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Original Article

SAMARAS OF *AUSTROPLENCKIA POPULNEA* (CELASTRACEAE): NEW CONSTITUENTS AND EFFECT OF EXTRACTS AND FRIEDELIN ON GERMINATION OF *BIDENS PILOSA* (ASTERACEAE)

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ABSTRACT

Objective: Evaluation of the impact of extracts and constituents from samaras of *Austroplenckia populnea* on percentage of seed germination (%SG), germination speed index (GSI), length of rootlets (LR), seedling length (SL), and on dry mass (DM) of *Bidens pilosa* L weed.

Methods: The powder of samaras was extracted with organic solvents providing the hexane (SAPEH), chloroform (SAPEC), ethyl acetate (SAPEAE) and ethanol (SAPEE) extracts. The terpene 1 was isolated from SAPEH by means of column and thin layer chromatography and identified through NMR spectroscopy. Each extract and 1 were subjected to growth inhibition assays evaluating the following parameters: %SG, GSI, LR, SL and DM, with five repetitions.

Results: The compounds Friedelin (**1**), 7-hydroxy-clerodan-3-en-16,15:18,20-diolide (**2**), 3,5,7,4'-tetrahydroxy-6-methoxy-8-prenylflavanone (**3**), tetradecanamide (**4**), and 4-hydroxy-1,6,15-acetyloxy-8,9-benzoyloxy-agarofurane (**5**) were isolated from hexane extract of samaras of *A. populnea* and identified by spectroscopic data. The compounds **2**, **3** and **5** were not previously described as being chemical constituents from Celastraceae family. In addition, the novel compounds **3** and **5** were described here for the first time. Substantial effect on the germination of *B. pilosa* L. (picão-preto) was observed after treatment of seeds with nonpolar extracts from Samaras of *A. populnea*. Friedelin inhibited the seed germination in the tested concentrations showing toxic properties against picão-preto.

Conclusion: The germination inhibition of seeds was higher using nonpolar extracts than polar extract. Friedelin inhibited the seed germination in the tested concentrations showing toxic properties against *B. pilosa.*

Keywords: *Austroplenckia populnea*, Samaras, Growth inhibition, *Bidens pilosa*, Allelopathy.

INTRODUCTION

During the last decades, natural products represent an adequate source of biologically active compounds that have been used to preserve or regenerate the human health. Many of these compounds have chemical properties associated to enzyme activity with consequent effects on the biosynthesis of essential endogenous substances and their ability to perform inherent functions in producing organisms [1]. Many of these natural compounds have been isolated from Celastraceae family species. Some of them have shown imunossupressor activities [2] and insecticide properties [3]. The Celastraceae family includes 98 genus with approximately 1210 species [4].

Austroplenckia populnea (Reissek), Brazilian species of the Celastraceae family, is popularly known as "mangabeira-brava", "mangabarana" and "marmelinho do campo". This plant has been found in Brazilian "Cerrado" (savanna region), mainly in Minas Gerais, São Paulo and Goiás states [5]. Leaves and roots of *A. populnea* have been used in traditional medicine to treat ulcers, dysenteries and rheumatism [6]. For this plant were also demonstrated larvicidal and molluscicidal properties [7]. Compounds from leaves of *A. populnea* were isolated and identified such as pentacyclic triterpenes of the oleanane [8, 9], friedelane [10, 11, 5], agarofuran sesquiterpenes [7, 12] and the polar constituents, epigalocathechin and pro-antocianidin A [12, 13, 14]. Despite the huge diversity of properties attributed to extracts or constituents isolated from A. populnea, no seed germination activity has been reported until now. Allelopathy effects have been considered a biological process in which plants, fungi and other microorganisms synthesize secondary metabolites, known as allelochemicals, modifying the growth, survival and reproduction of other organisms. Potential allelopathic interactions by different plants have often invoked why some invasive plants have become dominant inside plant communities [15]. Allelochemicals could have beneficial or detrimental properties on the target organisms [16, 17] and may alter plant competition also indirectly through changes in ecosystem properties [15]. Thus, allelochemicals have important biological relevance in plant resistance against herbivory [16]. Allelopathic interference has been considered an important factor to determine the distribution of some plant species and their abundance inside plant communities suggesting to be essential in success of many dominant plants [16, 17].

Bidens pilosa L. (Asteraceae) or hairy beggar stick is widely scattered mainly in tropical regions of the world, being endemically found in Central and South America. *B. pilosa* has been considered an invasive plant, commonly known in Brazil as "picão-preto", that is found in entire Brazilian territory, with major prevalence in the agricultural areas of the south-central region. In Brazil, *B. pilosa* is considered one of the most important unwanted plants in both annual and perennial crops [18]. In this work, it was described the preparation of extracts of different polarities from samaras of *A. populnea*. It was also performed the preparation of hexane extract to be submitted to phytochemical methods targeting to isolate friedelin, the main pentacyclic triterpene found in this type of extract. Aiming to contribute to control of B. pilosa, in parallel with chemical study, the extracts and friedelin isolated from Samaras of A. populnea and submitted to assays to evaluate their potential effect on percentage of seed germination (%SG), germination speed index (GSI), length of the rootlets (LR), seedling length (SL), and on dry mass (DM) of B. pilosa L. weed.

