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Volatile chemical composition of essential oil from *Bursera graveolens* (Kunth) Triana & Planch and their fumigant and repellent activities

Beatriz Eugenia Jaramillo-Colorado*, **Samyr Suarez-López and Vanessa Marrugo-Santander**

Programa de Graduação em Química, Grupo de Pesquisa em Agroquímica, Faculdade de Ciências Exatas e Naturais, Universidade de Cartagena, Rua Zaragocilla, 130014, Cartagena of Indias, Colombia. *Author for correspondence. E-mail: bjaramilloc@unicartagena.edu.co

ABSTRACT. The objectives of this work were the study of the volatile chemical composition of essential oils (EO's) from *Bursera graveolens* obtained in the locality of Malagana, municipality of Mahates, Bolívar, Colombia, as well as to evaluate their repellent and fumigant properties. EO's were extracted by hydro-distillation and characterized by gas chromatography-mass spectrometry (GC-MS). The major compounds found in *B. graveolens* were limonene (42.2%), pulegone (20.9%), carvone (7.5%), caryophyllene (4.1%), and *trans*-carveol (3.8%). The repellent activity of EO's was determined by the area preference method, where the EO of *B. graveolens* presented repellent activity against the *Tribolium castaneum* weevil at a concentration of 1 at 2% and 4 hours of exposure (88.1 and 88.6% respectively). *B. graveolens* essential oil was more effective in its fumigant activity with LC_{50} of 108.2 μg oil mL^{-1}. Also, the fumigant and repellent activities of two individual compounds present in the oil were evaluated, that is, limonene (majority) and caryophyllene. The results indicated that *B. graveolens* essential oil could be a promising alternative to new natural repellents and biocides.

Keywords: gas chromatography; bioactivity; essential oils; *Bursera graveolens*; *Tribolium castaneum*; terpenes.

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Introduction

Bursera graveolens (Kunth) Triana & Planch, (Burseraceae) is a tree (3-15 m) with a bark of reddish-brown color, alternate leaves grouped at the tips of their branches and unisexual flowers (Muñoz-Acevedo, Serrano-Uribe, Parra-Navas, Olivares-Escobar, & Niño-Porras, 2013). The tree can be seen in Figure 1. It is a native species of tropical America (from south Mexico down to Peru). The different parts of the plant, e.g., leaves, stems and resin, have been used in folk medicine (Peru, Costa Rica, Nicaragua, Guatemala, Cuba, Colombia), in different forms (smoked, infusions, cataplasms, compresses), as healing, abortive, anti-inflammatory, anti-tumor, analgesic, antidiarrheal, depurative, diaphoretic, expectorant, insecticidal, for the treatment of anemia, rheumatism, dermatitis, asthma and colic as well as a mosquito repellent (Nakanishi et al., 2005; Zúñiga et al., 2005; Monzote, Hill, Cuellar, Scull, & Setzer, 2012). Essential oil obtained from *B. graveolens* has shown diverse activities as an anti-inflammatory (Zuñiga et al., 2005; Manzano-Santana et al., 2009), antiproliferative (Monzote et al., 2012), antioxidant and antimicrobial agent (Andrade-Santiago et al., 2016; Sotelo-Mendez, Figueroa-Cornejo, Césare-Coral, & Alegría-Arnedo, 2017).

Essential oils (EO's) are a mixture of chemical compounds of an organic nature, which are found as end products of the secondary metabolism of aromatic plants, which are responsible for their characteristic aromas and bioactivities (Andres et al., 2017). Biological pesticides consisting mainly of essential oils have been shown to exert fungicidal, insecticidal and bactericidal effects, among others; therefore, their use in agroindustry has increased exponentially, what has been known as green alternatives in recent years (Prieto, Patiño, Delgado, Moreno, & Cuca, 2011; Benelli et al., 2017). Essential oils have been specially investigated as natural insecticides against several insects from stored products, and many studies have used the red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) as a biological model. It is a pest from stored products of great economic importance that is found in grain and flour at homes and stores. *T. castaneum* is a secondary pest of a wide variety of cereals, legumes, oilseeds, cakes, nuts, spices and products of animal origin. It abounds in warm and dry conditions (Manivannan, 2015).

Figure 1. *Bursera graveolens* tree.

There are various reports of the fumigant and repellent activities of essential oils on *T. castaneum* (Stefanazzi, Stadler, & Ferrero, 2011; Jaramillo-Colorado, Martelo, & Duarte, 2012; Taban, Saharkhiz, & Hooshmandi, 2017; Candy, Nicolas, Andriantsoanirina, Izri, & Durand, 2018; Hu, Wang, & Dai, 2019). However, there are no reports on *B. graveolens* and its components. This study aimed to determinate the volatile chemical composition of *B. graveolens* essential oil and evaluate its repellent and fumigant activities.

