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Article

Composition of essential oil and allelopathic activity of aromatic water of *Aster lanceolatus* Willd. (Asteraceae)

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The essential oil obtained from flowers of *Aster lanceolatus* was submitted the CG-MS and presented as result thirteen substances with largest concentration; among them, the caryophyllene oxide with the larger one. The aromatic water obtained during the extraction process of this essential oil was forwarded to allelopathic test, and demonstrated to be capable to inhibit the germination and growth of *Lactuca sativa*.

Uniterms: Essential oil. *Aster lanceolatus*. Caryuphyllene oxide. Aromatic water. Allelopathy.

O óleo essencial obtido das flores de *Aster lanceolatus* foi submetido a CG-EM e apresentou como resultado treze substâncias, entre elas o óxido de cariofileno com a maior concentração. A água aromática obtida durante o processo de extração do óleo essencial foi encaminhada para teste alelopático, a qual demonstrou ser capaz de inibir a germinação e crescimento de *Lactuca sativa.*

Unitermos: Óleo Essencial. *Aster lanceolatus.* Óxido de cariofileno. Água aromática. Alelopatia.

INTRODUCTION

The Asteraceae family compasses the largest family of angiosperms with approximately 23,000 species, 1,535 genera and represents approximately 10% of all world flora (Nakajima, Semir, 2001). The plants of Asteraceae family are studied respecting their chemical composition and biological activity (Verdi, Brighente, Pizzolatti, 2005) presenting varied constituents, between them essential oils (Cronquist, 1981) that could present as commercial importance for perfumes and liquors industry (Costa, Doni Filho, 2002, Agostini *et al.*, 2005) as pharmacological importance due to diverse biological activities: nematicide, genotoxic, larvicide, antispasmodic (Gazim *et al.*, 2007), modulation for macrophages activation (Lopes *et al.*, 2005), antimicrobial (Ferronatto *et al.*, 2007, Bozin *et al.*, 2008), antifungal (Gazim *et al.*, 2008) and allelopathic (Verdi, Brighente, Pizzolatti, 2005).

The genus *Aster* presents varied compounds, between them, flavones, saponines, sugars and esters in *A. ageratoides* (Cheng *et al.*, 1993); saponines in *A. batangensis* and *A. lingulatus* (Shao *et al.,* 1995a, Shao *et al.*, 1995b, Shao *et al.*,1995c, Shao *et al.*, 1997a, Shao, Ho and Chin, 1997); coumarins in *A. praealtus* (Wilzer *et al.*, 1989); 3,5-dicaffeoyl-*muco*-quinic in *A. scaber* (Kwon *et al.*, 2000); cyclic pentapeptides (Morita *et al.*, 1993), monoterpenic and triterpenic glicosides (Cheng, Shao, 1994), oligopeptides (Cheng *et al.*, 1994), acyclic peptide (Morita *et al.*, 1995) and pentapeptides (Cheng *et al.*, 1996) in *Aster tataricus*.

Known popularly as *margarida-de-são-miguel*, panicled aster and monte cassino, *Aster lanceolatus* is a cutting species (Ferronato, 2000) that presents extracts with antibacterial activity against *Streptococcus pyogenes, Salmonella typhimurium, Staphylococcus aureus* (Dias *et al.*, 2005a, 2006), antifungal activity against *Fusarium oxysporum* and *Cylindrocladium spathulatum* (Dias *et al.*, 2006) and allelopathic influence on the germination and growth of *Lactuca sativa* (Dias, Miguel, Miguel, 2007).

Many definitions were already presented for allelopathy, however, the definition accepted by the International Society of Allelopathy mentions allelopathy as a science that studies any process involving, mainly, secondary

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metabolites produced by plants, algae, bacteria and fungi, which influences the growth of biological systems with positive or negative effects (Pinto *et al.*, 2002).