MATERIALS AND METHODS

General

Column chromatography (CC) was performed on silica gel (Merck 60, 70-230 Mesh). Thin layer chromatography (TLC) was carried out using silica gel 60 F_{254} (Merck) plates. Melting points were recorded

on a Metler FP82 apparatus with processor Metler FP800. Gas chromatography coupled to mass spectroscopy (GC-MS) was realized using a GC-MSD 5975 Agilent equipment, with the following conditions: 240 °C injector temperature, 1.0 µL injected sample, 100:1 split, 1.4 psi helium pressure, column temperature program: 200 °C (1.0 min isotherm), 20 °C/min until 280 °C (40 min isotherm), with mass analysis range from 25 to 750 Da. ^{1}H and ^{13}C NMR spectra were measured on Bruker AVANCE DRX400 and AVANCE DPX200 MHz spectrometers in $CDCl_3$, with tetramethylsilane (TMS) as an internal standard.

Plant material

Samaras of *A. populnea* were collected closest to Miguelão Lake at Nova Lima, Minas Gerais, Brazil. The material was compared and identified with a voucher specimen (No 10473) deposited at the *Herbarium* of the Museum of Historia Natural of Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. Each plant material was dried over paper at room temperature (r. t.) And then fragmented using a knife mill.

Extract obtainment

The powder of samaras (286.97 g) was subjected to exhaustive extraction in a Soxhlet apparatus with organic solvents providing the hexane (SAPEH), chloroform (SAPEC), ethyl acetate (SAPEAE), and finally ethanol (SAPEE) extracts. Each solvent extractor was respectively recovered using a rotatory evaporator at temperature ≤ 40 ^{o}C and the respective extracts were dried in a vacuum desiccator. Targeting the isolation of friedelin, only the hexane extract was submitted to silica gel CC, TLC and other phytochemical methods phytochemical methods, as suggested by Matos (1980) [19] and Wagner & Bladt (1996) [20].

Phytochemical assay

The hexane extract of samaras (44.91g) was obtained as oil mixed with solid material, which was carefully separated under vacuum on a Buchner filter. Friedelin (**1**) (150.2 mg), melting range 260-263 ^{0}C, was isolated from this solid material in accordance to method previously described [19, 20]. The remaining of hexane extract was treated with ethyl acetate that induced the formation of another solid material (35.21 mg) which was separated, under vacuum on Büchner filter and dried on a dessicator. By TLC analysis, it was observed the presence of one constituent (Rf 0.8, $CHCl_3$) which was isolated using preparative TLC as a whitish solid material [**2** (9.28 mg)], melting range 189-192°C. The constituent **2** was subjected to ^{1}H and ^{13}C NMR spectroscopy, including DEPT experiment.

The oily fraction was subjected to fractionation on silica CC, eluted with hexane, chloroform, ethyl acetate and methanol, providing 100 fractions (EH-O) of 25 mL each. The eluent was recuperated using a rotary evaporator. The fractions were grouped in accordance to the TLC similar profile. By this process, the majority of fractions were considered as the complex mixture and not submitted to other assays. Two fractions, EH-O 55 and EH-O 59 were purified aiming to isolate pure constituents. The fraction EH-O 55 (152.2 mg) was subjected to preparative TLC eluted with a mixture of hexane, chloroform and ethyl acetate [35:50:15]. After treatment with iodine vapor, were observed three distinct spots. The constituent of greater concentration (Rf 0.6) was isolated by preparative TLC. After isolation, constituent **3** (11.5 mg) present a single spot when analyzed by TLC, even using eluents of different polarities. Due to this result, constituent **3** was submitted to ^{1}H and ^{13}C NMR spectroscopy including DEPT-135 experiment. The other two constituents were isolated in amounts ≤ 2 mg and disregarded. EH-O 59 (425.7 mg) was subjected to silica gel CC eluted with an isocratic mixture of chloroform and ethyl acetate [6:4], providing 26 fractions of 30 mL. After dried, the fraction 17, provides a pasty material **4** (15.4 mg). After evaporation of the eluent, the residue of fraction 18 was re-crystalized with chloroform-ethanol (8:2) to obtain compound **5** (12.08 mg) as a white amorphous solid, melting range 148-152 °C. Constituents **4** and **5** present a single spot when subjected to TLC, with eluent of different polarities, and then were submitted to ^{1}H and ^{13}C NMR spectroscopy including DEPT-135 experiment.

