhizomes of *A. donax* was initially tested with the model species *Lactuca sativa* L. (Asteraceae). The experimental design was completely randomized with five replications of ten seeds each that were assembled in Petri dishes with filter paper and 1.5 mL of each extract concentration (10, 5, 2.5, and 1.25%). Controls received distilled water. The petri dishes were kept in a germination chamber at 25 °C with 12 h photoperiod for seven days. After this period, germination was assessed and root and shoot length measured.

The phytotoxicity (inhibition of germination, root and shoot elongation) for species that occur in the Cerrado was then tested with aqueous extract from leaves and rhizomes of *A. donax*, to simulate what occurs in the field. The phytotoxicity of the aqueous extracts was tested at different concentrations (10, 5, 2.5, and 1.25%) in bioassays with seedlings of six species: *Megathyrsus maximus* (Jacq.) B.K.Simon and S.W.L.Jacobs (Poaceae), an invasive African grass co-occurring with *A. donax* in degraded Cerrado areas and with similar growth characteristics (Horowitz *et al.*, 2013) (Figure S1, Supplementary material); *Handroanthus impetiginosus* (Mart. ex DC.) Mattos (Bignoniaceae), *Eriotheca pubescens* (Mart. and Zucc.) Schott and Endl. (Malvaceae), *Pseudobombax tomentosum* (Mart. and Zucc.) A. Robiyns (Malvaceae), *Guazuma ulmifolia* Lam. (Sterculiaceae), and *Parkia platycephala* Benth. (Fabaceae), native tree species of the biome. These trees are commonly found in urban fragments of Cerrado vegetation, where the invasion of *A. donax* is favoured. The bioassays were assembled in Petri dishes (60 × 15 mm) with filter paper and 1.5 mL of each extract concentration.

The experimental design was completely randomized with four replications of ten seeds each. The plates were kept in a germination chamber at 25 °C with 12 h photoperiod for seven days for *M. maximus* and fourteen days for the remaining species. After this period, germination was scored, seedlings were photographed, and the shoot and radicle length measured with the program ImageJ®. Radicle length was scored as zero when the seed germinated (i. e. the radicle emerged from the seed coat) but the radicle failed to elongate.

Phytochemical screening

Phytochemical screening was performed for the main secondary metabolites from crude aqueous and methanolic extracts of *A. donax* leaves or rhizomes. Thin-layer chromatography, a classical qualitative method, was used to detect tannins and saponins (Pascual *et al.*, 2002), alkaloids (Peres *et al.*, 2012), flavonoids (Calina *et al.*, 2013), anthocyanins, triterpenoids, and steroids (Jork *et al.*, 1990).

Antioxidant activity

Samples of 5 mg of dried extracts of leaves and rhizomes were weighed and diluted in 10 mL of methanol. To test for antioxidant activity, two methods were used simultaneously, DPPH and Fe^{3+}-phenanthroline, both in triplicate (Martins *et al.*, 2014), with ascorbic acid as standard curve, and the results were expressed as ascorbic acid equivalent.

Nuclear magnetic resonance

^{1}H nuclear magnetic resonance (NMR) was used to identify functional groups and chemical classes present in aqueous and methanolic extracts of leaves and rhizomes. 25 mg of dried samples of aqueous extracts were diluted in deuterated water (D_2O), and 25 mg of dried samples of methanolic extracts were diluted in dimethylsulfoxide deuterated (DMSO-d_6). The NMR analyses were performed on a Bruker Fourier 300, operating at 300 MHz for the ^{1}H nucleus.

Toxicity test against Artemia salina

The cytotoxicity of the extracts was tested in bioassay against larvae of *Artemia salina* (Meyer *et al.*, 1982). Eggs were placed in a solution of salinized water for 48 hours for the hatching of the crustaceans. Aqueous extracts were tested at concentrations of 1000, 500, 250, 125, 60, and 30 $\mu g.mL^{-1}$ of salinized water. The lethality of the extracts was evaluated after incubation periods of 24 hours.