In this perspective, this work had the purpose to identify the essential oil composition by means of gas chromatography and evaluate the allelopathic activity of aromatic water from *A. lanceolatus*.

MATERIAL AND METHODS

A. lanceolatus was collected in the month of June/2003 at Holambra, state of São Paulo. The exsiccates were identified by the botanist Dr. Gerdt Hatchbach of Municipal Botanical Museum (*Museu Botânico Municipal* - MBM) of Curitiba, state of Paraná, and recorded in this museum under the number 287.063.

Utilizing hydrodistillation, 100 g of flowers were submitted to a "Clevenger" type apparatus for six hours, being obtained essential oil and aromatic water. The essential oil was analyzed by CG-MS (0.2 mL of oil being solubilized in 1 mL of methanol) utilizing an Agilent® 6890 gas chromatograph equipped with a HP-5 column (5% phenyl and 95% polydimethylsiloxane) with 0.25 mm of internal diameter and 15 cm of length, and an Agilent® 5973 mass spectrometer. Utilizing helium as carrier gas (0.8 mL per minute), the utilized temperature gradient was: initial temperature 40 °C with landing of 2 minutes and heating slope at 10 °C per minute up to 250 °C with landing of 20 minutes. The injector temperature was of 250 °C and the detector temperature of 280 °C. The volume of injection was of 20 µL with split of 100:1. The identification of constituents of essential oil was done by comparison of mass spectrum of referred constituent, with the existing spectra in the equipment databank (Mass Spectral Database NIST 98).

The aromatic water was forwarded to allelopathic assay utilizing the technique described by Macías, Castellano, Molinillo, 2000; Chon *et al.*, 2005; Dias *et al.*, 2005b with some modifications: the aromatic water was not taken to stove at 60 °C for 24 hours, but utilized directly on filter paper, being excluded, therefore, the use of distilled water to humidify the filter paper. The control utilized was distilled water under the same conditions as those of the assay. According to Farmacopéia (1988), aromatic water is the saturated solution of essential oil, therefore, the utilization of aromatic water had the purpose to establish the existing relationship between entre the essential oil constituents and the allelopathic activity. For statistical analysis it was employed the program SISVAR (Ferreira, 2000). The verification of differences of statistically significant averages was developed by means of Scott-Knott test with 5% of probability. The Scott-Knott test was chosen because it is clear, objective and exempted of ambiguities (commonly present in the majority of tests of multiple comparisons). The treatment was considered effective when all the repetitions were within the same group of averages.

RESULTS AND DISCUSSION

In 100g of flowers of *A. lanceolatus,* it was obtained 0.2 % of essential oil (w/v) and 5 mL of aromatic water.

The chromatogram evaluation has revealed the presence of thirteen substances (Table I), out of which the caryophyllene oxide (Figure 1) presented the higher concentration. At Figure 2, it is possible to visualize the mass spectrum comparison of caryophyllene oxide present in the sample, with the spectrum appearing at NIST Library and the peak of molecular ion in *m/z* 220, indicative of caryophyllene oxide structure (Moreira *et al.*, 2007).

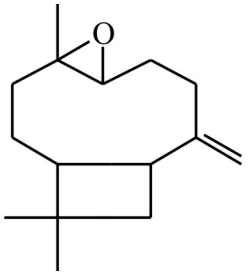

FIGURE 1 - Chemical structure of caryophyllene oxide (Sousa *et al.*, 2006)