Allelopathy assays

Seeds of *B. pilosa* L. (picão-preto) were collected at the experimental plantation of the Centro Universitário Norte do Espírito Santo (CEUNES), São Mateus, Brazil, and sterilized by immersion for 10 minutes in 2 % sodium hypochlorite aqueous solution.

The germination, development and growth of seedlings of *B. pilosa* were carried out treating seeds with the extracts and friedelin, both isolated from Samaras of *A. populnea*. The samples were dissolved in corresponding solvent to reach final concentration of 31.25; 62.5; 125; 250; 500; 750 and 1000 mg/L of extract (SAPEH, SAPEC, SAPEAE or SAPEE), and 6.25; 12.50; 25; 50; 100; 200 and 400 mg/L of fridelin (**1**). Due to the small isolated quantities, compounds **2** to **4** isolated from Samaras of *A. populnea* were not subjected to seed growth inhibition assays. Seeds of *B. pilosa* (picão-preto) were distributed in Petri plates on two filter paper discs, previously humidified with the extract or Fridelin (**1**), both in adequate concentration. Later the plates were conditioned in germination camera at 25 °C, equipped with fluorescent light (8 x 40 W) photoperiod of 12 hours, for twelve days.

At the end of an experimental period the following parameters were evaluated: percentage of seed germination (%SG), germination speed index (GSI), length of rootlets (LR), the seedling length (SL) and dry mass (DM) of *B. pilosa* L., with five repetitions [21]. The GSI was calculated as suggested by Maguire *et al*. (1962) [22], using the number of germinated seeds, divided by the day of germination and adding until the last day of germination according to the equation: $\mathbf{N_1/ D_1 + N_2/ D_2 + N_3/ D_3 +.... + N_n/ D_n}$. The terms N_1, N_2, N_3 and N_n represent the number of germinated seeds until enesimo day. And, D_1, D_2, D_3 e D_n represent the number of days in which the seed germination was evaluated. The inhibition percent were calculated basing on the experimental data obtained for control, realized without samples, maintaining constant the other parameters.

Statistical analysis

The experimental results were subjected to analysis of variance (ANOVA) and the significant variables established by *F* test were submitted to regression analysis and the test of averages, compared according Scott & Knott (1974) [23]. A completely randomized design was adopted, with the treatments arranged in factorial scheme (4x7), being four extracts (SAPEH, SAPEC, SAPEAE and SAPEE) and seven concentrations (0.00; 31.25; 62.50; 125.00; 250.00; 500.00; 750.00 and 1000.00 mg/L), with five repetitions. The data were analyzed using the System of Analysis of Variance for balanced data software (SISVAR) [24]. P values < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical assay

It was observed Friedelin (**1**) (fig. 1) was a principal pentacyclic triterpene found in the solid material isolated from hexane extract of samaras of *A. populnea*. The chemical structure of terpene **1** was identified by melting point, TLC analysis in comparison with authentic sample and NMR spectral data. The chemical shift assignments of **1** were in accordance with the NMR spectral data of friedelin previously reported [6, 25, 26].

The constituent **2** was subjected to ^{1}H and ^{13}C NMR spectroscopy, including DEPT experiment. Thus, it was possible to establish the chemical shift assignments correspondent to 2 methyl, 8 methylene, 5 methynic, being one bonded to hydroxyl (δ_c 72.39), and 5 non-hydrogenated carbon atoms. The signals at δ_c 169.91 and δ_c 174.74 were associated to carboxyl groups [27]. The presence of the double bond [δ_c 134.45 (CH) and δ_c 139.23 (C)], and the ^{1}H and ^{13}C NMR profiles suggested a structure of the clerodane diterpene class. For this reason, it was performed a comparison of the NMR data of compound **2** with published data for clerodanes (Table 1). The NMR spectral data of **2** were similar to the clerodane BT-CD isolated from *Bacharis trimera* (Asteraceae) [28, 29]. Based on the spectral data was attributed to **2** the structure of BT-CD [29] or 7-hydroxy-clerodan-3-en-16,15:18,20-diolide (**2**) (fig. 1).

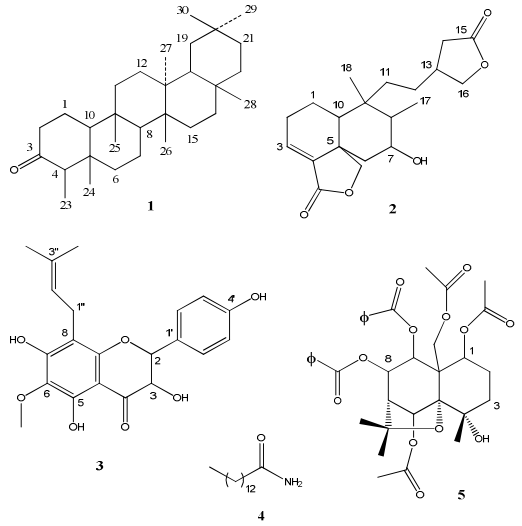

Fig. 1: Chemical structures of friedelin (1), 7-hydroxy-clerodan-3-ene-16,15:18,20-diolide (2), 3,5,7,4'-tetra-hydroxy-6-metoxy-8-prenylflavanone (3), tetradecanamide (4) and 4-hydroxy-1,6,15-tri-acetyloxi-8,9-di-benzyloxi-agarofurane (5).