Material and methods

Vegetal material

Fresh leaves from *B. graveolens,* were collected at the locality of Malagana, municipality of Mahates, Bolivar, Colombia. They were collected in January of 2016. Identification taxonomy was carried out at the Institute of Biology of the Faculty of Exact and Natural Sciences of the University of Antioquia. The EO's from *B. graveolens* were stored at the Agrochemical Research Laboratory according to the GIA 015 code.

Extraction of essential oils

The hydro-distillation method was used for the extraction for *B. graveolens*, using clevenger-type equipment, according to the procedure described by Jaramillo-Colorado, Duarte-Restrepo, and Jaimes (2016). 500 g of fresh leaves, stems, finely chopped and submerged in 2 L of water were used. The extraction time was 2 hours. Essential oil (EO) was separated from the water by decantation. The extractions were done three times. For each EO, an aliquot of 30 μL was taken and diluted in 1 mL of dichloromethane for chromatographic analyzes.

Gas chromatography analysis

Essential oils (20 μL mL^{-1}) were analyzed by means of *Agilent Technologies* 4890D (Palo Alto, California, USA) gas chromatography equipment (GC) equipped with a split/splitless (250°C, ratio split 1:30) injection port and a flame ionization detector (FID) (280°C). An Agilent 6890N GC coupled to an Agilent 5973N Mass Detector (electron ionization, 70 eV) was used for GC-MS analysis equipped with HP-5 capillary columns (phenyl-dimethylpolysiloxane, 0.2 μm film thickness) 25 m × 0.2 mm id). Working conditions were as follows: injector temperature 260°C; column temperature 70-190°C, 5°C $min.^{-1}$. The carrier gas was helium (99.995%, *Linde*, S.A) with a linear velocity of 35 cm s^{-1}. The temperature of the transfer line connected to the mass spectrometer was 280°C; Oven temperature 40 (15 min.) up to 250°C (15 min.) @ 5°C $min.^{-1}$. For identification of compounds, standard terpenes were used, by analyzing under the same instrumental conditions and by comparing the mass spectrum with those available in the Wiley Mass Spectral Database library. The Mass spectral and retention indexes obtained were compared with the literature reported (Adams, 2007).

Insects and bioassays

Adults of *T. castaneum* used in the experiments were collected seven days after hatching. Bioassays were carried out in the dark in incubators at 28-30°C and 70-80% relative humidity (r.h) at the Agrochemical Research laboratory of the University of Cartagena. Oat (*Avena sativa*) was employed to feed *T. castaneum.*

Repellent activity

The repellent activity was performed according to Tapondjou, Adler, Fontem, Bouda, & Reichmuth (2005). The experimental method was evaluated using the area preference method. Whatman No. 1 filter papers (diameter 9 cm) were cut in half. The essential oil from *B. graveolens* was dissolved in acetone.

The concentrations chosen to evaluate the repellent activity were 0.001, 0.01, 0.1 and 1%. A volume of 0.5 mL of each essential oil solution was applied slowly and uniformly to one half of each filter paper, while the other half was treated with an equal volume of acetone alone as a control. The treated and control half discs were dried at room temperature in order to allow evaporation of the solvent. Treated and untreated halves were attached using adhesive tape and placed in Petri dishes. Twenty adults (5-7 day old) of *T. castaneum* were released separately at the center of each filter paper disc. The dishes were then covered and transferred to an incubator at room temperature. Five replications were used for each concentration.

Fumigant activity

The toxic effect of *B. graveolens* EO's was tested on *T. castaneum* adults. Filter paper discs (Whatman No. 1, 2 cm diameter), deposited at the bottom of Petri dish covers (90 x 15 mm) were used. These were impregnated with oil at doses calculated in such a manner that equivalent fumigant concentrations of 500, 350, 250, 150, 50 μg of oil mL^{-1} air, respectively, were given. Twenty adult insects (1 to 10-d-old) were introduced and tightly capped (replicated four times for each concentration). Pirilan, a commercial insecticide containing methyl pirimiphos (organophosphorus compound, 300 μg mL^{-1} air) as an active ingredient was used as positive control. The mortality percentage was determined after 24 hours from the start of exposure (Prieto et al., 2011).