The caryophyllene oxide is a sesquiterpen present in essential oil of *Ageratum conyzoides* (known as wild mint) (Castro *et al.*, 2004), *Calyptranthes concinna*, *Calyptranthes lucida* and *Calyptranthes rubella* (Limberger *et al.*, 2002), *Piper arboreum* (Mesquita *et al.*, 2005), *Cinnamomun zeylanicum* (Lima *et al.*, 2005), *Baccharis articulata, Baccharis cognata, Baccharis uncinella, Baccharis milleflora* (Agostini *et al.*, 2005) propolis (Sousa *et al.*, 2006; Torres *et al*., 2008), *Chamomilla recutita* (Borsato *et al.*, 2007), *Calendula officinalis* (Gazim *et al.*, 2007), *Copaifera langsdorffii* (Lima Neto, Gramosa, Silveira, 2008), *Duguetia furfuracea* (Valter *et al.*, 2008) with antibacterial activity against *Staphylococcus aureus* (Ulubelen *et al.*, 1994) and anticarcinogenic activity (Sousa *et al.*, 2006)

The results of allelopathic assay have demonstrated statistically significant difference between the germination of *L. sativa* with aromatic water and with distilled water, indicating inhibition of germination (Table II). In

TABLE I - Constituents of essential oil of *A. lanceolatus*

PEAK	RETENTION TIME (min)	AREA (%)	NAME OF CONSTITUENT
1	8.27	2.58	Mirtenol
2	12.37	1.76	α-Murolen
3	12.94	3.47	Naphthalene, 1,2-dihydro-1,1,6-trimethyl
4	13.08	7.36	Bisabolene
5	13.26	1.51	β-Ionone
6	13.40	4.11	Spatulenol
7	**13.47**	**34.53**	**Caryophyllene oxide**
8	13.77	12.72	3-Cyclohexene-1-carboxaldehyde, 3,4
9	13.96	3.71	Cedren-13-ol, 8
10	14.22	2.48	Neoclovene-(I), dihydro
11	14.50	1.94	Azulene, 1-4-dimethyl-7-(1-methyl)
12	14.56	1.53	2*H*-Benzocyclohepten-2-one, 1,4a,5,6,7,8,9,9a-octahydro 4a methyl, *trans*
13	16.15	5.19	1, hexahydrofarnesyl acetone

NOTA: Data obtained by means of comparison to equipment databank (Database/NIST 98).

