



## Insecticidal activity of Chilean Rhamnaceae: *Talguenea quinquenervis* (Gill. et Hook)

[Actividad Insecticida de Rhamnaceas Chilenas: *Talguenea quinquenervis* (Gill. et Hook)]

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### Abstract

The insecticidal activity of extracts obtained from aerial parts of *Talguenea quinquenervis* (Gill. et Hook) were evaluated using bioassay against larvae of *Drosophila melanogaster*. All extracts tested had insecticidal activity, the most active being those that contain alkaloids. From the most active fraction, it was possible to identify the known alkaloids coclaurine, armepavine and N-methylcoclaurine

**Keywords:** Chilean Rhamnaceae; *Talguenea*; insecticidal activity; alkaloids

### Resumen

La actividad insecticida de diferentes extractos obtenidos desde la parte aérea de *Talguenea quinquenervis* (Gill. ex Hook) se evaluó utilizando bioensayos con larvas de *Drosophila melanogaster*. Todos los extractos ensayados presentan actividad insecticida, siendo las más activas aquellas fracciones que contienen alcaloides. Desde la fracción activa fue posible aislar e identificar tres conocidos alcaloides, coclaurina, armepavina y N-metilcoclaurina.

**Palabras Clave:** Rhamnaceas chilenas; *Talguenea*; actividad insecticida; alcaloides.

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## INTRODUCTION

The Rhamnaceae is a cosmopolitan family consisting of 40 genera and 900 species, commonly trees and shrubs, all with simple leaves. In Chile this family is represented by 16 species distributed in seven genera: *Colletia* (three species), *Condalia* (1 species) *Discaria* (4 species), *Retanilla* (2 species), *Rhamnus* (1 species) *Talguenea* (1 species) and *Trevoa* (5 species). These species occurs from the province of Aconcagua to the province of Llanquihue, mainly in central and south of Chile. An additional species is endemic to the Juan Fernández Islands (*Colletia spartioides* Colla) (Torres, 1992). Some species are used in folk medicine (Montes and Wilkomirsky, 1978; Delporte et al., 1997).

One of these species, *Talguenea quinquenervis*, is a shrub or small tree with deciduous pubescent leaves that occurs on sunny slopes in degraded soils. These plants produce small white flowers (5 petals) between August and October and subsequently small nuts about 5 mm long. Its fruits are small nuts about 5 mm long. The branches have many straight thorns. This species ranges from Region IV to VIII of Chile, from 500 to 2000 m in elevation. This geographical area is characterized by low rainfall (400-800 mm/year) concentrated in winter (June-August).

Previous phytochemical studies of Chilean rhamnaceous species revealed the presence of alkaloids of the benzyloquinoline, aporphine and cyclopeptide types. The cyclopeptide alkaloid crenatine A has been isolated from *Discaria crenata* and *Retanilla ephedra* (Silva et al., 1974) and a similar alkaloid integerresin from *R. ephedra*.

In contrast, a number of triterpenes including friedelin,  $\beta$ -sitosterol, lupeol, ursolic acid, betulinic acid and sapogenins derived from dammarane triterpenoids are well known from these plants. There are few other studies to date in the literature.

Alkaloids isolated from these plant species (Bhakuni et al., 1974; Pacheco et al., 1973; Silva et al., 1974; Otsuka et al., 1974; Bick et al., 1981; El-Seedi et al., 2007; Torres et al., 1979; Jollie and Richard, 2004) possess a variety of biological activities, including cardiovascular (Morales et al., 1998), sedative (Lee et al., 2001; Han et al., 1989), antifungal (Tschesche et al., 1974; Tschesche and Ammermann, 1974; Gournelis et al., 1997), and antibacterial activity (Tschesche et al., 1974; Tschesche and Ammermann, 1974; Gournelis et al., 1997). The insecticidal properties of other rhamnaceous species

have been reported by Ateyyat and Abu-Darwish, (2009) and Dwivedi and Shekhawat, (2004), but to date there are no reports on the insecticidal activities of Chilean members of this family.

