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Compounds from *Duguetia lanceolata* St.- Hil. (Annonaceae) bioactive against *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae)

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ABSTRACT

The variety of chemical compounds produced by members of Annonaceae exhibiting wide activity against pest organisms makes this family an interesting source for insecticidal compound identification. Accordingly, this study first aimed to evaluate the lethal and sublethal effects of ethanolic extracts of different parts of *Duguetia lanceolata* St.-Hil. against *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae), the primary pest of stored beans in tropical regions. Based on a screening bioassay, the ethanolic extract from *D. lanceolata* leaves was selected for chemical partitioning due to its high bioactivity against the weevil. The hexanic partition of the ethanolic leaf extract caused 98% mortality of *Z. subfasciatus* adults in a residual contact bioassay and completely eliminated the number of eggs per sample. Chromatography subfraction Flh2-5, a hexanic partition of *D. lanceolata* leaves, resulted in 100% mortality of *Z. subfasciatus* adults. An aromatic compound (2,4,5-trimethoxystyrene) and a mixture of sterols [(campesterol (8.44%) + stigmasterol (12.37%) + sitosterol (79.19%)] were isolated from subfraction Flh2-5 using various chromatographic procedures. The aromatic compound resulted in 60% weevil mortality, and the steroid mixture applied at 75 mg kg^{-1} promoted 28% mortality; in contrast, the positive control (K-Obiol® 2P, deltamethrin 2 g a.i./kg) at 375 mg kg^{-1} resulted in 100% mortality. Moreover, both 2,4,5-trimethoxystyrene and K-Obiol® 2P completely prevented oviposition on bean samples, demonstrating that, similar to a deltamethrin-based formulation, 2,4,5-trimethoxystyrene is able to act as a grain protector. Therefore, the aromatic compound and mixture of isolated sterols from *D. lanceolata* may be suitable management tools of stored-product pests and promising sources of insecticidal molecules with both lethal and sublethal effects on *Z. subfasciatus*.

1. Introduction

The pressure exerted by phytophagous insects has contributed to the selection of plant species endowed with complex defense mechanisms that are primarily characterized by the ability to synthesize and accumulate chemical compounds (allelochemicals) with adverse effects on insect biology and behavior (Wink, 2003). Thus, allelochemicals produced by secondary plant metabolism have been widely studied to provide a more thorough understanding of plant-insect interactions and to identify biologically active compounds with applications in many areas (Isman, 2006). With regard to insect pest management issues, the primary objectives of studying allelochemicals have been to identify new sources of bioinsecticides or molecules that can be used as model prototypes for synthesizing new analogous insecticides, particularly those that are highly effective and environmentally safe (Cantrell et al., 2012).

Fumigant and grain-protectant insecticides are important complementary tools for the chemical control of stored-product pests. However, the widespread occurrence of resistant insect pest populations in bean warehouses is currently a serious problem due to both the absence of new active ingredients and the lack of registered insecticide products for crops with less economic expressivity

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(Boyer et al., 2012; Mau et al., 2012; Opit et al., 2012; Nayak et al., 2013).

A key factor to overcoming insect resistance is discovering new compounds, primarily those with different modes of action. Within this context, the family Annonaceae is a rich source of insecticidal molecules with potential grain-protectant properties (Ribeiro et al., 2013). Indeed, this plant family is considered one of the most promising sources of allelochemicals with insecticidal action, especially alkaloids, acetogenins, terpenoids, lactones and essential oils (Santos and Salatino, 2000; Muhammad et al., 2001; Siqueira et al., 2001; Siqueira et al., 2003; Pérez et al., 2004; Pérez et al., 2005; Carollo et al., 2006; Maia et al., 2006; Silva et al., 2007; Pinheiro et al., 2009). A total of 417 acetogenins have been identified in 51 species of Annonaceae distributed among 13 genera (Bermejo et al., 2005) and 500 alkaloids in 138 species distributed among 43 genera (González-Esquinca et al., 2014). In addition, many sesquiterpenes and monoterpenes are found in Annonaceae species (Costa et al., 2013; Ferraz et al., 2013; Thang et al., 2014).

Annonaceae, which is systematically grouped into class Magnoliopsida and subclass Magnoliidae, includes many genera and a vast number of species of trees and shrubs that are native to tropical and subtropical regions (Maas et al., 2001). The genus *Duguetia* contains 90 species with 66 present in Brazil (29 endemic) (Maas et al., 2003). *D. lanceolata* St.- Hil., also commonly known as "pindaíba", naturally occurs in the Middle-West, South and Southeast regions of Brazil, primarily in semideciduous seasonal forests and in forest formations of the Atlantic complex (Maas et al., 2001; Sousa et al., 2008).

