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Fungicidal activity and constituents of *Ageratum conyzoides* essential oil from three regions in São Paulo state, Brazil

Atividade fungicida e constituintes químicos do óleo essencial de **Ageratum conyzoides** *de três regiões do estado de São Paulo, Brasil*

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ABSTRACT: The chemical composition and antifungal activity of the *Ageratum conyzoides* essential oils, obtained from the leaves collected in Ibiúna (1), Ribeirão Pires (2) and Campinas (3) in the São Paulo state, Brazil, were investigated. The essential oils were obtained from *A. conyzoides* leaves by hydrodestilation and analyzed by GC/MS. The chemical composition of the *A. conyzoides* oil collected in Ribeirão Pires and Ibiúna consisted mainly of precocene I and II. The essential oil from leaves collected in Campinas had only traces of precocene II and a highest proportion of precocene I, α-humulene and (E)-caryophyllene than the oils (1) and (2). The *Aspergillus flavus* growth was inhibited by essential oils (1) and (2) at 60 and 64%, respectively, and the oil (3) was inactive. On the other hand, the three essential oils inhibited the sporulation of the fungus for more than 120 days. The essential oils of leaves collected in sites that show similarities in the latitude, altitude and average temperatures, as Ribeirão Pires and Ibiúna, showed chemical composition and antifungal activity similar, either, which showed the importance of geo-ecological factors in production of metabolites of the plant.

KEYWORDS: *Ageratum conyzoides*; essential oil; *Aspergillus flavus*; antifungal activity; Asteraceae.

RESUMO: A composição química e a atividade antifúngica do óleo essencial de folhas de *Ageratum conyzoides*, coletadas em Ibiúna (1), Ribeirão Pires (2) e Campinas (3) no estado de São Paulo, foram investigadas. Os óleos essenciais foram obtidos a partir de folhas de *A. conyzoides* por hidrodestilação e analisados por CG/EM. A composição química do óleo essencial de *A. conyzoides* coletadas em Ribeirão Pires e Ibiúna consiste principalmente de precoceno I e II. O óleo essencial das folhas coletadas em Campinas possui apenas traços de precoceno II e uma maior proporção de precoceno I, α-humuleno e (E)-cariofileno quando comparado com os óleos (1) e (2). O crescimento de *Aspergillus flavus* foi inibido pelos óleos essenciais (1) e (2) em 60 e 64%, respectivamente, enquanto que o óleo (3) foi inativo. Por outro lado, os três óleos essenciais inibiram a esporulação do fungo por mais de 120 dias. Os óleos essenciais de folhas coletadas em locais que mostram semelhanças na latitude, altitude e temperatura média, como Ribeirão Pires e Ibiúna, mostraram composição química e atividade antifúngica semelhante. Isso mostrou a importância dos fatores geo-ecológicos na produção de metabólitos da planta.

PALAVRAS-CHAVE: *Ageratum conyzoides*; óleo essencial; *Aspergillus flavus*; atividade fungicida; Asteraceae.

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The genus *Ageratum* consists of approximately 30 species but only a few species have been phytochemically investigated (Okunade, 2002; Vieira et al.; 2009). *Ageratum conyzoides* L. (mentrasto) have been used in folk medicine for the treatment of pneumonia, infections, asthma, fevers, inflammation, diarrhea etc. (Almagboul et al., 1985; Marques-Neto et al., 1988; Magalhães et al., 1998; Moura et al., 2005).

The antimicrobial and insecticidal activities are one of the most important biological activities of the *A. conyzoides* (Martins et al., 2005).

A. conyzoides essential oil is reported to contain mainly terpinene-4-ol, bornyl acetate, E-caryophyllene, γ-muroleno, δ-cadinene, α-muroleno, caryophyllene oxide, α-humulene, precocene I and II. The precocenes are major compounds of oil with variations from 30 to 93% (Castro et al., 2004).

A. conyzoides essential oil possesses antifungal activities against *Aspergillus flavus* and inhibitory effect on aflatoxin production (Nogueira et al., 2010). *A. flavus* can produce aflatoxins B_1 and B_2 (AFB_1, AFB_2) and cyclopiazonic acid (CPA), and the co-production of these mycotoxins can result in an additive or synergistic toxic effect on consumers (Gqaleni et al., 1997). Aflatoxins are considered carcinogens (Class 1 group), especially AFB_1 (Iarc, 1993). The literature showed that the yield of essential oils, their chemical constituent and their biological activity are influenced by genetic and environmental factors (Xie et al., 2012).