Based on previously published information on other members of this family and our observation that these plants appeared to be highly resistant to both insect and pathogen attack in the field, we undertook examination of Chilean members of the family. In this paper we report the insecticidal effects of methanolic extracts and alkaloid-containing fractions from aerial parts of *Talguenea quinquenervis* against *Drosophila melanogaster*. Our goal was to correlate phytochemical composition and insecticidal activity to identify biopesticides of botanical origin for insect control studies (Torres et al., 2003; Cespedes et al., 2004; Cespedes et al., 2005; Cespedes et al., 2006; Urzua et al., 2010; Cespedes and Alarcon, 2011).

## MATERIAL AND METHODS

### *Plant material*

*Talguenea quinquenervis* (Gill. et Hook), was collected at the roadside to pass at 4.7 km NW of Portezuelo on the road to Ninhue (S 36° 34.105', W 72° 26.26.865), VIII Región, Chile, in June, 2010. Voucher specimens have been deposited in the Herbarium of the Basic Science Department, University of Bío-Bío (Voucher DS-2010/05-16246) and the Herbarium of the University of Illinois, at Urbana-Champaign, Illinois, USA, (ILL, Voucher DS-16246).

### *Extraction and Isolation*

Air-dried aerial portions of *Talguenea quinquenervis* (1592 g) were exhaustively extracted with MeOH in a Soxhlet apparatus for 12 h. The resulting MeOH extract was filtered and concentrated under vacuum to obtain a crude residue (139 g). The total extract (TqFT) of *T. quinquenervis* was divided in two parts. Part A (76.82 g), was solvent partitioned using *n*-hexane (TqFH, 5.43 g), EtOAc (TqFAE, 7.51 g) and H<sub>2</sub>O (TqFW). Part B (62 g) was dissolved in H<sub>2</sub>O (50 ml) and acidified to pH 2–3. The acidic solution was exhaustively extracted with Et<sub>2</sub>O (5 x 50 ml) to yield an acidic ether extract (TqFEA, 13.4 g). The aqueous solution was then made basic (pH 8–9) to yield a basic ether extract (TqFEB, 2.5 g), mainly composed of alkaloids (Table 1). A portion of the basic ether extract (1.0 g) was applied to a silica gel column (80 g) and eluted with CHCl<sub>3</sub> containing increasing amounts of MeOH (up to 20%) to give 10 fractions. Fractions 2–3

(CHCl<sub>3</sub>:MeOH, 99:1) were combined (20 mg) and submitted to preparative TLC (CHCl<sub>3</sub>:MeOH, 99:1, two elutions) to yield 2 (12 mg). Fractions 4 - 5 (CHCl<sub>3</sub>:MeOH, 99:2) were recombined and concentrated *in vacuo* to give a yellow solid material (50 mg) that was applied to a silica gel column (2.0 g) eluted with CHCl<sub>3</sub> containing increasing amounts of MeOH (up to 5%) to give 3 (15 mg), and 4 (10 mg). Fractions 7–8 (CHCl<sub>3</sub>:MeOH, 95:5), consisting of one alkaloid, were combined and concentrated *in vacuo* to give 1 (850 mg).

#### Spectrometric data

**N-methylcoclaurine (1):** MP: 181-182°C. [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -92° (c 0.5, MeOH). IR (CHCl<sub>3</sub>): 3600, 2850 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 2.46 (s, 3H), 3.80 (s, 3H), 5.90 (bs, 2H), 6.50 (s, 1H), 6.60-7.00 (m, 4H). MS (EI, 70 eV): m/z (%) = 299 (0.2), 193 (4), 192 (100), 178 (4), 177 (6), 176 (0.4), 148 (2), 107 (2).

**Compound (2):** still unidentified

**Coclaurine (3):** MP: 221-224°C. [ $\alpha$ ]<sub>D</sub><sup>20</sup>: + 4.7 (c 0.48, MeOH). IR (nujol): 3530, 3470, 3300 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 2.70-3.36 (m, 6H), 3.83 (s, 3H), 4.39 (d, 1H, J = 10.2 Hz), 6.59 (d, 1H, J = 4.1 Hz), 6.63 (d, 2H, J = 8.4 Hz), 6.79 (s, 1H), 7.02 (d, 2H, J = 8.4). MS (EI, 70 eV): m/z (%) = 285 (0.1), 178 (100), 163 (20), 107 (8).