Antimicrobial activity

The microorganisms were donated by the Laboratory of Reference for Microorganisms of the National Institute of Quality Control in Health, from the Oswaldo Cruz Foundation: *Acinetobacter baumannii* ATCC 19606; *Aeromonas hydrophila* IOC/FDA 110-36; *Candida albicans* ATCC 10231, CBS 6431; CBS 604; *Citrobacter freundii* ATCC 8090; *Edwardsiella tarda* ATCC 15947; *Enterobacter cloacae* ATCC 13047; *Eschericia coli* ATCC 11775; *Klebsiella pneumoniae* ATCC 13883; *Morganella morganii* ATCC 00082; *Pseudomonas aeruginosa* ATCC 10145; *Pseudomonas fluorescens* ATCC 13525 (NCTC 10038); *Salmonella enterica* ATCC 13076; *Serratia marcescens* ATCC 13880; *Staphylococcus aureus* ATCC 12600. The bacterial colonies were inoculated from pure 24-hour cultures in Müeller Hinton broth and homogenized. The turbidity of the inoculum was adjusted to match a 0.5 MacFarland standard, corresponding to a suspension containing 1-2 x 10^8 colony-forming units ($CFU.mL^{-1}$). The test microorganisms were diluted (1:20), and 10 mL of each were inoculated in 96 well plates. The antimicrobial activity was evaluated with different fractions of the extracts (1000, 500, 250, 125, 62.25, 31.12, and 15.5 $\mu g.mL^{-1}$) and the determination of microbial growth was performed by spectrophotometer at 625 nm. The positive control was oxytetracycline 125 $\mu g.mL^{-1}$ and the negative control was the culture medium itself.

Statistical analyses

ANOVA followed by Tukey's HSD test for pairwise comparisons was used for the statistical analyses after checking for normality with the Shapiro-Wilk test and homogeneity of variances with the Levene test. The Kruskal-Wallis test followed by Dunn's tests was applied, when the data did not follow a normal distribution or were heteroscedastic. Differences were considered to be significant at $P < 0.05$. The program RStudio 1.3 was used for the statistical analysis.

Results

Aqueous and methanolic extracts from leaves or rhizomes caused a significant reduction in *L. sativa* seed germination at higher concentrations. Both extracts inhibited seedling growth in a dose-dependent manner (Table 1).

Table 1. The effect of different concentrations of aqueous and methanolic extracts (%) from leaves and rhizomes of *Arundo donax* on percentage seed germination, root and shoot growth of seedlings of *Lactuca sativa* L. (Asteraceae)

Concentrations of extracts	Germination (%)		Shoot length (cm)		Root length (cm)	
	Aqueous	Methanolic	Aqueous	Methanolic	Aqueous	Methanolic
Leaf extracts						
Control	94.5± 2.7 a	97.5 ± 2.3 a	1.26 ± 0.29 a	1.30 ± 0.06 a	3.68 ± 0.37 a	4.38 ± 0.27 a
1.25%	92.3 ± 6.9 a	82.5 ± 4.8 ab	1.16 ± 0.03 a	1.33 ± 0.08 a	2.87 ± 0.37 a	3.99 ± 0.20 a
2.50%	95.0 ±2.6 a	60.0 ± 7.8 b	0.86 ± 0.06 ab	1.09 ± 0.11 ab	1.58 ± 0.08 b	1.73 ± 0.13 b
5%	75.3 ± 1.5 b	22.5 ± 4.6 c	0.61 ± 0.10 bc	0.76 ± 0.14 b	0.91 ± 0.18 bc	0.75 ± 0.12 c
10%	39.0 ± 3.0 c	10.0 ± 6.5 c	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c	0 .00 ± 0.00 c
Rhizome extracts						
Control	95.5 ± 2.3 a	97.5 ± 2.3 a	1.25 ± 0.29 a	1.30 ± 0.06 a	3.68 ± 0.37 a	4.38 ± 0.27 a
1.25%	95.0 ± 4.6 a	85.0 ± 5.6 ab	1.23 ± 0.05 a	1.09 ± 0.13 ab	3.04 ± 0.21 ab	1.73 ± 0.16 b
2.50%	95.6 ± 5.1 a	80.0 ± 1.4 b	1.16 ± 0.06 a	1.00 ± 0.03 b	2.98 ± 0.18 b	1.02 ± 0.03 bc
5%	92.2 ± 4.4 a	65.0 ± 9.1 b	0.73 ± 0.15 b	0.27 ± 0.01 c	1.21 ± 0.36 c	0.32 ± 0.01 cd
10%	43.4 ± 2.0 b	12.5 ± 6.9 c	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d

Controls received distilled water. Different letters within a single column of leaf or rhizome extracts denote significant differences at $P < 0.05$ between concentration levels. Data expressed as mean ± standard error.

Similarly, at the highest concentrations, aqueous extracts from leaves of *A. donax* strongly reduced seed germination and seedling growth on the other tested species when compared to untreated controls (Table 2, Figure 1 and Table S1, Supplementary material).