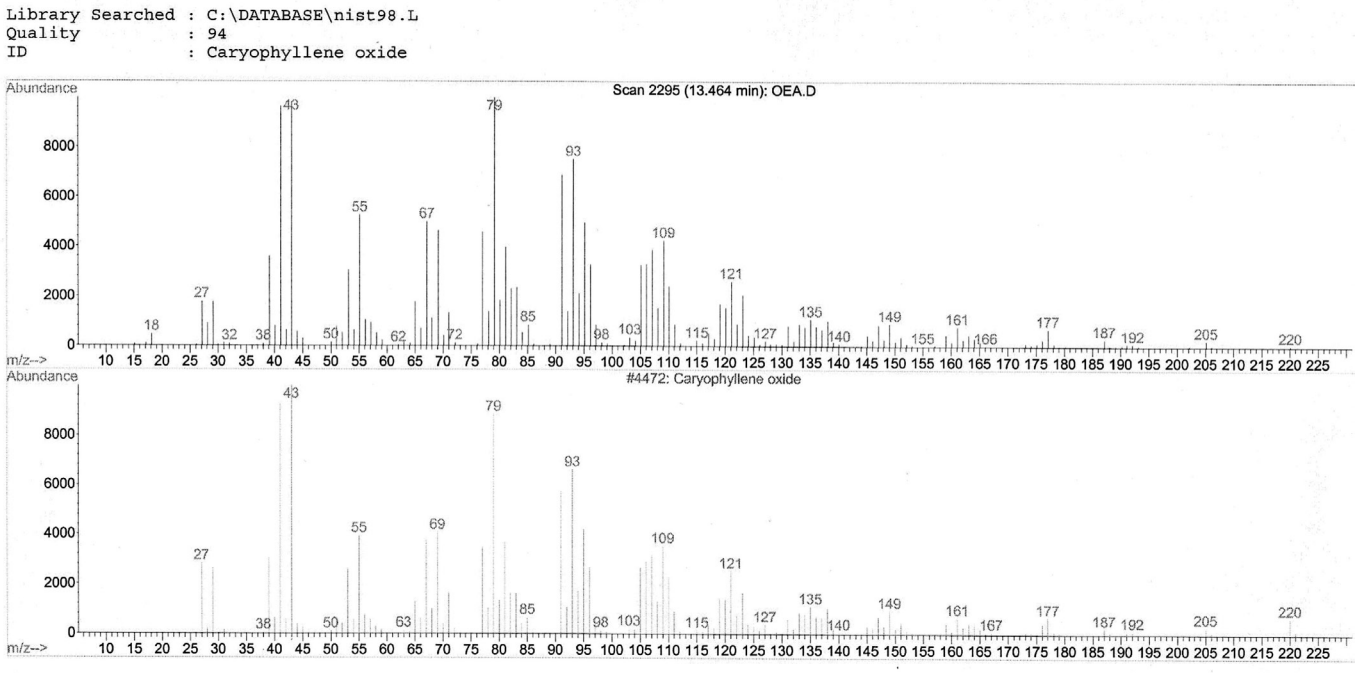

FIGURE 2 - Mass spectrum of caryophyllene oxide.

the growth assay it was observed that the radicles of *L. sativa* did not suffer influence of aromatic water from *A. lanceolatus*, but, the hypocotyls have suffered such inhibition (Table II).

When evaluating the growth, it is known that it results from the germination. The hypocotyl and the radicle are originated from the embryonic axis, a vital part of seed containing meristematic tissue in both of its two extremities, with growth conditions for both directions, that of the roots (radicle) and the stalk one (hypocotyl), originating a plantule with fixation conditions to soil and ability to photosynthesize necessary substances (Carvalho, Nakagawa,

TABLE II - Evaluation of allelopathic activity of aromatic water from *A. lanceolatus*

GERMINATION –Scott-Knott Test (P<0.05)					
SAMPLE	GVI (GERMINATION VELOCITY INDEX)				
Aromatic water	3.6625 a				
Control	7.5425 b				
GROWTH –Scott Knott Test (P<0.05)					
Radicle			Hypocotyl		
Treatment	Repetition	Average (mm)	Treatment	Repetition	Average (mm)
Aromatic water	1	16.9 a	Aromatic water	1	10.3 a
Aromatic water	2	7.9 a	Aromatic water	2	8.2 a
Aromatic water	3	13.2 a	Aromatic water	3	10.7 a
Aromatic water	4	19.9 a	Aromatic water	4	13.8 a
Control	1	20.6 a	Control	1	22.4 b
Control	2	23.6 a	Control	2	29.8 b
Control	3	21.2 a	Control	3	20.6 b
Control	4	24.2 a	Control	4	28.4 b

*Averages followed by the same letter in the same column, do not statistically differ between each other.

1983). The growth of embryonic axis results from its cells increase of and multiplication by means of mitotic divisions (Carvalho, Nakagawa, 1988), therefore, plantules with lower rates of growth present lower incorporation of tissues' reserve supplies by the embryonic axis, thanks to their lower transformation and reserve supplying capacities (Krzyzanowski, Vieira, França Neto, 1999). In this perspective, alterations occurred in the hypocotyl growth of *L. sativa* could be originated by germination or processes involved in the phase of embryonic axis growth, justifying so the utilization of growth and germination assays.

It is concluded, therefore, that the essential oil of *A. lanceolatus* possesses in its composition, a higher concentration of caryophyllene oxide, which is present in different vegetable species and possesses biological activities. The aromatic water obtained during the process of extraction of essential oil, presented significant allelopathic property, which is already enough to induce abnormal development of plantules of *L. sativa.* The results here in presented indicate the biotechnological potential of the species.

New assays with aromatic water should be developed with the purpose to verify its composition and the presence of caryophyllene oxide, establishing with reasonable sureness the allelopathic influence of caryophyllene oxide.

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