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Chemical composition of essential oils of *Drimys angustifolia* Miers and *Drimys brasiliensis* Miers and their repellency to drywood termite *Cryptotermes brevis* (Isoptera: Kalotermitidae)

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ABSTRACT

The essential oils extracted from leaves of the Southern Brazilian native species *Drimys angustifolia* Miers (DA) and *Drimys brasiliensis* Miers (DB) by Clevenger distillation were analyzed by gas chromatography/mass spectrometry (GC/MS). The oils of DA and DB consisted predominantly of monoterpenoids and sesquiterpenoids. The largest components of DA oil were bicyclogermacrene (19.6%), sabinene (9.7%) and myrcene (5.2%), while DB oil was characterized by cyclocolorenone (18.2%), followed by terpinen-4-ol (8.7%) and alpha-gurjunene (6.9%). Laboratory tests were carried out to determine the repellency of the essential oils to the drywood termite *Cryptotermes brevis* (Isoptera: Kalotermitidae). It was observed that the oil showed repellency at the concentrations 25, 12.5, and 6.25 µg/mL. The oils of both species exhibited a negative repellency index, which represents repellent activity, except for DA oil at the highest concentration, which was attractive. Five deaths (11% of the termite sample) were observed at 25 µg/mL DA, in the four-hour repellency test, while four deaths occurred at 12.5 µg/mL (approximately 9%). The essential oil of DB did not cause any termite deaths.
Keywords: Monoterpenoids. Sesquiterpenoids. *Drimys angustifolia*. *Drimys brasiliensis*. *Cryptotermes brevis*.

INTRODUCTION

In Brazil, the Neotropical woody evergreen plant genus *Drimys* (Winteraceae) occurs in two species, *D. angustifolia* (South Brazil) and *D. brasiliensis* (South, Southeast and parts of Midwest and Northeast Brazil) (Mello-Silva, 2012). *Drimys* is popularly known in Brazil as *casca d'anta* (tapir's bark) and *cataia* (peppery leaf) (Trinta & Santos, 1997). Among insects of economic interest that require alternative forms of control, the drywood termites (Kalotermitidae) occupy a prominent position, especially the species *Cryptotermes brevis*. These wood-boring insects live exclusively inside the timber that they devour, forming long hidden aerated galleries and disposing of their six-sided fecal pellets through small holes, forming mounds. Only the juveniles leave to form new niches in other pieces of wood (Gonzaga, 2006). Although the economic losses caused by termites in urban areas are certainly underestimated, *C. brevis* colonies are known to be responsible for much damage to buildings and furniture. The economic losses caused by termites around the world are estimated at 10 billion dollars a year (Fontes & Berti-Filho, 1998; Ibrahim & Adebote, 2012).

Natural products are considered a potentially valuable alternative control measure. Among them, essential oil components are known to possess repellent, chemosterilant, antifeeding and biocidal activities against various insects and have a promising potential as products for tick control, since some of them are selective and have few or no harmful effects on non-target organisms (Facey et al., 2005; Dietrich et al., 2006; Tunon et al., 2006). Many oils have proven to be more effective than conventional fumigants (Singh et al., 1998; Il-kwon & Sang-Chul, 2005; Seon-Mi et al., 2009). Repellents are substances that act locally or at a distance, deterring an arthropod from flying to, landing on or biting human or animal skin (or a surface in general) (Blackwell et al., 2003; Choochote et al., 2007).

The South Chilean tree *Drimys winteri* J.R. Forst & G. Forst (known in the UK as winter's bark) also showed repellent activity against the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), one of the most widespread and destructive stored-product pests in the world (Zapata & Smagghe, 2010). Therefore, it is important to study this plant genus for its biological activity.

Our research group had already tested the essential oil of DB for its toxicity to the larvae of the cattle tick *Rhipicephalus* (*Boophilus) microplus* and the brown dog tick *Rhipicephalus sanguineus*, by larval immersion.

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The results proved interesting, which impelled us to test the essential oils of DA and DB against insect species, specifically the drywood termite, at the same concentrations as in our previous study (Ribeiro et al., 2008).

MATERIALS AND METHODS

Termites

Termites collected from infested furniture were identified and kept in the Laboratory of Pest Control at the Institute of Biotechnology, University of Caxias do Sul, Rio Grande do Sul, Brazil. The termites were removed and placed in Petri dishes (9 cm in diameter), fed with wooden blocks of *Pinus elliotii* Engelm and maintained at 24 ± 2 °C and relative humidity (R.H.) 70% and not exposed to light until the bioassay.