Table 2. Percentage seed germination of five Cerrado tree species and one invasive African grass (*Megathyrsus maximus*) treated with different concentrations of *Arundo donax* leaf and rhizome aqueous extracts, plus the control

Concentrations of extracts	*Handroanthus impetiginosus*	*Megathyrsus maximus*	*Eriotheca pubescens*	*Guazuma ulmifolia*	*Parkia platycephala*	*Pseudobombax tomentosum*
	Seeds treated with leaf extracts					
Control	90.0 ± 5.0 a	45.0 ± 7.4 a	77.5 ± 5.4 a	47.5 ±3.8 a	45.0 ± 2.3 a	32.5 ± 1.5 a
1.25%	57.5 ± 13.4 ab	23.0 ± 2.8 ab	60.0 ± 11.1 ab	35.0 ± 3.5 ab	35.0 ± 6.3 ab	30.0 ± 0.9 a
2.50%	47.5± 9.2 bc	17.5 ± 5.5 b	27.5 ± 8.4 bc	25.0 ± 2.4 bc	25.0 ± 2.3 bc	20.0 ± 2.7 bc
5%	40.0 ± 1.4 bc	7.5 ± 0.8 b	20.0 ± 3.6 c	15.0 ± 3.5 cd	15.0 ± 1.5 cd	20.0 ± 3.0 bc
10%	22.5 ± 3.3 c	2.5 ± 0.2 b	20.0 ± 0.4 c	10.0 ± 1.7 d	10.0 ± 1.1 d	10.0 ± 2.7 c
	Seeds treated with rhizome extracts					
Control	90.0 ± 5.0 a	45.0 ± 7.4 a	77.5 ± 5.4 a	47.5 ± 3.8 a	45.0 ± 4.6 a	32.5 ± 1.5 ab
1.25%	55.0 ± 14.7 a	35.0 ± 6.1 ab	77.5 ± 5.5 a	32.5 ± 3.6 b	32.5 ± 3.9 b	37.5 ± 1.9 a
2.50%	57.5 ± 14.5 a	27.5 ± 0.7 ab	77.5 ± 5.9 a	42.5 ± 5.2 ab	42.5 ± 4.7 ab	37.5 ± 1.3 a
5%	72.5 ± 10.6 a	20.0 ± 0.2 b	82.5 ± 7.2 a	35.0 ± 0.4 ab	35.0 ± 6.2 ab	27.5 ± 0.6 b
10%	55.0 ± 11.5 a	37.5 ± 6.4 ab	97.5 ± 1.8 a	15.0 ± 4.2 c	15.0 ± 2.4 c	10.0 ± 3.0 c

Different letters within a single column of leaf or rhizome extracts denote significant differences at $p<0.05$ between concentration levels. Data expressed as mean ± standard error.

Aqueous rhizome extracts had detrimental effects in germination of *G. ulmifolia*, *M. maximus*, *P. platycephala* and *P. tomentosum*, in the root and shoot growth of seedlings of *E. pubescens* and *P. tomentosum*, and in the root growth of *P. platycephala* (Table 2, Figure 2).