**Armepavine (4):** MP: 145-146°C. [ $\alpha$ ]<sub>D</sub><sup>20</sup>: -105 (c 0.9, MeOH); IR (CHCl<sub>3</sub>): 3600, 2850 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 2.53 (s, 3H), 3.56 (s, 3H), 6.59 (s, 1H), 6.60-6.97 (m, 4H). MS (EI, 70 eV): m/z (%) = 313 (0.2), 207 (12), 206 (100), 191 (6), 190 (9), 162 (5), 132 (2), 107 (2).

#### Bioassay for insecticidal activity against larvae of *D. melanogaster*

The bioassay for insecticidal activity against larvae of *D. melanogaster* was carried out as follows (Miyazawa et al., 2000): three concentrations (10.0, 50.0, and 100.0 ppm of sample) were used for determining LD<sub>50</sub> values. Test compounds were dissolved in 50 µL of EtOH and mixed in 1 mL of artificial diet [brewers' yeast (60 g), glucose (80 g), agar (12 g), and propionic acid (8 mL) in water (1000 mL)]. A control diet was treated with 50 µL of EtOH only. About 100 adults from the colonies of *D. melanogaster* were introduced into a new culture bottle, into which artificial diet had been poured into a

Petri dish, and allowed to oviposit at 25 °C and relative humidity > 60% for 3 h. The diet was taken out of the bottle, and 10 new eggs were collected and transplanted onto each diet (1 mL) in glass tubes and reared at 25 °C and relative humidity > 90% for 8 days. One day after transplantation, the larvae were hatched and fed each test compound with the artificial diet. At 25 °C, larvae generally change to pupae after 7 days. In each instance, the developmental stage was observed, and the numbers of pupae were recorded and compared with those of a control. Ten new eggs were used in each of three replicates. The LD<sub>50</sub>, the concentration that produces 50% mortality, was determined by log-probit analysis.

#### Chemicals and solvents

All reagents used were either analytical grade or chromatographic grade, tris-hydrochloride buffer, phosphate buffered saline (PBS), propionic acid, sorbitol, brewers' yeast, glucose, tricine, and trizma-hydrochloride were purchased from Sigma-Aldrich Química, S.A., Santiago, Chile, or Sigma, St. Louis, MO. Methanol, CH<sub>2</sub>Cl<sub>2</sub>, CHCl<sub>3</sub>, NaOH, KOH, HCl, sodium acetate trihydrate, glacial acetic acid, silica gel GF254 analytical chromatoplates, Sephadex LH-20, silica gel grade 60, (70–230, 60A°) for column chromatography, n-hexane, and ethyl acetate were purchased from Merck-Chile, S.A., Santiago, Chile.

#### Apparatus

Nuclear magnetic resonance (NMR) spectra were recorded at <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained with a Bruker Avance III spectrometer operated at 300 MHz for <sup>1</sup>H and at 75 MHz for the <sup>13</sup>C nucleus in CDCl<sub>3</sub> (Merck Chemical Co., Inc.), chemical shifts (ppm) are related to (CH<sub>3</sub>)<sub>4</sub>Si as the internal reference. CD<sub>3</sub>OD, CDCl<sub>3</sub> and acetone-d<sub>6</sub> from Merck Chemical Co. were used as solvents. Coupling constants are quoted in Hertz. IR spectra were obtained on a Perkin-Elmer 283-B and a FT-IR Nicolet Magna 750 spectrophotometers. A UV Spectronic model Genesys 5 spectrophotometer was used for biological and spectrophotometric analyses.

#### Statistical analyses

Data shown in figures are average results obtained by means of five replicates and are presented as average ± standard errors of the mean (SEM). Data were subjected to analysis of variance (ANOVA) with significant differences between means identified by GLM procedures. Results are given in the text as

probability values, with  $p < 0.05$  adopted as the criterion of significance. Differences between treatment means were established with a Student–Newman–Keuls (SNK) test. The  $LD_{50}$  values for each activity were calculated by PROBIT analysis based on percentage of mortality obtained at each concentration of the samples.  $LD_{50}$  is the concentration producing 50% of mortality. Complete statistical analysis was performed by means of the MicroCal Origin 6.0 statistical and graphs PC program.