Leaves of *D. angustifolia* and *D. brasiliensis*

Leaves of DA were collected at the Research and Nature Conservation Center Pró-Mata (CPCN Pró-Mata), São Francisco de Paula, Rio Grande do Sul, Brazil and DB leaves were collected in São Jerônimo, Rio Grande do Sul, Brazil, on June 15th 2009. Specimens were identified by the botanist Ph D. Sérgio A. de L. Bordignon. Voucher specimens were deposited in the ICN Herbarium (UFRGS, Porto Alegre), under numbers ICN 123644 and ICN167795, respectively.

Essential oils of *D. angustifolia* and *D. brasiliensis*

The essential oils were extracted from DA and DB fresh leaves (100 g), separately, by hydrodistillation for 4 hours in a Clevenger apparatus. The yields were calculated for both oils. The extraction was done only once for this analysis.

Oil constituents

Quantitative and qualitative analyses were performed by gas chromatography/mass spectrometry (GC/MS). The GC analysis was performed in a gas chromatograph (Shimadzu GC-17A), equipped with Shimadzu GC 10 software, using a DB-5 fused silica capillary column (30m×0.25mm×0.25µm) coated with (5%-phenyl)-methylpolysiloxane. Injector and detector were set at 220 and 250 °C, respectively, and the column was heated in the oven from 60 to 300 °C at 3 °C/min. The carrier gas was helium flowing at 1 mL/min. The mass spectrometer was a quadrupole MS system (QP 5000) operating at 70 eV. Compounds were identified by comparing their retention indices (their retention times normalized to those of a series of n-alkanes – C10 to C22) and mass spectra with those of authentic samples and/or with literature data (Barrero et al., 2000; Adams, 2007; Limberger et al., 2007).

Repellency test

This test was based on the method proposed by McDonald et al. (1970). Filter paper discs, 9.0 cm in diameter (63.6 cm^2), were cut into two halves, which were placed together in a Petri dish, one being treated with 1 mL of ethanol (the control), and the other with 1mL of an essential oil. Three concentrations (25.0, 12.5 and 6.25 µg/mL) of each oil dissolved in ethanol were tested, to observe the minimum effective concentration (threshold value). The solvent was evaporated at room temperature and 15 termite workers were placed in each Petri dish and maintained at 25 ± 2 ºC and relative humidity 70% in the dark. Each treatment was performed in triplicate, plus control. The 15 insects were observed for the first four hours of testing and their attraction to the control or to the treated paper in the plate was periodically assessed by analyzing their displacement to one portion or the other. From the data recorded in the test, the Preference Index (PI) cited by Procópio et al. (2003) was calculated**:**

$$PI = (\%ITP - \%IC)/(\%ITP + \%IC),$$

where %ITP =% of insects on the test paper,

%IC =% of insects on the control paper.

The index was graded as follows: P.I.: -1.00 to-0.10 means repellent oil, -0.10 to +0.10 means neutral oil and +0.10 to +1.00 means attractive oil.

DATA ANALYSIS

All bioassays were performed in triplicate and were compared by ANOVA and Tukey's test, significance being accepted when $p<0.05$.

RESULTS

The essential oil distilled from the leaves of DA and DB yielded 0.4% and 0.3% of the fresh leaf weight, respectively, and the chemical composition is presented in Table 1. The constituents identified account for 82.9% of the total essential oil obtained from DA and 85.6% of that from DB. The main constituents of the oil from DA were bicyclogermacrene (19.6%), the most abundant, followed by sabinene (9.7%) and myrcene (5.2%). The oil of DB was characterized by cyclocolorenone (18.2%), followed by terpinen-4-ol (8.7%) and alpha-gurjunene (6.9%).

It was observed that the first two concentrations of DA were repellent, as indicated by negative indices, whereas the highest concentration was mildly attractive, with positive values, in the course of 4 hours (Figure 1). It is interesting that, at the highest concentration, the DA solution was also lethal. Also, the lowest concentration apparently became more repellent during the 4-hour exposure.

Table 1. Percent composition obtained by normalization of GC/MS analysis* of essential oils from fresh leaves of Southern Brazilian *D. angustifolia* and *D. brasiliensis.*