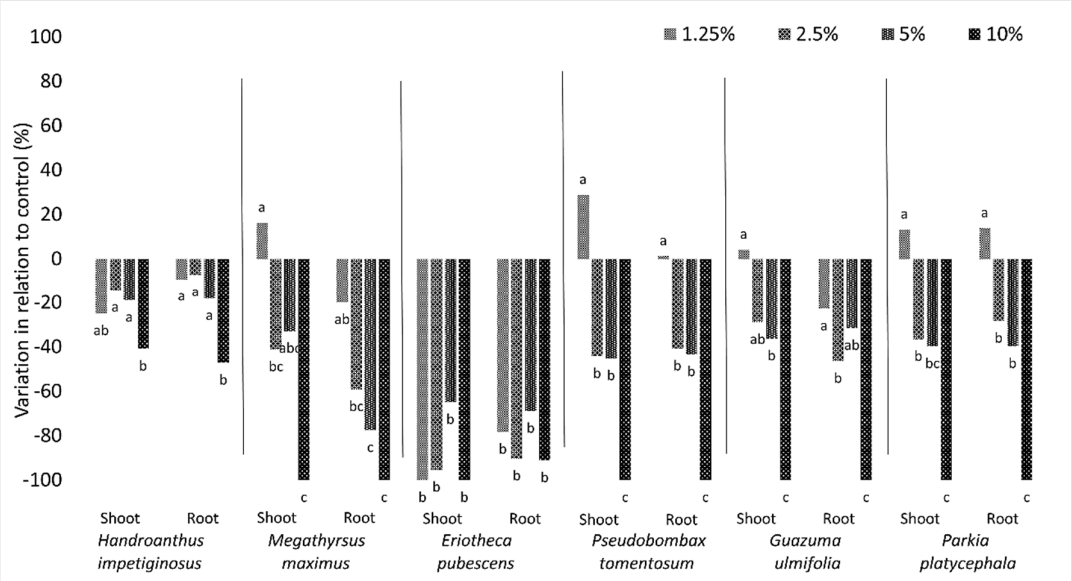

Figure 1. Effects of different concentrations (%) of aqueous leaf extracts from *Arundo donax* on shoot and root length of seedlings of the invasive African grass *Megathyrsus maximus* and five native Cerrado tree species Values are expressed as a percentage difference from control. Different letters denote significant differences at $p<0.05$ between concentration levels. Concentration levels with letter "a" do not differ from controls.

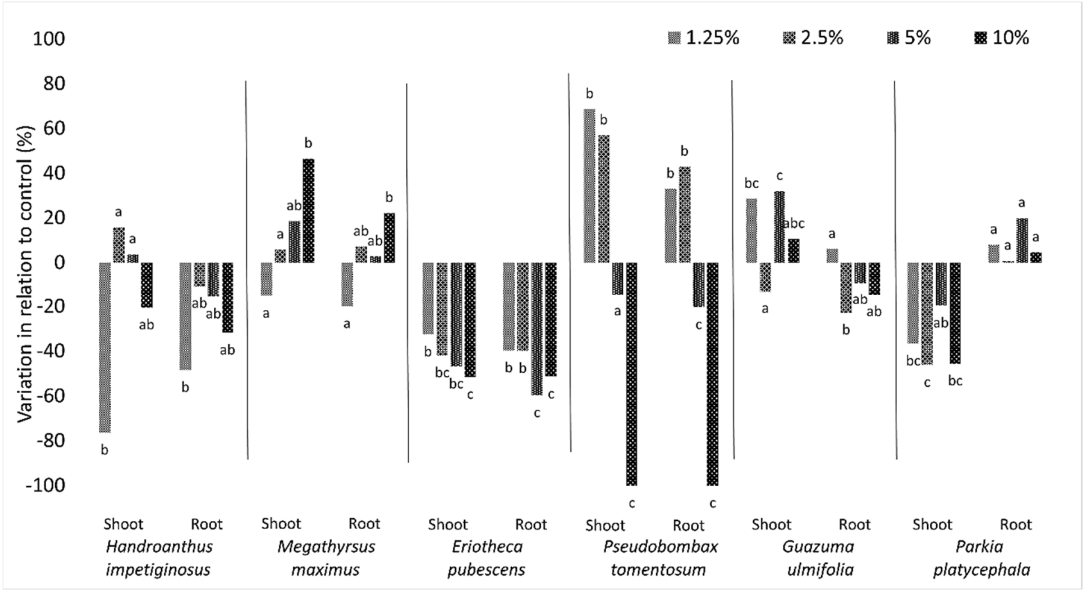

Figure 2. Effects of different concentrations (%) of aqueous rhizome extracts from *Arundo donax* on shoot and root length of seedlings of the invasive grass *Megathyrsus maximus* and five native Cerrado tree species Values are expressed as a percentage difference from control. Different letters denote significant differences at $p<0.05$ between concentration levels. Concentration levels with letter "a" do not differ from controls.

Thin-layer chromatography analysis (Table 3) revealed the presence of terpenes in the aqueous and methanolic extracts of leaves and rhizomes and flavonoids in the leaf aqueous extracts. Phenolic compounds, alkaloids, and flavonoids were detected in the leaf methanolic extracts of *A. donax*.