## RESULTS AND DISCUSSION

The methanol extract of aerial parts from *T. quinquenervis* was fractionated in two parts A and B as described in the Experimental section. The insecticidal activity of the different fraction obtained (Table 1) was evaluated using bioassay for insecticidal activity against larvae of *D. melanogaster*. The mortality at 24, 48 and 72 h for all samples was recorded, in Figures 1 and 2. The results at 72 h and  $LD_{50}$  are shown in Table 1. The total fractions present larvicidal activity at 72 h. Surviving larvae pupate and undergo eclosion, giving rise to normal insects.

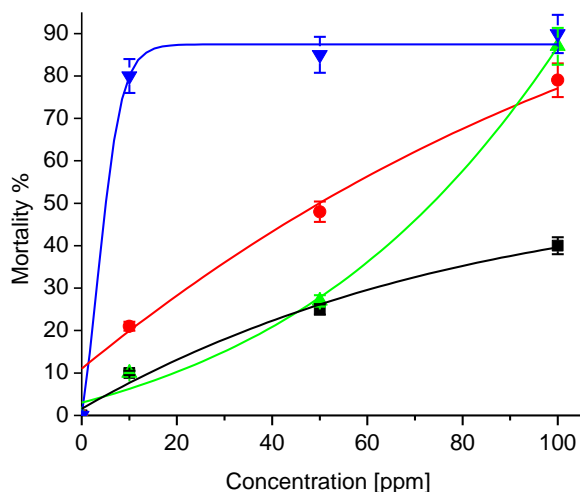


Figure 1. Mortality effects of *T. quinquenervis* extracts at 72 hrs against *D. melanogaster* produced by extracts TqFT (■), TqFH (●), TqFAE (▲), and TqFW (▼).

The bioassay was evaluated for 6 days, because the passage from larva to pupa in *D. melanogaster* requires about 7 days. Fractions TqFW and TqFEB exhibited the lowest  $LD_{50}$  (Table 1, Figures 1 and 2). These fractions showed 80% and 77% mortality, respectively at 72 h at the lowest concentration tested (10 ppm) (data not shown).

Moreover, it is possible to observe that the larvicidal effect is dose dependent in both figures. However, this effect is not observed in all fractions tested. From a chemical standpoint, alkaloids were found in fractions TqFW and TqFEB.

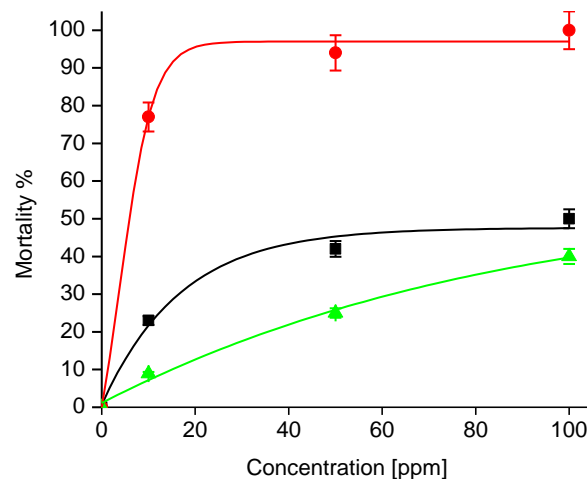


Figure 2. Mortality effects of *T. quinquenervis* extracts at 72 hrs. against *D. melanogaster* larvae produced by extracts TqFT (▲), TqFEA (■), and TqFEB (●).

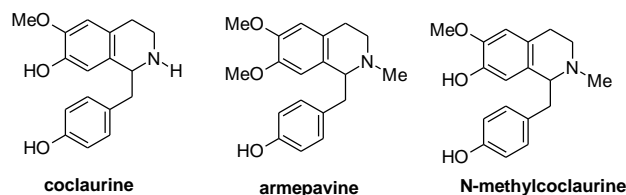


Figure 3 Alkaloids isolated from aerial parts of *Talguenea quinquenervis*.

The  $Et_2O$ -soluble basic fraction after acid–basic extraction of fraction TqFEB (originally from the methanolic extract of the aerial parts of *T. quinquenervis*) was further fractionated by column chromatography to afford three known alkaloids: coclaurine, armepavine and *N*-methylcoclaurine. These alkaloids (Figure 3) were obtained previously by Torres (1992). In addition, one presently unidentified alkaloid is under spectroscopic analysis for structural determination.