Theoretical AI/ Calculated AI	Compound name	D. angustifolia	D. brasiliensis
		Monoterpene hydrocarbons %	
932/926	alpha-pinene	3.3	3.2
969/965	sabinene	9.7	3.6
974/968	beta-pinene	3.9	3.3
988/985	myrcene	5.2	6.5
1032/1031	(*Z*)-beta-ocimene	4.6	0.6
1054/1050	gamma-terpinene	3.0	3.2
1086/1080	terpinolene	1.4	1.8
	partial 1	31.1	22.2
		Oxygenated monoterpenes %	
1026/1023	1,8-cineole	0.6	4.4
1095/1094	linalool	1.3	1.8
1174/1168	terpinen-4-ol	6.4	8.7
1186/1181	alpha-terpineol	0.9	2.1
	partial 2	9.2	17.1
		Sesquiterpene hydrocarbons %	
1409/1400	alpha-gurjunene	-	6.9
1408/1406	beta-caryophyllene	0.8	0.1
1439/1426	aromadendrene	0.9	0.4
1484/1469	germacrene D	1.5	0.4
1500/1487	bicyclogermacrene	19.6	6.5
	partial 3	22.8	14.3
		Oxygenated sesquiterpenes %	
1577/1566	spathulenol	1.4	2.9
1582/1572	caryophyllene oxide II	1.4	-
1590/1579	globulol	3.4	1.3
-/1585	epiglobulol	2.8	0.8
1607/1590	5-epi-7-epi-alpha-eudesmol	1.1	0.1
1766/1745	drimenol	1.2	0.8
1759/1748	cyclocolorenone	-	18.2
	partial 4	11.3	24.0
		Phenylpropanoids %	
1285/1278	safrole	3.5	1.4
1517/1511	myristicin	5.0	6.5
	partial 5	8.5	7.9
	Total	82.9	85.6

*Based on GC/MS retention times on DB-5 column; AI: Arithmetic retention index

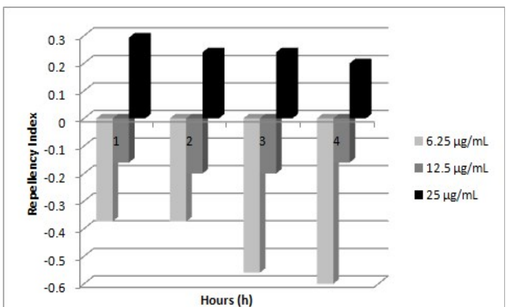

Figure 1. Repellency Index of essential oil of *Drimys angustifolia* tested at three different concentrations over a period of four hours.

DB did not cause deaths, but the repellency rates were much higher than those of the DA essential oil (Figure 2).

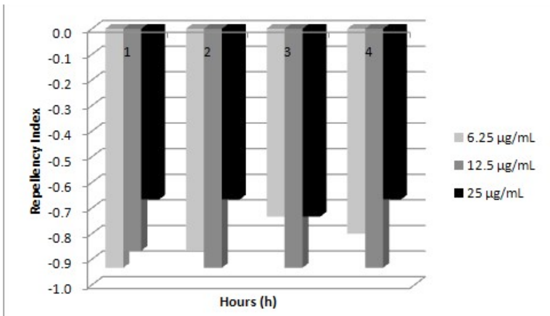

Figure 2. Repellency Index of essential oil of *Drimys brasiliensis* tested at three different concentrations over a period of four hours

DISCUSSION

Plant essential oils and their constituent metabolites have demonstrated great potential for repellent activity against several species of insects and other arthropods (Nerio et al., 2010). Usually, insect repellents work by providing a vapor barrier that deters the arthropod from coming into contact with the surface (Brown & Hebert, 1997).

The composition of the hydrodistilled oils indicated monoterpenoid, sesquiterpenoid and phenylpropanoid biosynthetic pathways. The oil of DA was characterized by bicyclogermacrene (19.6%), which was the most abundant constituent, followed by sabinene (9.7%) and myrcene (5.2%). The most abundant compound in the DB oil was cyclocolorenone (18.2%), followed by terpinen-4-ol (8.7%) and alpha-gurjunene (6.9%). The repellent properties of a range of essential oils often appear to be associated with the presence of monoterpenes and sesquiterpenes (Kiran & Devi, 2007; Jaenson et al., 2006; Sukumar et al., 1991).

The DB oil had 41.2% of oxygenated compounds (mono- and sesquiterpenes) in its composition, while the DA oil had 20.5% of these compounds (Table 1). Besides terpenoids, a small quantity (up to 8.5%) of the phenylpropanoids safrole and myristicin were detected in DA and DB oils. The presence of phenylpropanes has also been demonstrated in other *Drimys* species, *D. granadensis* (Cicció, 1997) and *D. winteri* (Barrero et al., 2000), as well as in the Australasian sister genus *Tasmannia* (Southwell & Brophy, 1992).

The predominance of cyclocolorenone, observed in the essential oil from *D. brasiliensis*, has not previously been reported in other *Drimys* oils and thus could be used as a chemical aid to its taxonomy. This ketone has a restricted occurrence, having been reported in *Pseudowintera colorata* (Corbett & Speden, 1958) and *Tasmannia* (Hisashi et al., 2002) among the family Winteraceae, *Solidago Canadensis* (goldenrod) (Jacyno & Montemurro, 1991), *Ledum palustre* ('Labrador tea') (Mikhailova et al., 1978), *Magnolia grandiflora* (Southern magnolia) (Schünly et al., 2001) and in liverworts such as *Porella* (Asakawa et al., 1976), *Frullania* and *Schusterella* (Asakawa et al., 2003) species and *Bazzania trilobata* (Nagashima et al., 1996), and can easily be isolated (by hydrodistillation or solvent extraction) and characterized by GC or GC/MS (Limberger et al. 2007).