Table 3. Main chemical classes of compounds detected by thin-layer chromatography (TLC) analysis in aqueous and methanolic extracts of leaves and rhizomes of *Arundo donax*

Extract	Dragendorff (alkaloids)	NP-PEG (flavonoids)	Presence of persistent foam (saponins)	Ferric chloride (phenolics)	Anisaldehyde (Terpenes)
Aqueous					
Rhizome	-	-	-	-	+
Leaves	-	+	-	-	+
Methanolic					
Rhizome	-	-	-	-	+
Leaves	+	+	-	+	+

Note: Presence (+) or absence (-)

No antioxidant activity was found in any of the extracts, by DPPH or Fe^{3+}-phenanthroline. Similarly, none of the treatments by *A. donax* extracts reduced the number of larvae of the crustacean *Artemia salina* in the cytotoxicity test. Inhibitory effect on proliferation of microorganisms was slight in the antimicrobial assay, being observed only in the methanolic extract of rhizomes in two of the thirteen subjects tested. While some microorganisms did not react to the treatment, the most expressive result was the stimulus to proliferation, observed in several cultures (Table 4).

Table 4. Antimicrobial test results with thirteen different microorganisms treated with aqueous and methanolic extracts of leaves and rhizomes of *Arundo donax*

Microorganism	Aqueous		Methanolic	
	Leaves	Rhizome	Leaves	Rhizome
Acinetobacter baumannii	0	0	+	0
Aeromonas hydrophila	0	0	+	0
Candida albicans	0	0	+	0
Citrobacter freundii	+	+	+	0
Edwardsiella tarda	0	+	+	0
Enterobacter cloacae	0	0	+	0
Eschericia coli	+	0	+	0
Klebsiella pneumoniae	+	0	+	-
Morganella morganii	0	0	+	-
Pseudomonas aeruginosa	+	0	+	0
Salmonella enterica	0	+	+	0
Serratia marcescens	+	0	+	0
Staphylococcus aureus	0	0	+	0

Note: (-) indicates inhibitory effect, (+) indicates stimulatory effect, and (0) indicates that there was no effect

The nuclear magnetic resonance spectra showed a large number of signals in the chemical shift range between 3 and 5 ppm, a region characterized by the presence of sugars, in all analyzed extracts (Figure 3). In all extracts, it can be found the presence of aromatic hydrogen of glucose in alpha position (around 5.2 ppm and with J = 3.8 Hz). Between 6 and 8 ppm, in all extracts, we can observe signals related to aromatic substances, but in less amount in the aqueous extract of rhizomes (Figure 3). The presence of signals in the aromatic region confirms the results of the TLC analysis that indicated the presence of flavonoids. The high concentration of sugars in all extracts, and especially in the methanol ones, suggest that they are probably linked to sugars (flavonoid glucosides). The presence of terpenes is also confirmed by several signals between 0.5 and 1.4 ppm (Figure 3) in all extracts, characteristic of methyl groups of this class. But is still soon to confirm if they are triterpenes or other terpene classes. Small signals in the range of 11 ppm are discernible in the ^{1}H-NMR spectra of methanolic extracts from leaves and rhizomes (Figure 4). These are related to indolic alkaloid that was previously isolated from *A. donax* (Khuzhaev *et al.*, 2004), and expected to occur between 10 and 11 ppm.