The presence of clerodanes diterpenes was also found in species of the family Flacourtiaceae [30], Asteraceae [29] and Labiatae [31]. Properties such as antifungal, antibacterial, insect repellence and others were attributed for compounds of the clerodane class by bio-monitored fractionations [30, 31].

The constituent **3** was isolated as an amorphous solid, melting point 85-88 °C. By the ^{13}C NMR and DEPT-135 spectral data were observed three signals correspondents to methyl, one of them attributed to methoxyl, one methylene, seven methine groups, and ten signals associated to non-hydrogenated carbon. The signal at δ_C 195.91 was attributed to ketone carbonyl and the signals at δ_C 121.30 and δ_C 132.54 were associated to double bond. In the ^1H NMR spectrum were observed signals correspondent to aromatic hydrogen atoms in the region between δ_H 6.00 and δ_H 8.00. The signal centered at δ_H 3.97 was attributed to hydroxyl linked to CH (δc 73.33) (Table 2).

A comparison of the NMR spectral data of **3** with those available in the literature was performed. Our results demonstrated the high similarity with the flavonoid 3,5,7,2'-tetrahydroxy-6-methoxy-8-prenylflavanone isolated from *Dioclea grandiflora* by Lemos *et al.* (2002) [32]. Based on this comparison it was possible to propose for constituent **3** the chemical structure of 3,5,7,4'-tetrahydroxy-6-methoxy-8-prenylflavanone (**3**) ($C_{21}H_{22}O_7$, MW 386 g/mol) (fig. 1), an unpublished flavanoid.

Table 1: 1D NMR spectral data ($CDCl_3$, 200 MHz) of 7-hydroxy-clerodan-3-en-16,15:18,20-diolide (2) and comparison of its δc with reported data.

Carbon Number	7-hydroxy-clerodan-3-ene-16,15:18,20-diolide (2)			Reported δ_C	
	δ_C	Attribution	δ_H [*J* (Hz)]	(Werner *et al.*, 1977)	(Januário *et al.*, 2004)
1	19.35	CH_3	0.86 *s*	19.4	19.3
2	27.65	CH_2	-	27.6	27.6
3	134.45	CH	3.9	139.2	135.1
4	139.23	C	6.72 *d*	134.7	139.0
5	44.90	C	-	44.9	44.9
6	40.44	CH	-	40.5	40.4
7	72.39	CH	1.63 *s*	72.21	72.3
8	40.47	CH_2	-	40.5	40.4
9	38.56	C	-	38.4	38.3
10	48.19	CH	-	48.3	48.1
11	36.22	CH_2	-	36.2	32.2
12	26.56	CH_2	-	26.5	26.5
13	36.13	CH	-	36.1	36.1
14	34.18	CH_2	-	34.6	34.7
15	176.74	C	-	176.6	176.9
16	73.23	CH_2	3.9-5.3 *m*	73.1	73.3
17	11.63	CH_3	1.03 *d* [7.2]	11.9	12.0
18	19.26	CH_2	1.00 *d*	19.1	19.3
19	72.71	CH_2	4.11 *s*	72.7	72.8
20	169.91	C	-	169.8	170.1

Through the analysis of ^1H-NNR spectrum of constituent **4**, the signal at δ_H 0.80 was attributed to hydrogen of methyl group and the intense signal between δ_H 1.20 and δ_H 1.90 was correlated to hydrogen atoms of long chain methylene groups. In the ^{13}C NMR spectrum, signals were predominant in the region between the δ_C 10.00 and δ_C 35.00. These signals were associated to long chain containing methylene and methyl groups [27]. These observations were proved by the sub-spectrum DEPT-135, in which the signal at δ_C 14.75 was attributed to methyl group and the intense signals observed between δ_C 28.16 and δ_C 33.02 was associated to a long-chain methylene groups. The signal at δ_C 175.10 was attributed to a carboxyl-terminal amide [27]. Based on the correlation between the number of hydrogen and carbon atoms was possible to establish the presence of 14 carbon atoms and suggested for compound **4** the structure of tetradecanamide (fig. 1).