In the DA test, 11% of the termites died at the concentration of 25 µg/mL, implying that the increase in concentration led to a change in action, from repellent to fumigant activity. Similarly, at the intermediate concentration (12.5 µg/mL), 9% of the termites died (data not shown). This may represent a dose-response relationship between the concentration and the number of deaths. On the other hand, DB caused no deaths. Termites live in galleries, unlike insects that live outdoors. The saturation of the environment caused by the volatilization of the oil may have favored the death of the insects with gradually increasing concentration (Verma et al., 2009). The high volatility of essential oils enables them to create a barrier, which is essential to prevent infestation by termites.

Statistical analysis showed no significant difference in repellence between concentrations at each time for each species ($p > 0.05$). However, there were significant differences between the species at the greatest concentration (25 µg/mL) in the first hour ($p < 0.019$) and in the second hour ($p < 0.021$). The data may explain the higher repellent activity of DB, considering that the majority of metabolites isolated (cyclocolorenone) from essential oils with the best repellent activity are oxygenated (41.2 %). The activity of essential oils is usually attributed to their major components and the presence of oxygenates increases repellency (Nerio et al., 2010), as shown in Figures 1 and 2 and Table 1.

Although essential oils are effective repellents when freshly applied, their protective effects usually dissipate relatively quickly (Trongtokit et al., 2005). In the case of mosquitoes, essential oils commonly act in the vapor phase (Zhu et al., 2001), which is effective for a relatively short period of time. From the analysis of the repellency observed at all concentrations, in relation to the composition of the two extracts, the volatilization rate was much higher in DA than in DB. This may be explained by the greater presence of hydrogenated compounds in DA, which have lower boiling points than the oxygenated compounds present in greater quantities in DB.

The study of the synergistic effects among constituents of essential oils and mixtures of oils, as well as the search for new additives that could prolong the protection period, is a promising line of research. It may allow the replacement of synthetic repellents currently in use by natural products effective even at low concentrations, offering biodegradability and reducing the resistance acquired by the insects, bioaccumulation and environmental pollution (Viegas Júnior, 2003; Nerio et al., 2010).

The genus *Drimys* has shown promising activity as a repellent against arthropods, especially insects, because of the complex chemical composition of its essential oils. Previous work in our research group (Ribeiro et al., 2008) has demonstrated its importance in combating two major arthropod pests (ticks) in the veterinary field. Other plant species containing essential oils have shown repellent and toxic activity against species of termite (Cheng et al., 2007; Nisar et al., 2012).

In the present study, testing the repellency of two related species revealed two different activities, the repellent action of DB and the fumigant/toxic activity of DA. Thus, this study has pointed to new essential oil applications and shown a possible alternative way to combat insects with a biodegradable and natural source.

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RESUMO

Composição química e repelência de óleos essenciais de Drimys angustifolia Miers e Drimys brasiliensis Miers em cupins de madeira Cryptotermes brevis (Isoptera: Kalotermitidae)

Os óleos essenciais obtidos das folhas das árvores nativas do sul do Brasil *Drimys angustifolia* Miers (DA) e *Drimys brasiliensis* Miers (DB) foram analisados por cromatografia gasosa acoplada a espectrometria de massas (CG/EM). O óleo de DA foi caracterizado pela presença de monoterpenoides e sesquiterpenoides, biciclogermacreno (19,6%), seguido por sabineno (9,7%) e mirceno (5,2%). O óleo de DB foi caracterizado por sesquiterpenóides e monoterpenóides, ciclocolorenona (18,2%), seguido por terpinen-4-ol (8,7%) e alfa-gurjuneno (6,9%). Os testes foram realizados a fim de se determinar o potencial repelente dos óleos essenciais contra o cupim de madeira seca *Cryptotermes brevis* (Isoptera: Kalotermitidae) nas doses de 25, 12,5, e 6,25 µg/mL. Ambas as espécies mostraram índice de repelência negativo, o que representa a ocorrência da atividade repelente, exceto na maior concentração de DA, na qual o óleo mostrou-se atraente. Cinco mortes foram observadas na concentração de 25 µg/mL de DA, correspondendo a 11% do total em quatro horas de análise, sendo que 9% ocorreram na concentração de 12,5 µg/mL. No caso do óleo essencial de DB não foram observadas mortes de cupins.

Palavras-chave: Monoterpenoides. Sesquiterpenoides. *Drimys angustifolia*. *Drimys brasiliensis*. *Cryptotermes brevis*.

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