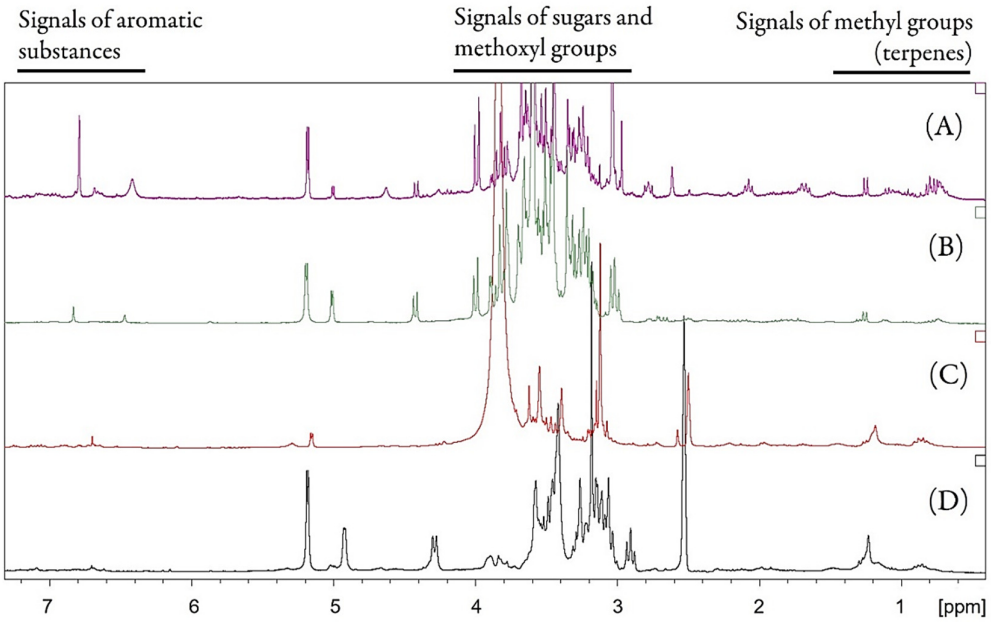

Figure 3. Nuclear magnetic expansion spectra of the region between 1 and 7 ppm of the four different crude *Arundo donax* extracts: A) aqueous leaf (D_2O), B) aqueous rhizome (D_2O), C) methanolic leaf (DMSO-d_6) and D) methanolic rhizome (DMSO-d_6) (300 MHz)

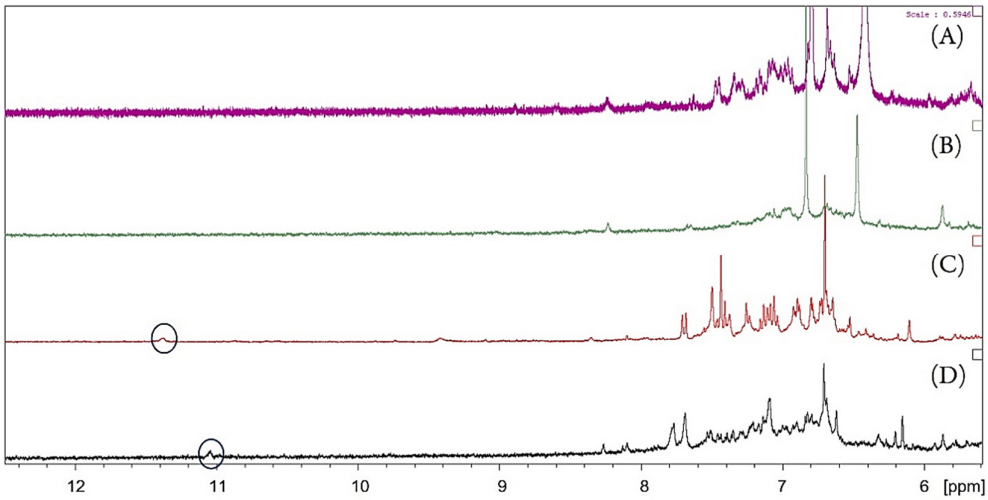

Figure 4. Nuclear magnetic resonance expansion spectra of the region between 6 and 12 ppm of the four different crude *Arundo donax* extracts: a) aqueous leaf (D_2O), b) aqueous rhizome (D_2O), c) methanolic leaf (DMSO-d_6) and d) methanolic rhizome (DMSO-d_6) (300 MHz)

Discussion

Arundo donax is a large perennial invasive grass that established and rapidly propagated in Cerrado areas of Central Brazil in the last decade (Simões *et al.*, 2013; IABIN, 2019). The lateral propagation of the plant, which occurs through the growth and regrowth of the rhizome and fragments of the stem, gives rise to clonal populations that form large monospecific stands (Simões *et al.*, 2013).

We provide evidence that its invasive success can be attributed, in part, to the production of allelopathic compounds that could potentially inhibit seedling establishment of native species. Our results show that aqueous extracts of leaves and rhizomes of *A. donax* have an allelopathic effect on Cerrado woody species impairing germination and seedling growth. This has implications for the eradication of invasive stands of this species, especially when applying mechanical or chemical methods.

The rhizome is the main structure of propagation of the species and remains buried in the soil after cutting the aerial part, which is the main method used for controlling the spread of this species (Boose and Holt, 1999; Simões *et al.*, 2013). Effective control of this plant is managed through complete removal of the rhizome (Bell, 1997), which is laborious, involves high costs, and is often not done. Applied weed control methods relying on cutting the plant back to soil level will probably result in plant resprouting and, particularly if the plant material is not removed, might also contribute to the release of allelochemicals into the soil in a continuous and lasting way, potentially inhibiting seed germination and establishment of other species.

The fragmentation of Cerrado environments, transforming the natural vegetation into patches surrounded by pastures and crop cultures facilitates the spread of different invader grasses. Leaf aqueous extracts of *A. donax* negatively affected germination and seedling growth of the invasive grass *Megathyrsus maximus*, which is widely distributed in the ecosystems of Cerrado (Filgueiras, 2015). Some authors attribute the success of the colonization of *M. maximus* to its rapid germination and seedling establishment, which increases its competitiveness relative to other species (Ferreira *et al.*, 2008), than to its allelopathic potential which is low (Hartmann *et al.*, 2017). We are not aware of studies addressing competitive interactions between *A. donax* and other invasive species and whether allelochemicals are involved. Although our results are not conclusive and require additional studies, allelopathy may enhance the competitive success of *A. donax* not only against native species but also against other invasive species.

