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Original Article

Dendranthema grandiflorum, a hybrid ornamental plant, is a source of larvicidal compounds against *Aedes aegypti* larvae

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ABSTRACT

In hybrid cultivated form, *Dendranthema grandiflorum* (Ramat.) Kitam., Asteraceae, flowers (*Chrysanthemum morifolium* Ramat.) were utilized in the production of extracts, which were analyzed for larvicidal activity against *Aedes aegypti* third instar larvae. Methanol and dichloromethane extracts showed LC_{50} values of 5.02 and 5.93 ppm, respectively. Using GC–MS, phytochemical analyses of the dichloromethane extract showed the presence of triterpenoids and fatty acids, while flavonoids and caffeoylquinic acids were shown to occur in the methanol extract by ESI Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS). Triterpenoids and fatty acids are well known insecticidal compounds. From this study, it can be concluded that *D. grandiflorum* grown for floriculture, as an agribusiness, can have additional applications as raw material for the production of insecticidal products.

Introduction

Known for over 2000 years, the chrysanthemum is an ornamental plant native to China. It belongs to the genus *Dendranthema*, Asteraceae, and numerous cultivars have been produced (Kim et al., 2015). The cut chrysanthemum is one of the most commercially successful flowers by the diversity of its inflorescence (Brackmann et al., 2005). Floriculture is a representative activity in Brazilian agribusiness and, as such, it has made an outstanding economic and technological contribution. In particular, the chrysanthemum culture (*Dendranthema grandiflorum* (Ramat.) Kitam.) has great importance in Brazilian floriculture, and its cultivation has increased throughout the country (Polanczyk et al., 2008). Climate is an important factor that favors the expansion of floriculture, as an agribusiness, in Brazil. Specifically, it allows for the cultivation of temperate and tropical flowers with low cost and high production all year long (Da Silva Junior et al., 2011).

Dengue is a major public health problem in Brazil. The transmission of the dengue virus to humans occurs through the bite of the mosquito *Aedes aegypti*. The disease can be temporarily disabling, but in its hemorrhagic form, it can result in death. Although public policies exist for mosquito control, resistance to conventional insecticides is concerning to health authorities (Liu, 2015). Therefore, this paper describes a natural product, extracts of *D. grandiflorum*, which are obtained from the cultivar "yellow sheena", as a source of compounds with larvicidal activity against *A. aegypti* third instar larvae. The studied plant is organically cultivated in the State of Rio de Janeiro, Nova Friburgo City. In view of its larvicidal potential, the cut flower should also support the establishment of floriculture as an important agribusiness in Brazil.

Materials and methods

Plant material

Dendranthema grandiflorum (Ramat.) Kitam., Asteraceae, yellow sheena, a hybrid under organic cultivation, was obtained from farmers in Nova Friburgo, State of Rio de Janeiro, in September 2013 and botanically identified by Dr. Mariana Machado Saavedra from

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the Rio de Janeiro Botanical Garden (voucher specimen deposited at the herbarium Prof. Jorge Pedro Pereira Carauta under number HUNI 3531).

Preparation of extracts

The flowers were dried in an oven at 50 °C for 7 days (223 g). After that, they were submitted to methanol extraction over a period of 15 days. The extract was filtered and the solvent removed by rotative evaporation under vacuum to obtain the first product, a dry MeOH extract (80 g). Seventy grams were suspended in water, and after solvent removal, the suspension was partitioned with dichloromethane to yield the second product, a dry CH_2Cl_2 extract (29 g).

Phytochemical analysis

Electron Impact Ionization/Mass Spectrometry (EI/MS) was obtained using a Shimadzu QP5050 GC–MS on a DB-5 column (30 m × 0.25 mm and film thickness of 0.25 μm, J&W Scientific) with an ionizing energy of 70 eV. Programming of the oven temperature started at 80 °C, which was maintained for 2 min. Then, the temperature was raised to 260 °C at a rate of 15 °C/min and again increased to 320 °C at 5 °C/min. An isotherm during 10 min was performed at this final temperature. The temperatures of injector and ion source were kept at 250 °C and 280 °C, respectively. Helium was used as the carrier gas with a constant flow of 1.1 ml min^{-1}. One microliter of the sample was injected with a split ratio of 70:1. Mass spectra of extract compounds were compared with the NIST Mass Spectral Database (MS Search 2.0) and with literature data (Assimoupoulou and Papageorgiou, 2005).