In the ^1H-NMR spectrum of **5** it was observed a set of hydrogen signals between δ_H 1.26 to δ_H 1.67 and δ_H 2.08 to δ_H 2.11, which was associated to aliphatic carbon chain. The signals observed between δ_H 7.38 to δ_H 7.67, and δ_H 7.93 to δ_H 8.15, was correlated to hydrogen atoms bonded to aromatic carbon. Singlet signals were observed at δ_H 1.47; 1.52; 2.11; 2.08 and δ_H 2.26. Through integration of this hydrogen signals it was possible to establish the presence of methyl groups bonded to non-hydrogenated carbons. The integration of the signal at δ_H 1.56 was correlated with hydrogen atoms of two methyl groups and the signal at δ_H 4.01 was attributed to a hydroxyl bonded to CH. By the ^{13}C NMR spectrum and DEPT-135 of **5** it was established the presence of signals associated to 6 methyls, 3 methylenes, 15 methynes, and 11 non-hydrogenated carbon atoms, and the signal at δ_C 170.55 was attributed to C=O of acetyl group bonded to methylene carbon. Through the analysis of NMR data it was possible to establish the presence of hydroxyl, methyl, acetyl and benzoyl groups bonded to the basic structure of **5** (Table 3).

Similarities were found between NMR spectral data of **5** with published data of 4-hydroxy-1,2,6,15-tetrahydro-9-acetyloxy-benzoyloxy-agarofurane or populnane isolated from leaves of *A. populnea* [26]. ^{13}C NMR data of **5** (Table 3) were also similar to carbon chemical shifts of agarofurans known as reissantines FH1, FH2 and FH3, isolated from *Reissantia buchananii*, species of the Celastraceae family [33].

Table 2: ^{1}H and ^{13}C NMR (400 MHz, CDCl3) data of 3,5,7,4'-tetrahydroxy-6-methoxy-8-trocar prenil por prenyl (3) and comparison with previously reported ^{13}C data of an isomer*

Carbon Number	3,5,7,4'-Tetra-hydroxy-6-metoxy-8-prenylflavone (3)			δ_C of isomer*
	δ_C	Attribution	δ_H	
2	79.00	CH	6.99	79.2
3	73.33	CH	3.97	72.6
4	195.91	C	-	199.6
5	151.09	C	11.19 (OH)	153.9
6	128.72	C	-	129.9
7	156.82	C	-	158.7
8	108.24	C	-	108.4
9	155.59	C	-	156.4
10	100.38	C	-	101.4
1′	124.10	C	-	124.9
2′	126.94	CH	7.26	157.3
3′	130.09	CH	7.0	116.3
4′	153.96	C	-	129.9
5′	121.46	CH	7.03	119.5
6′	118.53	CH	7.55	129.0
1″	21.094	CH_2	3.27	22.4
2″	121.30	CH	5.43	123.4
3″	132.54	C	-	130.8
4″	17.76	CH_3	1.66	17.7
5″	25.76	CH_3	1.25	25.7
OCH_3	61.09	CH_3	3.30	60.2

* 3,5,7,2'-Tetrahydroxy-6-methoxy-8-prenylflavanone (Lemos *et al*., 2002).

Table 3: 1D NMR spectral data ($CDCl_3$, 200 MHz) of 4-hydroxy-1,6,15-tri-acetyloxi-8,9-di-benzyloxi-agarofurane (5) and comparison of its δ_C with the published data for reissantines [26]

4-hydroxy-1,6,15-tri-acetyloxi-8,9-di-benzyloxi-agarofurane (5)			δ_c Reissantines ($CDCl_3$, 125 MHz) (Chang *et al*., 2006)		
C	**Attribution**	**δ_c**	**FH1**	**FH2**	**FH3**
1	CH	75.23	76.39	77.32	-
2	CH_2	22.69	25.06	27.49	-
3	CH_2	37.79	37.99	39.11	39.84
4	C	70.58	70.22	70.95	70.16
5	C	91.95	92.51	91.32	93.87
6	CH	78.25	75.11	78.54	74.87
7	CH	53.45	52.07	53.71	53.74
8	CH	74.02	75.50	68.74	70.88
9	CH	72.85	75.04	72.68	-
10	C	52.03	50.71	51.08	-
11	C	82.65	83.97	84.47	83.82
12	CH_3	24.40	25.79	30.41	24.00
13	CH_3	29.59	29.62	26.51	29.37
14	CH_3	22.48	23.41	23.78	23.29
15	CH_2	61.04	61.03	-	-
CH_3COO-**C1**					
C=O		169.74	169.82	169.24	
CH_3		21.53	21.43	20.80	
CH_3COO-**C6**			-	-	-
C=O		169.92	-	-	-
CH_3		21.28	-	-	-
ΦCOO-**C8**					
C=O		164.81	-	-	-
C (C1)		129.98	-	-	-
CH (C2-C6)		128.60	-	-	-
CH (C3-C5)		128.54	-	-	-
CH (C4)		133,24	-	-	-
ΦCOO-C9					
C=O		166.08	165.62	165.69	166.27
C (C1)		129.64	128.38	128.83	129.28
CH (C2-C6)		129.81	129.02	129.90	130.16
CH (C3-C5)		129.27	128.38	128.67	128.82
CH (C4)		133.38	133.35	133.37	133.82
CH_3COO-C15					
C=O		170.55	169.82	169.24	-
CH_3		20.70	21.43	20.80	-