Nonetheless, *D. lanceolata* has not been widely studied to date for its phytochemistry or the biological activity of its derivatives, with biological activity mainly being determined for a few disease agents and in the treatment of human pathologies. Both an ethanolic extract prepared from its leaves (Sousa et al., 2004) and an essential oil from its fruit peel were found to have antinociceptive and anti-inflammatory properties in mice (Sousa et al., 2008). Furthermore, the partition of alkaloids from an ethanolic extract of *D. lanceolata* leaves also exhibited antiplasmidic activity against *Plasmodium falciparum* (Fisher, 2004), and an essential oil from *D. lanceolata* branch bark exerted antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Candida albicans* (Sousa, 2012). Production of alkaloids and flavonoids has been reported for a few species of *Duguetia*. *D. furfuracea* (St. Hil.) Benth and Hook f. (1862) and *D. flagellaris* Huber present alkaloids (Fechine et al., 2002a,b; Carollo et al., 2006), and Santos and Salatino (2000) identified 76 flavonoids in 31 species of Annonaceae, including *D. bahiensis* Maas, *D. furfuracea* and *D. chrysocarpa* Maas. Moreover, some studies were done in relation to insecticide and acaricide activities of extracts, fractions and pure compounds from *D. lanceolata* (Ribeiro et al., 2013; Alves et al., 2015; Ansante et al., 2015; Alves et al., 2016; Ribeiro et al., 2016).

The present study aimed to evaluate the lethal and sublethal effects of ethanolic extracts prepared from different parts of *D. lanceolata* against *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae), which is the primary pest of stored beans in tropical regions (Barbosa et al., 2002). Additionally, bioassay-guided partitioning using different chromatographic techniques was performed to isolate and characterize the compounds responsible for the observed bioactivities.

2. Material and methods

2.1. Test insects

The colonies of *Z. subfasciatus* used in bioassays were established by collecting specimens from warehouses of Piracicaba municipality, SP, Brazil. A *Z. subfasciatus* colony was maintained under controlled laboratory conditions (25 ± 2 °C, 60 ± 10% RH and a photoperiod of 14L: 10 D h) using *Phaseolus vulgaris* grains cv. Bolinha as a rearing substrate, which was previously exposed to −10 °C for at least 48 h in a freezer to remove possible contaminant insects (Aguayo et al., 2006).

2.2. Collection of plant material and preparation of crude extracts

The plant parts used in this study [fruit peels (676 g), leaves (1214 g), branches (752 g), and seeds (598 g)] were collected on March 2011 from *D. lanceolata* trees grown at the campus of "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil (22°42′41.5 "S, 47°38′00.2" W and 556 m elevation). A voucher specimen of the collected specimen was deposited in the ESA herbarium (registration number: 121204) in Piracicaba, SP, Brazil. After collection, the plant parts were dried in a drying chamber with air circulation at 38 °C for a period between 48 and 72 h. The parts were then ground separately using a knife mill (Model 090, Marconi, Piracicaba, SP, Brazil) to obtain powders, which were stored in sealed glass containers.

The plant powders were subjected to a cold maceration technique in ethanol (analytical grade, 99.5%) to obtain organic extracts for bioassay-guided fractionation. For this, the plant powders [fruit peels (676.0 g), leaves (1214.0 g), branches (752.0 g) and seeds (598.0 g)] were immersed in ethanol at a ratio of 5:1 (w v^{-1}), stirred for 10 min and stored at 25 °C for 72 h without agitation. After this period, the ethanolic solution (ethanol with extracted compounds) was filtered through filter paper to remove the plant powder, and the remaining ethanol in the filtered solution was eliminated using a rotary evaporator (Model 550, São Paulo, SP, Brazil) at 50 °C and −600 mmHg. After complete evaporation of the ethanol in a chamber with airflow, the extraction yield for each part of *D. lanceolata* was determined [fruit peels (24.3 g, 3.59%), leaves (203.1 g, 16.73%), branches (47.4 g, 6.30%), and seeds (59.5 g, 9.96%)]. The remaining plant powders from each filtration process were subjected another three times to the same cold maceration technique in ethanol.

2.3. Bioassay procedures

All bioassays were performed in an acclimatized room at a temperature of 25 ± 2 °C, 60 ± 10% RH and a photoperiod of 14 L: 10 D hours. A completely randomized experimental design was used.

To apply the extract to bean grain surfaces (100 g sample for each treatment), a microatomizer coupled to a pneumatic pump adjusted to provide a spray pressure of 0.5 kgf cm^{-2} with a volume of 30 L t^{-1} was used, as determined in preliminary tests. With this approach, 3.0 mL solution [solvent (acetone:methanol (1:1)) and/or treatment (extract, fraction or compound)] was sprayed onto each 100 g bean sample. After spraying, the samples were lightly shaken for one minute in plastic bags with a capacity of 2 L to equalize the distribution and adhere the treatment to the bean surface. The treated bean samples were placed in an airflow chamber for 2 h to evaporate the solvent used in the suspension of the respective extract.

The effects of *D. lanceolata* crude extracts (at 1500 mg kg^{-1}) on *Z. subfasciatus* were evaluated using the following variables: adult mortality, oviposition (number of eggs $sample^{-1}$), F1 progeny (number of adults emerged $sample^{-1}$), sex ratio, viability of the egg to the adult stage and the percentage of damaged grains in each sample. For this, bean samples (10 g) treated with the respective treatments were placed in Petri dishes (6.5 cm diameter × 2 cm high) and infested with five *Z. subfasciatus* pairs aged between 0 and 24 h. For each treatment, 10 repetitions were used; for each bioassay, a control consisting of the solvent used for suspension of the respective derivative was included.