The methanolic extract of *D. grandiflorum* was analyzed in a mass spectrometer (Model 9.4 T Solarix, Bruker Daltonics, Bremen, Germany), which was set to operate in negative ion mode, ESI(-), over a mass range of *m/z* 200–1300. The parameters of the ESI(-) source were as follows: nebulizer gas pressure of 0.5–1.0 bar, capillary voltage of 3–3.5 kV, and transfer capillary temperature of 250 °C. The mass spectrum was processed using the Compass Data Analysis software package (Bruker Daltonics, Bremen, Germany). A resolving power, $m/\Delta m_{50\%} \cong 500{,}000$, in which $\Delta m_{50\%}$ is the full peak width at half-maximum peak height of $m/z \cong 400$ and a mass accuracy of <1 ppm, provided the unambiguous molecular formula assignments for singly charged molecular ions. Elemental compositions of the compounds were determined by measuring the *m/z* values. The unsaturation level of each molecule could be deduced directly from its double bond equivalent (DBE), following the equation DBE = c − h/2 + n/2 + 1, where c, h, and n are the numbers of carbon, hydrogen, and nitrogen atoms, respectively (Ferreira et al., 2014; Costa et al., 2014; De Sá et al., 2015; Nascimento et al., 2015). Molecular formula, measured *m/z* values, DBE, and mass error are shown in Table 1. Tandem mass spectrometry (MS^2) experiments were also performed on a quadrupole analyzer coupled to the FT-ICR mass spectrometer, and quadrupole Fourier transform ion cyclotron resonance mass spectrometry (Q-FT-ICR MS) was performed for ions of *m/z* 353, 431, 447, 449 and 515. The fragments produced (*m/z* values) from ESI(-)-MS/MS spectra are shown in Table 3.

Larvicidal activity

A. aegypti larvae that originated from the NPPN strain were reared in the laboratory under controlled photoperiod (12 h light and 12 h dark) at 27 °C and 80 ± 10% rel. humidity in trays filled with dechlorinated tap water and canine food.

Larvicidal activity was conducted following the method adapted from WHO (1970). For each treatment and control, five larvae of third stage instars, or early fourth stage instars, were transferred into 20 ml glasses in 14.9 ml of distilled water. The solutions of crude extracts, or fractions, were prepared in ethanol and added (0.1 ml) to the treatment glasses with a pipette to give the final assay concentrations. Controls received aqueous solution with 0.1 ml of ethanol only. After 24 h, the number of dead larvae in each glass was counted. All treatments were replicated three times. The 50% lethal concentration (LC_{50}) was calculated with a 95% confidence limit by Probit analysis.

Results and discussion

Phytochemical analysis

As shown in Fig. 1, methanol extract of *D. grandiflorum* by ESI(-)FT-ICR MS yielded 11 compounds identified as fatty acids, phenylpropanoids and flavonoids (Table 1). All compounds were detected as deprotonated molecules, *i.e.*, adduct ion, as represented by $[M-H]^-$ ion, formed by the interaction of a molecule with a proton, or hydrogen (Table 1). Among fatty acids, palmitic acid, *m/z* 255.2330, was the major compound found in the crude extract. Phenylpropanoids, such as monocaffeoylquinic acids,

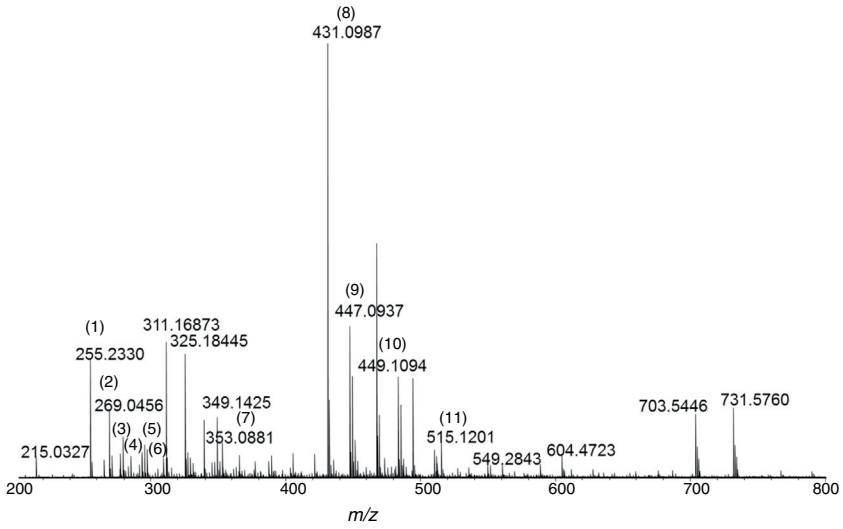

Fig. 1. ESI(-)FT-ICR mass spectrum of MeOH extract of *Dendranthema grandiflorum* flowers.