Thus, it was proposed the basic structure of agarofuran for **5**. To confirm this hypothesis, constituent **5** was subjected to GC-MS. In the mass spectra obtained for the main constituent [retention time 39.19 min (76.02 %)], it was observed a peak at *m/z* 592 associated with [M^+ - acetic acid], *m/z* 105, concerning to the loss of C_7H_5O (ø–CO^-) and *m/z* 43 corresponding to CH_3-C=O. Based on the spectral data, it was suggested for **5** the structure of 4-hydroxy-1,6,15-tri-acetyloxi-8,9-di-benzyloxi-agarofurane [$C_{35}H_{40}O_{12}$, MW 652 g/mol] (fig. 1). Sesquiterpene esters with β-dihydro-agrofuran structure were suggested as a taxonomic marker of the species of the Celastraceae family [34]. From the hexane extract of samaras were isolated and identified by spectroscopy data the constituents: friedelin (**1**); 7-hydroxy-clerodan-3-en-16,15:18,20-diolide (**2**); 3,5,7,4'-tetrahydroxy-6-methoxy-8-prenylflavanone (**3**); tetradecanamide (**4**) and 4-hydroxy-1,6,15- -acetyloxy-8,9-benzoyloxy-agarofurane (**5**) (fig. 1). Compounds **2**, **3** and **5** were not previously identified in species of the family Celastraceae. In addition, it was not found reports in the literature regarding the compounds **3** and **5**.

Allelopathy assay

The triterpenoids have been shown one of the main groups of secondary compounds with allelopathic activity [35]. Pentacyclic triterpenes have been considered as a class of promising secondary metabolites which arising from cyclization of squalene [36]. Several biological activities have been attributed to this group of secondary metabolites, such as bactericidal, fungicidal, antiviral, cytotoxic, analgesic, anticancer, spermicidal, cardiovascular and antiallergic [37]. Waller (1999) [35] suggested triterpenoids as being the principal group of secondary compounds with allelopatic properties. Among the pentacyclic triterpenes, fridelin was cited as being potent allelochemical when it is used along with epifridelinol against weeds malice (*Mimosa pudica*) and obtusifolia (*Senna obtusifolia*) [38]. This pentacyclic triterpene was also isolated from leaves of *A. populnea* [26] and from other species of the Celastraceae family, such as *M. gonoclada* [38].

The potential allelochemical effect has been associated to the nature of secondary metabolite and the sensitivity of the type that assesses development [39]. The structural diversity of triterpenes with potential antiprotozoal activity involved in different mechanisms of action has stimulated the interest to identify other natural compounds that may provide new types of antimicrobials and also for weed control [40].

Then, the main objective associated to the studies of allelopathic properties has been based on the resistance or tolerance acquisition to secondary metabolites which act as allelochemicals being more or less specific [39]. The hexane (SAPEH), chloroform (SAPEC), ethyl acetate (SAPEAE), and ethanol (SAPEE) extracts from Samaras of *A. populnea* were subjected to assays to evaluate their allelochemicals properties through %SG, GSI, LR, SL and DM of *B. pilosa* L.

Percentage of seed germination (%SG)

In this work the most efficient extract was the SAPEH. At concentration of 1000 mg/L, this extract inhibited 50% of seed germination, while SAPEE, the least efficient, had no significant inhibition at the same concentration. These extracts from samaras displayed more phytotoxic effect against *B. pilosa* L comparing with the other extracts. Therefore, the extracts SAPEE and SAPEAE showed higher germination percentage and consequently lower inhibitory effect on seed germination. No statistical differences between these extracts were observed (Table 4). Similar results related to the reduction of the percentage of germination were observed in previous studies developed by Hoffmann *et al*. (2007) [41], Azambuja *et al*. (2010) [42] and Haida *et al*. (2010) [43] using seeds of *B. pilosa*.

Germination speed index (GSI)

SAPEH and SAPEAE extract displayed high inhibition comparing with the other extracts (Table 5).

