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Assessment of insecticidal responses of extracts and compounds of *Drimys winteri*, *Lobelia tupa, Viola portalesia* and *Vestia foetida* against the granary weevil *Sitophilus granarius*

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ABSTRACT

Extracts and compounds from the Chilean plants canelo, *Drimys winteri* J.R. Forst. & G. Forst. (Winteraceae), tabaco del diablo, *Lobelia tupa* L. (Campanulaceae), huevil, *Vestia foetida* Hoffmans. (Solanaceae) and violeta, *Viola portalesia* Gay (Violaceae) were evaluated against *Sitophilus granarius* L. (Coleoptera: Curculionidae), one of the most widespread and destructive primary pests of stored cereals. Total extracts at concentrations of 2.5%$^{w}/_{w}$ in diets, over 6-days, display insecticidal effects against *S. granarius*. *D. winteri* caused the mortality of 87.5% of insects; *L. tupa* 80%, *V. foetida* 56% whereas *V. portalesia* killed 45% of insects under the same conditions. In an effort to determine the active compounds, the extracts of *Lobelia tupa* and *Drimys winteri* were purified by preparative chromatography. The piperidine alkaloid lobelanidine was isolated from *L. tupa* and the drimane sesquiterpenes drimenin, drimenol and polygodial were isolated as the major components in the extract from *D. winteri*. The purified compounds compounds displayed insecticidal activity against *S. granarius* in a concentration/dependent-time manner (% mortality at 0.5%$^{w}/_{w}$ over 6-days): polygodial 80%, drimenol 60%, lobelanidine 47%, and drimenin 20%. In agreement with these results, grains treated with polygodial showed greater protection against the feeding attack by the granary weevil. These results provide evidence of the importance of elements of the native Chilean flora as new potential sources of botanical pesticides for the insect pest control.

1. Introduction

Cereals are a major source of dietary proteins for humans (Shewry, 2007), but these food commodities are often infested by various stored-product pests, mainly of the order Coleoptera, which can cause both quantitative and qualitative losses and can negatively influence food safety (Athanassiou et al., 2011). Grain losses during storage can reach 50% of total production (Fornal et al., 2007) and the proper treatment of the grain and harvest is essential to reduce damage caused by insects. This is especially true for the wheat weevil *Sitophilus granarius* L. (Coleoptera: Curculionidae), which is a strong and persistent pest of stored grains. This insect mainly affects the quality of grain and yields of corn, wheat, and rice. There are several chemical fumigants with a broad activity spectrum for this purpose, but there also is a global concern about their negative side effects such as ozone depletion (many of them are halogenated), environmental pollution, toxicity to non-target organisms, pest resistance, and pesticide residues (Benhalima et al., 2004; Bughio and Wilkins, 2004). Because of these problems it is desirable to search for new bioactive compounds against insect attack. Natural products from plant sources often have an advantage over conventional fumigants because of their low toxicity and ready degradation.

Chile is surrounded by the Atacama Desert, the Pacific Ocean, the Andes Mountains and the Antarctic. The Chilean flora is composed of approximately 6000 often endemic plant species. Native people have used them to treat ailments of people, animals and plants. For theses reasons, it seems that they can serve as new sources for biologically

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active natural compounds that include environmentally friendly insecticides, with low mammalian toxicity and desirable biodegradation characteristics (Cespedes et al., 2013, 2015, 2016; Isman, 2006; Muñoz et al., 2013). We have concentrated on a search for ecologically sound alternative options against *S. granaries.* Our attention has been focused on four native Chilean: *Lobelia tupa* L. (Campanulaceae), *Drimys winteri* J.R. Forst. & G. Forst. (Winteraceae), *Viola portalesia* Gay (Violaceae) and *Vestia foetida* Hoffmans. (Solanaceae). The current study deals with evaluation of the toxicity of extracts and components of these species against *S. granarius.* We have isolated, purified and determined the molecular structure of the most active compounds isolated from *Lobelia tupa* and *Drimys winteri* and assessed the dose dependent toxicity of pure active compounds from these species against *Sitophilus granarius*.

2. Materials and methods

2.1. Plant material and extraction

2.1.1. Lobelia tupa

Fresh aerial parts (10 kg) were collected on December 2015 in Concepción, Region VIII, Chile (S 36°50′00″ W 73°01′46″). Dried and milled plant material was macerated for three days in acidic water (20 L, pH 3, HCl, RT) and then filtered. The aqueous layer was basified with NaOH to pH 10 and extracted with EtOAc (3 × 5 L), the organic layer was concentrated *in vacuo* to yield a crude alkaloid fraction (LTA1) (extract OH^-: 140 g), storage at −80 °C.

2.1.2. Drimys winteri

Tree bark (4.5 kg) was collected on February 2016 in Temuco, Region IX, Chile (S38°48′25″ W 72°32′56″). The bark was initially crushed and extracted by maceration with ethyl acetate for three days. The organic layer was evaporated *in vacuo* to yield a crude product (DWM1) (60 g), storage at −80 °C.