The aim of the present study was to evaluate the main chemical composition and activity in *A. flavus* growth of the essential oils from the leaves of *A. conyzoides* collected at three different localities in São Paulo state.

Leaves of *A. conyzoides* were collected at three different localities in São Paulo state (Ibiúna, Campinas and Ribeirão Pires) in June 2008. The plant was deposited in the herbarium of the City of São Paulo (PMSP 9686). Fresh leaves (300 g) were submitted to the hydro-distillation, so the essential oil was obtained. It was used by test in *A. flavus* and GC/MS analyses.

GC/MS analyses of the main components of the essential oils were done in a Shimadzu QP-5000 equipped with an OV-5 (30 m × 0.25 mm × 0.25 µm, Ohio Valley Specialty Chemical, Inc.) capillary column. Operating conditions were undertaken at oven temperature from 60 to 240ºC at 3ºC/min, injector and detector temperatures of 240 and 230ºC, respectively, at 70 eV. The oil components were identified using retention indices with those of authentic compounds or with literature data (McLafferty; Stauffer, 1989).

The strain of *A. flavus* producing AFB_1 was donated by the Reference Laboratory of Microbiology located at the Instituto de Tecnologia de Alimentos (ITAL), Campinas, São Paulo, Brazil. The strains are inoculated into tubes containing potato dextrose agar (Difco Laboratories, for 10 days at 25ºC). The spore suspension used as inoculum was prepared by washing cultures with sterile 0.01% solution of Tween 80.

For antifungal assay, filter paper disks (6 mm diameter) containing 5.0 µL of each essential oil of *A. conyzoides* were applied on the potato dextrose agar medium in Petri dishes previously inoculated with *A. flavus* inoculum on the surface. The inoculated plates were incubated at 25ºC for 5 days. At the end of the period, antifungal activity was evaluated by measuring the zone of inhibition (mm) against the test fungus (Yin; Tsao, 1999); 5.0 µL of the commercial fungicide (1 mg/mL) were used as a positive control. All treatments consisted of three replicates, and the averages of the experimental results were determined. The fungal growth was assessed using analysis of variance (ANOVA) with significance level of $p < 0.05$ and Tukey-Kramer analysis of variance for multiple comparisons with significance level $p < 0.05$.

The essential oil yield [w/w, fresh weight (f.w.)] of three *A. conyzoides* varied from 0.11 to 0.19%. The highest yield was obtained in the plant collected in Ibiúna. The latitude, altitude, longitude and average temperatures are presented in Table 1.

The identification of compounds represented 98.58; 96.46; and 95.57% for essential oil collected in Ribeirão Pires, Campinas and Ibiúna, respectively. The three oil essential have shown some differences in chemical constitutions (Table 2). Essential oil from leaves collected in Campinas is composed at 81.25% of precocene I and only traces of precocene II. On the other hand, the precoceno II from Ribeirão Pires and Ibiuna samples appeared in greater proportions 10.39% and 54.99%, respectively. Precocene I is the main constituent in the sample from Campinas and Ribeirão Pires; on the order hand, precocene II is the main constituent of essential oil of leaves collected in Ibiúna (Table 2). (E)-caryophyllene and α-humulene were also identified in the three oils, but in higher concentrations in the essential oil from Campinas. Higher concentrations of (E)-caryophyllene in the essential oil of *A. conyzoides* were also related by literature (Rana; Blazquez, 2003).

The results confirmed that the geographical variation may influence the composition of oil in a quantitative or qualitative extent. The environmental conditions, to which the plant was submitted, have major implications in their chemical composition. The differences in chemical composition of

Table 1. Latitude, longitude, altitude, average maximum temperature and average minimum temperature of local of collection*.

	Ibiúna	Ribeirão Pires	Campinas
Latitude	-23°23'	-23°25'	-22°31'
Longitude	47°7'	46°15'	47°02'
Altitude (m)	880	800	680
AMaxT (°C)	22.8	22.8	29.0
AMinT (°C)	8.5	9.0	11.0

*Taken from the site www.cpa.unicamp.br/outras-informacoes/clima-dos-municipios-paulistas.html.
AMaxT: average maximum temperature; AMinT: average minimum temperature.

Table 2. Chemical composition of essential oils of leaves of *A. conyzoides* collected in Campinas, Ribeirão Pires and Ibiúna.