The other fractions obtained showed lower activity than alkaloid-containing extracts. Fractions TqFH and TqFAE had activity of 30% and 16.7%,

respectively, at the lowest concentration tested. Triterpenes were identified from both fractions.

Table 1. LD<sub>50</sub> of extracts of *T. quinquenervis* assayed against *D. melanogaster*.

Sample/meaning	Alkaloid presence**	Value in ppm
TqFW/Aqueous residue	+++	5.0
TqFH/n-Hexane partition		51.0
TqFAE/Ethyl acetate partition		73.7
TqFT/Total MeOH extract	+***	w/d*
TqFEA/Acidic ether extract		w/d*
TqFEB/Basic ether extract	+++	5.9

\* Alkaloids below the range of detection.

\*\* Three signals indicate presence of alkaloid with the Dragendorff test.

\*\*\* The Dragendorff test weakly indicates the presence of alkaloids.

## CONCLUSIONS

An initial, partial phytochemical profile of *T. quinquenervis* from the present study is reported here. The most potent insecticidal activity is associated with fractions TqFW and TqFEB, probably due to alkaloids in these extracts. Fractions TqFEA, TqFAE, and TqFH lack alkaloids and also lack insecticidal activity.

Based on the results of the present study, we suggest that the insect growth inhibition caused by TqFEB and TqFW could be due to the presence of the benzyloisoquinoline alkaloids present and that this species may serve as a source of useful insecticides. Interestingly, they have inhibition of growth activity similar to the triperpene toosendanin (Cespedes et al., 2001). We are continuing studies of the effects of these compounds on the growth of insects and their mechanism of action.

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## REFERENCES

- Ateyyat MA, Abu-Darwish MS. 2009. Short communication. Insecticidal activity of different extracts of *Rhamnus dispermus* (Rhamnaceae) against peach trunk aphid, *Pterochloroides persicae* (Homoptera: Lachnidae). **Spanish J Agric Res** 7: 160 - 164.
- Bhakuni D, Gonzalez C, Sammes PG, Silva M. 1974. The alkaloids of *Retanilla ephedra* (Vent) Brogh. **Rev Latinoamer Quim** 5: 158 - 162.
- Bick IRC, Sevenet Th, Sinchai W, Skelton BW, White AH. 1981. Alkaloids of *Cryptocarya longifolia*: X-ray crystal structure of thalifoline and longifolone. **Aust J Chem** 34: 195 - 207.
- Cespedes CL, Alarcón J. 2011. Biopesticidas de origen botánico, fitoquímicos y extractos de Celastraceae, Rhamnaceae y Scrophulariaceae. **Bol Latinoam Caribe Plant Med Aromat** 10: 175 - 181.
- Cespedes CL, Alarcon J, Aranda E, Becerra J, Silva M. 2001. Insect growth regulator and insecticidal activity of  $\beta$ -dihydroagarofurans from *Maytenus spp.* **Z Naturforschung** 56: 603 - 613.
- Cespedes CL, Torres P, Marin JC, Arciniegas A, Romo de Vivar A, Perez-Castorena AL, Aranda E. 2004. Insect growth inhibition by tocotrienols and hydroquinones from *Roldana barba-johannis*. **Phytochemistry** 65: 1963 - 1975.
- Cespedes CL, Salazar JR, Martinez M, Aranda E. 2005. Insect growth regulatory effects of some extracts and sterols from *Myrtillocactus geometrizans* (Cactaceae) against *Spodoptera frugiperda* and *Tenebrio molitor*. **Phytochemistry** 66: 2481 - 2493.
- Cespedes CL, Avila JG, Marin JC, Dominguez M, Torres P, Aranda E. 2006. **Natural compounds as antioxidant and moulting inhibitors can play a role as a model for search of new botanical pesticides**. In: *Naturally occurring bioactive compounds*. Cecilia Carpinella, Mahendra Rai. Eds. Advances in Phytomedicine Series, Vol III. Elsevier, The Netherlands, pp. 1-27.
- Delporte C, Backhouse CN, Erazo S, Negrete R, Silva C, Hess A, Muñoz O, Garcia-Avalos MD, San Feliciano A. 1997. Biological activities and metabolites from *Trevoa trinervis* Miers. **Phyther Res** 11: 504 - 507.
- Dwivedi SC, Shekhawat NB. 2004. Repellent effect of some indigenous plant extracts against *Trogoderma granarium* (Everts). **Asian J Exp Sci** 18: 47 - 51.