2.1.3. Viola portalesia

Fresh aerial parts (6.5 kg) were collected on December 2015 in Concepción, Region VIII, Chile (S 36°46′33″ W 73°11′40″). Dried and milled plant material was macerated for three days in ethyl acetate. The organic layer was evaporated *in vacuo* to produce a crude product (VPM1) (55 g), storage at −80 °C.

2.1.4. Vestia foetida

Fresh aerial parts (7.2 kg) were collected on December 2015 in Concepción, RegionVIII, Chile (S 36°46′29″ W 73°12′09″). Dried and milled plant material was macerated for three days in ethyl acetate. The organic layer was evaporated *in vacuo* giving a crude product (VFM1) (45 g); storage at −80 °C.

2.2. Chemicals and solvents

Column chromatography was performed on Merck silica gel 60 (0.063–0.200 mm). Size-exclusion chromatography was performed using Sigma Aldrich Sephadex LH-20. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 PF254 plates. Solvents used in this study were previously distilled and dried over appropriate drying agents.

2.3. Purification of alkaloids from Lobelia tupa

The crude OH^- extract was chromatographed over a silica gel column (200–300 mesh) with solvents of increasing solvent polarity from hexane to ethyl acetate and ethyl acetate/methanol 1:1 $^{v}/_{v}$, giving fractions B1–B7 which were monitored by TLC (silica gel) and visualized with UV light and Dragendorff's reagent. Fractions B1–B4 contained only sterols and chlorophylls, but no alkaloids. Fraction B7 (5.3 g) was applied to a Sephadex LH-20 column and eluted with methanol/ethyl acetate (1:1 v/v). This procedure afforded a white solid, which was recrystallized from methanol/EtOAc 1:1 to yield colorless crystals of lobelanidine (Fig. 2). The identity of the compound was confirmed by spectroscopic methods, see Tables 2 and 3 for NMR analyses.

2.4. Purification of drimane sesquiterpenoids from Drimys winteri

The drimane sesquiterpenoids drimenin, drimenol and polygodial (Fig. 3) were isolated from DWM1, which was further purified by preparative column chromatography on silica gel. Primary fractionation of eight fractions (F1–F8) by using increasing polarity solvents from hexane to ethyl acetate was evaluated by TLC. A subsequent chromatographic purification of F3 with hexane/ethyl acetate (9:1 v/v) produced a solid identified as drimenin. A second crystalline compound with different retention time on TLC was subsequently obtained. This compound was identified by NMR spectroscopy as drimenol. Purification of F4 with hexane/ethyl acetate (8:2 v/v) afforded a yellow oil that proved to be polygodial. The identities of these compounds were confirmed by TLC and NMR using pure standards previously characterized by spectroscopic methods; see Tables 2 and 3 for NMR analysis.

2.5. Apparatus

Melting points were determined on a Melting Point SMP10 (Stuart) uncorrected. The ^{1}H- and ^{13}C NMR spectra were recorded in $CDCl_3$ solution in 5 mm tubes at room temperature on a Bruker Avance III spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) at 600.13 (^{1}H) and 150.61 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS (for ^{1}H) or the solvent (for ^{13}C) as internal standard. All spectra (^{1}H NMR, ^{13}C NMR) were acquired and processed with the standard Bruker software.

2.6. Insect source

Individuals of *Sitophilus granarius* were provided By Dr. R. Rebolledo. The insects were cultured in the laboratory on wheat grain and maintained in the dark in incubation chambers (Thermo Scientific™ Forma™ Environmental Chambers, Thermo Fischer Scientific, Series 3960), set at 25 ± 1 °C, 70% r.h., 16:8 (L:D) photoperiod. All experiments were carried out under the same environmental conditions as the cultures.

2.7. Insecticidal bioassay

The experiments used for the insecticidal activity bioassay against *S. granarius* were carried out according to Broussalis et al., 1999; and Athanassiou et al., 2004; with few modifications. Each bioassay consisted of three triplicates of 10 insects, chosen completely at random from first generation offspring, and subjected to a 24 h previous fasting (or pre-starved for 24 h). The insects were placed into a plastic cup with 10 grains of wheat, with no superficial damage as determined by inspection under a microscope and previously not treated (control) or previously treated with the compounds to be tested. These compounds were impregnated into the grains in weight percent concentrations based on the weight of 10 grains. The analytes were added in aliquots of a stock solution in acetone to obtain the desire concentration on the wheat grains. For analysis of total extracts four different concentrations were evaluated: 2.5, 5.0, 7.5 and 10%$^{w}/_{w}$. For pure compounds, lower concentrations were evaluated: 0.5, 1.0, 2.0 and 3.0%$^{w}/_{w}$. In each case, after addition of the acetone solution to the grains, the parcels were kept for 12 h at 25 °C in an oven with forced air to remove the solvent. A control experiment was carried out with only acetone to assess natural mortality and solvent influence. Experiments were carried out in a chamber at 25 °C and 70% humidity. Mortality was evaluated based on the number of dead insects as a function of time, from 1 to 6 days,