Chemical compounds	EOC (%)	EORP (%)	EOI (%)	IR*	IR**
n-tetradecane	0.60	tr	tr	1400	1400
(E)-caryophyllene	13.36	8.39	11.45	1416	1418
α-humulene	1.25	0.69	tr	1449	1454
Dimethoxi ageratocromene (precocene I)	81.25	79.11	29.13	1454	1463
Ageratocromene (precocene II)	tr	10.39	54.99	1659	1660

*Experimental retention index; **retention index literature (Adams, 2007).
EOC: Campinas; EORP: Ribeirão Pires; EOI: Ibiúna; tr: trace of the substance (tr ≤ 0.59).

three essential oils probably cannot be attributed to latitude and longitude of the three cities, because there are no major differences. The biggest difference among the three locations is the average of the maximum and minimum temperatures. The temperatures of Ribeirão Pires and Ibiúna are lower and similar; the average temperatures in Campinas are higher (Table 1). The proportion of precocene II increases with the increase of altitude, while constituents like precocene I and α-humulene increase in quantity with a decrease in altitude. Haider et al. (2010) related the increase of α-humulene in the *Artemisia nilagirica* var. septentrionalis essential oil with the decrease in altitude, a fact that was also observed by us. *A. conyzoides* essential oil from Ibiúna (880 m) contains only trace of α-humulene, while *A. conyzoides* essential oil from Campinas (680 m) contains 1.25% of α-humulene.

The fungal growth inhibition assessed by the disk diffusion test has been used in the evaluation of plant extracts and essential oils by several authors. The influence of the essential oils on the inhibitory zone against *A. flavus* was measured at 2.5, 1.6 and 1.5 cm for commercial fungicide, essential oils of leaves collected in Ribeirão Pires and in Ibiúna respectively; on the other hand, the essential oil from Campinas did not form halo of inhibition. The percentages of the fungal growth inhibition were 64 and 60% for essential oils of leaves collected in Ribeirão Pires and Ibiúna, respectively (Table 3). The *A. conyzoides* essential oils from Ribeirão Pires and Ibiúna inhibited *A. flavus* growth, were similar and did not have statistically significant difference ($p > 0.05$).

A. flavus sporulation was inhibited by three essential oils and this inhibition persisted for more 120 days, showing fungistatic activity for the three essential oils.

Ribeirão Pires and Ibiúna essential oils showed similar chemical composition and antifungal activity, an expected result since biological activities are correlated to the presence of secondary metabolites. The season, the soil, even the number of hours that plants receive sunlight or rain may influence the phytochemistry of the plant since some compounds may be accumulated in response to environmental changes (Kamatou et al., 2010). The significant variation in the composition of the essential oils of *A. conyzoides* leaves collected in Ibiúna and Ribeirão Pires and of the essential oil of *A.conyzoides* leaves collected in Campinas can be attributed to different

Table 3. Essential oils of leaves of *A. conyzoides* collected in Campinas, Ribeirão Pires and Ibiúna, size of inhibition zone, and *A. flavus* growth inhibition.

Essential oils (5.0 µL)	Size of inhibition zone (cm)	A. flavus growth inhibition (%)
Control	2.15 ± 0.115	100
EOC	0	0
EORP	1.6 ± 0.073	64
EOI	1.5 ± 0.083	60

EOC: Campinas; EORP: Ribeirão Pires; EOI: Ibiúna.

geographical locations, environmental conditions and abiotic factors to which the plants were exposed.

The *A. flavus* growth inhibition by essential oil could be due to precocene II, confirming the hypothesis of Nogueira et al. (2010), who attributed the antifungal activity of *A. conyzoides* to the presence of this compound. However, sporulation inhibition can be related with synergism of essential oil's substances.

Furlan et al. (2010) reported chemical variability of the *Cymbopogon citratus* essential oils collected in different regions of São Paulo state. They attributed this variation to the occurrence of climatic factors and the phytogeography. Moreover, the environmental and the genetic factors are very important. Other authors also reported the chemical variation of the essential oils of plants from different ecological regions in several countries as China, Iran and Argentina (Rahimmalek et al., 2009; Xie et al., 2012).

These results suggested that the difference in chemical composition of essential oils can be attributed to climatic variations proper of different locations, which showed the importance of geo-ecological factors in the production of metabolites of the plant.

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