El-Seedi HR, Zahra MH, Goransson N, Veerpoorte R. 2007. Cyclopeptide alkaloid. **Phytochem Rev** 6: 143 - 165.

Gournelis DC, Laskarisb GG, Verpoorte R. **The Alkaloids**, ed. A. Brossi, Academic Press, New York, 1985, vol. 26, pp. 299-306.

Han BH, Park MH, Park JH. 1989. Chemical and Pharmacological studies on sedative cyclopeptide alkaloids in some Rhamnaceae plants. **Pure Appl Chem** 61: 443 - 448.

Johnston MC, 1962. Revision of *Condalia* including *Microrhamnus* (Rhamnaceae). **Brittonia** 14: 332 - 338.

Joullie MM, Richard DJ. **Cyclopeptide alkaloids: Chemistry and Biology**. Department of Chemistry, University of Pennsylvania, Philadelphia, USA. August 2004.

Lee SS, Su WC, Liu KCS, 2001. Cyclopeptide alkaloids from stems of *Paliurus ramossissimus*. **Phytochemistry** 58: 1271 - 1276.

Miyazawa M, Nakamura Y, Ishikawa Y. 2000. Insecticidal sesquiterpene from *Alpinia oxyphylla* against *Drosophila melanogaster*. **J Agric Food Chem** 48: 3639 - 3641.

Montes M, Wilkomirsky T. 1978. **Plantas chilenas en medicina popular: Ciencia y Folklore**. Escuela de Química y Farmacia y Bioquímica. Universidad de Concepción, Concepción, Chile.

Morales MA, Bustamante SE, Brito G, Paz D, Cassels BK. 1998. Cardiovascular effects of plant metabolites norarmepavine, coclaurine and norcoclaurine. **Phytother Res** 12: 103 - 109.

Otsuka H, Ogihara Y, Shibata S. 1974. Isolation of coclaurine from *Zizyphus jujuba* by

droplet counter-current chromatography. **Phytochemistry** 13: 2016.

Pacheco P, Albonico SM, Silva M, 1973. Alkaloids, triterpenes and other constituents of *Discaria crenata*. **Phytochemistry** 12: 954 - 955.

Silva M, Bhakuni D, Sammes PG, Pais M, Jarreau FX. 1974. A new peptide alkaloid from *Discaria crenata*. **Phytochemistry** 13: 861 - 863.

Torres R. 1992. **Rhamnaceae**. Cap. 9, en *Química de la Flora de Chile*. Orlando Muñoz Ed. Universidad de Chile. Pp. 167 - 176.

Torres R, Delle Monache F, Marini-Bettolo GB. 1979. Alkaloids from *Discaria serratifolia*. **J Nat Prod** 42: 430 - 431.

Torres P, Avila JG, Romo de Vivar A, Grcia AM, Marin JC, Aranda E, Cespedes CL. 2003. Antioxidant and insect growth regulatory activities of stilbenes and extracts from *Yucca periculosa*. **Phytochemistry** 64: 463 - 473.

Tschesche R, Davis ST, Zerbes R, Radloff M, Kaußmann EU, Eckhardt G, 1974. Mucronin-E, -F, -G und -H sowie Abissenin-A, -B und -C, weitere 15 gliedrige Cyclopeptidalkaloid. **Liebigs Annalen der Chemie** pp 1915-1928.

Tschesche R, Ammermann E, 1974. Scutianine-C, -D und -E, drei weitere cyclopeptidalkaloid aus *Scutia buxifolia* Reuiss. **Chemische Berichte** 107: 2274 - 2283.

Urzúa A, Santander R, Echeverría J, Villalobos C, Palacios SM, Rossi Y. 2010. Insecticidal properties of *Peumus boldus* Mol. Essential oil on the house fly, *Musca domestica* L. **Bol Latinoam Caribe Plant Med Aromat** 9: 465 - 469.