Table 4: Effect of the concentration of extracts from samaras of *A. populnea* on percentage of seed germination (%SG) of *Bidens pilosa* L

Extract	SG % after treatment with extracts			
(mg/L)	SAPEH	SAPEC	SAPEAE	SAPEE
0	83.32 Ba	88.32 Aa	81.68 Ba	90.02 Aa
31.25	73.36 Ba	86.66 Aa	80.00 Ba	88.36 Aa
62.50	71.66 Bb	83.34 Ab	70.00 Bb	85.00 Ab
125	70.00 Bb	80.00 Ab	68.32 Bb	83.36 Ab
250	66.66 Bb	76.66 Ab	66.66 Bb	81.66 Ab
500	61.68 Bc	73.34 Ac	60.00 Bc	79.98 Ac
750	51.66 Bc	70.00 Ac	58.34 Bc	78.34 Ac
1000	41.68 Bc	70.00 Ac	50.00 Bc	73.36 Ac

* S = Samaras, A = *Austroplenckia*, P = *populnea*, H = hexane, C = Chloroform, EA = ethyl acetate, and E = ethanol. ** Means followed by the same letter, being this tiny on the column, and capital in same line, do not differ by Scott-Knott test at 5 % significance.

Table 5: Effect of the concentration of extracts from samaras of A. *populnea* on germination speed index (GSI) of *Bidens pilosa* L

Extract	GSI after treatment with extracts			
(mg/L)	SAPEH	SAPEC	SAPEAE	SAPEE
0	20.70 Ba	23.26 Aa	19.78 Ba	21.88 Aa
31.25	18.88 Bb	21.24 Ab	18.46 Bb	20.56 Ab
62.50	18.06 Bb	21.30 Ab	17.14 Bb	21.32 Ab
125	17.94 Bb	19.26 Ab	15.92 Bb	19.90 Ab
250	16.70 Bc	18.32 Ac	14.40 Bc	19.68 Ac
500	15.94 Bc	17.68 Ac	13.24 Bc	18.66 Ac
750	13.12 Bd	16.04 Ad	14.10 Bd	18.74 Ad
1000	10.60 Bd	17.34 Ad	12.20 Bd	16.78 Ad

* S = Samaras, A = *Austroplenckia*, P = *populnea*, H = hexane, C = Chloroform, EA = ethyl acetate, and E = ethanol. ** Means followed by the same letter, being this tiny on the column, and capital in same line, do not differ by Scott-Knott test at 5 % significance.

Length of rootlets (LR)

The hexane extract of *A. populnea* samaras, at concentrations ≥ 25 mg/L induced strong allelopathic effect on the length of the rootlets of *B. pilosa* L., in accordance with the quadratic model. It was observed that SAPEH behaved significantly different from other extracts and their activity increased with higher concentrations. Regarding extracts SAPEC and SAPEE were not observed significant differences related to length of rootlets. On the other hand, SAPEAE induced a significant difference on length of rootlets (Table 6).

Table 6: Effect of the concentration of extracts from samaras of A. *populnea* on length of rootlets (LR) (mm) of *Bidens pilosa L*

Extract (mg/L)	LR (mm) after treatment with extracts			
	SAPEH	SAPEC	SAPEAE	SAPEE
0	28.54 Ca	37.96 Aa	30.96 Ba	36.12 Aa
31.25	28.08 Ca	37.14 Aa	30.54 Ba	35.68 Aa
62.50	23.86 Cb	36.82 Ab	29.26 Bb	34.52 Ab
125	21.14 Cb	35.98 Ab	29.26 Bb	33.22 Ab
250	21.04 Cb	35.34 Ab	29.20 Bb	32.64 Ab
500	20.70 Cc	32.06 Ac	26.88 Bc	31.44 Ac
750	19.42 Cc	31.92 Ac	26.32 Bc	29.84 Ac
1000	18.76 Cc	30.32 Ac	25.76 Bc	29.56 Ac

* S = Samaras, A = *Austroplenckia*, P = *populnea*, H = hexane, C = Chloroform, EA = ethyl acetate, and E = ethanol. ** Means followed by the same letter, being this tiny on the column, and capital in same line, do not differ by Scott-Knott test at 5 % significance.

According Gussman *et al*. (1994) [44] and Hoffman *et al*. (2007) [41], the elongation of shoots and roots are dependent on the intensity of cellular division, training and exchange xilematic vessels. These structures are dependent on the nutrient uptake and nutrient partitioning by seedling. As a result, the root tissues of plants are the most sensitive to the action of allelochemicals. Thus, the lower germination speed index showed more difficult for the plant to stretch, as observed after treatment with SAPEE. Our results were in accordance with previous studies developed by Alves *et al*. (2004) [45], related to length of rootlets of lettuce seedlings observed after the treatment of seeds with oils isolated from cinnamon, rosemary, peppermint and citronella grass.

Seedling length (SL)

In relation to the seedlings length, the hexane (SAPEH) and ethyl acetate (SAPEAE) extracts from samaras it was not observed significant differences among them. These extracts reduced the shoot length of hairy beggartick seeds (*B. pilosa*) at low concentrations ≤ 62.50 mg/L. The extracts SAPEC and SAPEE exhibited similar behavior and also did not differ statistically, but induced less allelopathic effect comparing with other extracts from samaras (Table 7).