TLC analysis showed that the molecules identified in the methanolic extracts belonged to the classes of alkaloids, flavonoids, and other phenolics, and are produced mostly in the leaves, which corroborates what was observed by other authors regarding the allelopathic efficiency of the extracts obtained from this organ (Hong *et al.*, 2010; Temiz *et al.*, 2013; Ahrar *et al.*, 2017). In contrast, TLC analysis did not detect flavonoids and any other phenolic compounds in the rhizomes. Terpenes, which were also present in the leaves, were the main secondary metabolites in the crude aqueous and methanolic extracts of *A. donax* rhizomes. The presence of terpenes in rhizomes has been reported in other plants, *e.g.* species in the Zingiberaceae family (Afzal *et al.*, 2013). By TLC we detected alkaloids only in the methanolic extract of leaves, despite a report of the presence of alkaloids in leaves, roots, and rhizomes of this species collected in Asia (Khuzhaev *et al.*, 1994; 2004). However, we detected the presence of signs of indole alkaloids in both methanolic extracts (from rhizomes and leaves) by ^{1}H-NMR analysis. It was possible to obtain indications of the presence of alkaloids in the signals with chemical shift in the range of 10-11 ppm in a spectrum of ^{1}H, as found by other authors (Dijkstra *et al.*, 1989; Dijkstra *et al.*, 1990).

The absence of antioxidant activity in the analyzed extracts is a result similar to that observed by other authors (Menezes *et al.*, 2004). However, molecules that have antioxidant activity when in isolated state or in fractions of extracts may not be active when diluted in crude extracts, such as those used in this study. Thus, the results found in the tests performed are not conclusive on the presence of molecules with antioxidant properties in *A. donax*.

Arundo donax is a species often found in humid habitats such as the shores of rivers and lakes. Active compounds present in the rhizomes and shoot fragments can be washed into the water bodies and interfere through the liquid medium in other plant species and microorganisms (Gopal and Goel, 1993). However, the test with *Artemia salina* showed that the aqueous extract of *A. donax*, in the analyzed concentrations, had low or no toxicity. This microcrustacean is a good preliminary indicator of the cytotoxicity of the material (Lhullier *et al.*, 2006). The analysis is based on the median lethal concentration, or LC_{50}, the concentration in μg/mL that causes the death of half of the organisms tested. To confirm the cytotoxicity of the sample in the test of *A. salina* the LC_{50} should be equal to or less than 30 μg/mL (Meyer *et al.*, 1982). This value was not achieved by

any of the extracts. The results may indicate that *A. donax* does not have biological activity on the aquatic fauna and that it has negative effects only on other plants, or that the concentrations analyzed were low to cause toxicity.

In the results of the antimicrobial activity, only the methanolic extracts of the rhizome showed inhibitory effects on some microorganisms, *Klebsiella pneumoniae* and *Morganella morganii*. There was no significant variation in the growth of microorganisms in most treatments. The stimulus to the growth of some microorganisms especially in the methanolic extract of leaves possibly occurred due to the high carbohydrate content, observed in the chromatographic test with Anisaldehyde and in the ^{1}H-NMR analyses.

Conclusions

We were able to show that clonal populations of *A. donax* that were successfully established in Cerrado ecosystems conserved the ability to produce a variety of secondary metabolites. We also provide evidence that the aqueous extracts of leaves and rhizomes were detrimental to germination and seedling development of several Cerrado tree species and an invasive African grass, *Megathyrsus maximus*. Leaf extracts were more effective than rhizome extracts. This information is important not only for better understanding the invasive potential of the species and for developing measurements for its effective control, but also for bioprospecting studies to isolate bioactive compounds in this species.