Dry mass (DM)

The inhibition of germination of "picão-preto" (*B. pilosa* L.) seeds was higher after treatment with nonpolar extracts SAPEH and SAPEC. At 1000 mg/L, SAPEH inhibited 50 % germination of the seeds, while SAPEE, the least efficient, had no significant inhibition at the same concentration. These results were also reflected through the results of dry mass (Table 8).

Fridelin (**1**) (fig. 1), isolated from hexane extract of Samaras reduced the percentage of germination (%SG) even at lower concentrations (12.50 mg/L) and also reduce the germination speed index (GSI) in concentration higher than 25 mg/L (Table 9). This triterpene did not induce differences in the length of rootlets (LR) and induced a small reduction in the seedlings length (SL) in concentrations higher than 50.0 mg/L (Table 9).

Table 7: Effect of the concentration of extracts from samaras of A. *populnea* on seedling length (SL) (mm) of *Bidens pilosa L*

Extract (mg/L)	SL (mm) after treatment with extracts			
	SAPEH	SAPEC	SAPEAE	SAPEE
0	58.06 Ba	65.72 Aa	50.10 Ba	63.16 Aa
31.25	41.00 Bb	63.54 Ab	44.32 Bb	61.74 Ab
62.50	37.00 Bc	62.70 Ac	40.94 Bc	60.48 Ac
125	36.64 Bc	60.82 Ac	40.80 Bc	59.08 Ac
250	36.12 Bc	60.54 Ac	39.66 Bc	58.10 Ac
500	35.56 Bc	60.76 Ac	37.56 Bc	57.28 Ac
750	35.22 Bc	58.54 Ac	37.44 Bc	56.92 Ac
1000	33.22 Bd	50.54 Ad	36.02 Bd	48.66 Ad

* S = Samaras, A = *Austroplenckia*, P = *populnea*, H = hexane, C = Chloroform, EA = ethyl acetate, and E = ethanol. ** Means followed by the same letter, being this tiny on the column, and capital in same line, do not differ by Scott-Knott test at 5 % significance.

Table 8: Effect of the extract concentration of extracts from samaras of A. *populnea* on dry mass (mg) of *Bidens pilosa* L

Extract (mg/L)	DM (mg) after treatment with extracts			
	SAPEH	SAPEC	SAPEAE	SAPEE
0	59,48 Ba	62,98 Aa	58,58 Ba	61,00 Ba
31.25	56,42 Bb	61,82 Ab	56,34 Bb	56,74 Bb
62.50	54,94 Bb	61,26 Ab	56,00 Bb	55,32 Bb
125	53,88 Bb	59,26 Ab	55,00 Bb	55,20 Bb
250	53,40 Bb	58,76 Ab	52,94 Bb	54,12 Bb
500	50,72 Ba	57,34 Aa	49,38 Ba	52,40 Ba
750	48,26 Ba	56,02 Aa	47,08 Ba	51,66 Ba
1000	45,68 Ba	55,46 Aa	42,62 Ba	51,00 Ba

* S = Samaras, A = *Austroplenckia*, P = *populnea*, H = hexane, C = Chloroform, EA = ethyl acetate, and E = ethanol. ** Means followed by the same letter, being this tiny on the column, and capital in same line, do not differ by Scott-Knott test at 5 % significance.

The inhibition of germination of "picão-preto" (*B. pilosa*) seeds was higher using nonpolar extracts. Friedelin inhibited the seed germination in the tested concentrations showing toxic properties against "picão-preto". This study contributed to the researches of allelopathic effects and open perspectives for the production of natural substances that can be used in weeds control. And also it

stimulates the accomplishment of new experiments using other invasive plants, others constituents isolated from other species of the Celastraceae family and respective derivatives in order to establish chemical structure *versus* allelopatic activity relationships.

Table 9: Effect of fridelin concentration on percentage of seed germination (%SG), germination speed index (GSI), length of rootlets (LR) and seedling length (SL) of *Bidens pilosa* L

Friedelin	Parameter evaluated			
(mg/L)	SG (%)	GSI	LR (mm)	SL (mm)
0	85.00 A	17.00 A	45.60 A	44.14 A
6.25	76.66 A	17.04 A	44.32 A	42.10 A
12.50	58.34 B	15.62 A	41.90 A	42.10 A
25	58.32 B	11.60 B	38.96 A	39.76 A
50	58.34 B	15.62 A	38.92 A	35.50 B
100	56.64 B	12.02 B	33.86 A	33.00 B
200	53.34 B	12.42 B	33.70 A	31.96 B
400	41.66 B	9.90 B	33.02 A	31.54 B

* Means followed by the same letter, being this tiny on the column, and capital in the same line, do not differ by Scott-Knott test at 5 % significance.

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CONFLICT OF INTERESTS

Declared None

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