Authors' Contributions

LG, CSF, and SCCO conceived and designed the study. LG performed the experiments and collected the data (with assistance of CVN, MCSS, MTFE). CSF, ACF, LG, and CVN analyzed and interpreted the data. CSF, ACF, LG wrote the first draft. All authors discussed the results and contributed comments to the manuscript. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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Supplementary Table S1. The effect of different concentrations of aqueous extracts (%) from leaves and rhizomes of *Arundo donax* on the length (cm) of shoots and roots of seedlings of the invasive African grass *Megathyrsus maximus* and five native Cerrado tree species

Treatment	*Handroanthus impetiginosus*		*Megathyrsus maximus*		*Eriotheca pubescens*		*Pseudobombax tomentosum*		*Guazuma ulmifolia*		*Parkia platycephala*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Leaf extract												
Control	1.45 ± 0.12 a	2.64 ± 0.17 a	1.53 ± 0.10 ab	2.76 ± 0.19 a	1.89 ± 0.12a	4.02 ± 0.32 a	0.83 ± 0.06 a	1.03 ± 0.09 a	1.17 ± 0.08 a	1.98 ± 0.17 a	2.02 ± 0.19 ab	1.52 ± 0.07 a
1.25%	1.09 ± 0.11 ab	2.39 ± 0.20 a	1.78 ± 0.25 a	2.21 ± 0.39 ab	0.00 ± 0 b	0.87 ± 0.22 b	1.06 ± 0.09 a	1.04 ± 0.14 a	1.22 ± 0.05 a	1.53 ± 0.12 a	2.29 ± 0.20 a	1.73 ± 0.06 a
2.50%	1.24 ± 0.11 ab	2.45 ± 0.17 a	0.90 ± 0.21 bc	1.13 ± 0.30 bc	0.09 ± 0.05 b	0.39 ± 0.10 b	0.46 ± 0.02 b	0.61 ± 0.05 b	0.83 ± 0.09 ab	1.06 ± 0.12 b	1.28 ± 0.17 b	1.09 ± 0.11 b
5%	1.18 ± 0.11 ab	2.16 ± 0.18 a	1.03 ± 0.42 abc	0.62 ± 0.25 c	0.66 ± 0.20 b	1.25 ± 0.21 b	0.45 ± 0.03 b	0.58 ± 0.04 b	0.75 ± 0.23 b	1.35 ± 0.54 b	1.22 ± 0.38 bc	0.92 ± 0.24 b
10%	0.86 ± 0.09 b	1.39 ± 0.12 b	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 b	0.36 ± 0.15 b	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 c
Rhizome extract												
Control	1.20 ± 0.13 a	2.66 ± 0.23 a	1.53 ± 0.10 a	2.76 ± 0.19 ab	1.89 ± 0.12 a	4.02 ± 0.32 a	0.83 ± 0.06 a	1.03 ± 0.09 ab	1.17 ± 0.08 ab	1.98 ± 0.17 a	2.02 ± 0.19 a	1.52 ± 0.07 a
1.25%	0.28 ± 0.09 b	1.37 ± 0.14 b	1.30 ± 0.29 a	2.21 ± 0.48 a	1.27 ± 0.07 b	2.43 ± 0.16 b	1.39 ± 0.14 b	1.37 ± 0.13 bc	1.51 ± 0.09 bc	2.10 ± 0.15 a	1.28 ± 0.06 bc	1.65 ± 0.15 a
2.50%	1.39 ± 0.23 a	2.37 ± 0.36 ab	1.62 ± 0.33 a	2.96 ± 0.59 ab	1.10 ± 0.06 bc	2.42 ± 0.16 b	1.30 ± 0.09 b	1.47 ± 0.11 c	1.02 ± 0.06 a	1.53 ± 0.19 b	1.09 ± 0.05 c	1.54 ± 0.14 a
5%	1.24 ± 0.15 a	2.25 ± 0.24 ab	1.82 ± 0.27 ab	2.84 ± 0.43 ab	1.00 ± 0.10 bc	1.62 ± 0.20 c	0.71 ± 0.08 a	0.82 ± 0.09 a	1.55 ± 0.10 c	1.80 ± 0.16 ab	1.62 ± 0.08 ab	1.83 ± 0.17 a
10%	0.95 ± 0.14 ab	1.82 ± 0.28 ab	2.24 ± 0.18 b	3.37 ± 0.38 b	0.92 ± 0.07 c	1.96 ± 0.16 c	0.00 ± 0 c	0.00 ± 0 d	1.30 ± 0.12 abc	1.69 ± 0.24 ab	1.10 ± 0.08 bc	1.59 ± 0.25 a

Mean ± standard error. Different letters denote significant differences at $p<0.05$ between concentration levels.

Figure S1. *Megathyrsus maximus* (linear arrow) co-occurring with *Arundo donax* (dashed arrow) in degraded Cerrado in Brasilia, Federal District, Brazil