Statistical analysis

The data presented in terms of repellent activity are submitted as the mean ± standard deviation through an ANOVA statistical formula and a t-test of for students; as for the fumigant activity, the mean lethal concentration (LC_{50} = dose at which 50% mortality of the insect population is produced) was calculated by a linear regression analysis using the STATGRAPHIC Centurion XVI statistical program, version 16.1.03.

Results

The yield of essential oil from *B. graveolens* was 0.3%. The main components found in the oil were monoterpenes: Limonene (42.2%), pulegone (20.9%), carvone (7.5%), Caryophyllene (4.1%), and *trans*-carveol (3.8%); see Table 1.

Repellent activity of essential oil

The results of the repellent activity from *B. graveolens* EO's are presented in Table 2. The oil from *B. graveolens* exhibited repellent activity against the *Tribolium castaneum* weevil at a concentration of 1% at 2 and 4 hours of exposure (88.1 and 88.6% respectively). Limonene (standard), the major compound present in the oil, had the highest repellent activity at a concentration of 1% at 2 and 4 hours of exposure (100%); this had a repellent capacity greater than that presented by the commercial repellent, Ethylbutylacetylaminopropionate (approx. 78%). Caryophyllene exhibited a weak repellent effect, as shown in Table 2.

Fumigant activity

EO from *B graveolens* exhibited 95 and 100% of mortality on *T. tribolium* at 350 and 500 μg EO mL^{-1} of air, respectively, which is similar to the commercial pesticide methyl-pirimiphos (see Figure 2). However, the essential oil from *B. graveolens* (LC_{50} = 108.2 μg mL^{-1} of air) is not more toxic than methyl-pirimiphos (LC_{50} = 87.4 μg m^{-1} of air) versus *T. castaneum* (as deduced by the LC_{50} confidence intervals in Table 3). On the other hand, limonene, the main compound in the EO of *B. graveolens* was more toxic (LC_{50} = 189.6 μL L^{-1}) than caryophyllene (LC_{50} = 472.6) on *T. castaneum* (Table 3).

Table 1. Chemical composition of essential oil from leaves of *Bursera graveolens*, obtained by hydro-distillation.

No	Name of compound[a]	LR HP-5[b]	LR HP-5[c]	Relative area[d], %
1	Limonene	1028	1031	42.0±2.40
2	*cis-p*-Mentha-2,8-dien-1-ol	1118	1120	1.6±0.50
3	*cis*-Limonene oxide	1131	1134	1.1±0.50
4	Menthone	1159	1154	1.7±0.36
5	Isomenthone	1166	1165	3.4±0.22
6	α-Terpineol	1171	1189	1.0±0.08
7	*trans*-Carveol	1221	1217	3.8±0.08
8	*cis*-Carveol	1230	1229	2.2±0.50
9	Pulegone	1232	1237	20.9±1.40
10	Carvone	1235	1242	7.5±0.50
11	Carvenone	1247	1252	1.8±1.20
12	Carvone oxide	1270	1277	2.3±0.30
13	Isomenthone-2-ethyl	1277	1289	1.4±0.42
14	α-Copaene	1375	1376	1.1±0.50
15	β-Elemene	1388	1391	1.9±0.60
16	Caryophyllene	1410	1404	4.1±0.88
17	α-Humulene	1452	1454	1.7±0.30
				99.7%

[a]Identification made by mass spectrum (EI: electron impact ionization, 70 eV; peak matching > 90%), [b]Experimental retention indices on HP-5 column; [c]Literature retention indices. [d]Averages of three independent extractions.

Table 2. Repellent activity of essential oils from *B. graveolens* versus *T. castaneum*.

Sample	Concentration (μg EO cm^{-2} air)	Repellent, %	
		2 hours	4 hours
B. graveolens EO	0.001	41.0±10.2	-20.0±10.0
	0.01	47.0±9.2	47.5±8.9
	0.1	70.5±5.5	74.4±8.2
	1.0	88.1±6.4	88.6±5.9
Limonene	0.001	52.5±5.5	58.7±3.3
	0.01	77.8±8.2	79.0±1.0
	0.1	98.2±2.2	98.3±4.4
	1.0	100±0	100±0
Caryophyllene	0.001	30.5±5.0	37.2±9.8
	0.01	34.6±9.6	42.0±3.2
	0.1	40.8±6.4	47.5±5.5
	1.0	55.2±3.5	63.0±6.0
Commercial repellent (Ethylbutylacetylaminopropionate)	0.001	25.0±1.5	18.0±3.0
	0.01	39.2±3.0	45.5±5.5
	0.1	50.0±5.0	54.6±1.2
	1.0	78.4±2.2	78.8±2.4

Statistically significant difference between the number of organisms in treated and untreated areas, using the paired t test (p < 0.001).

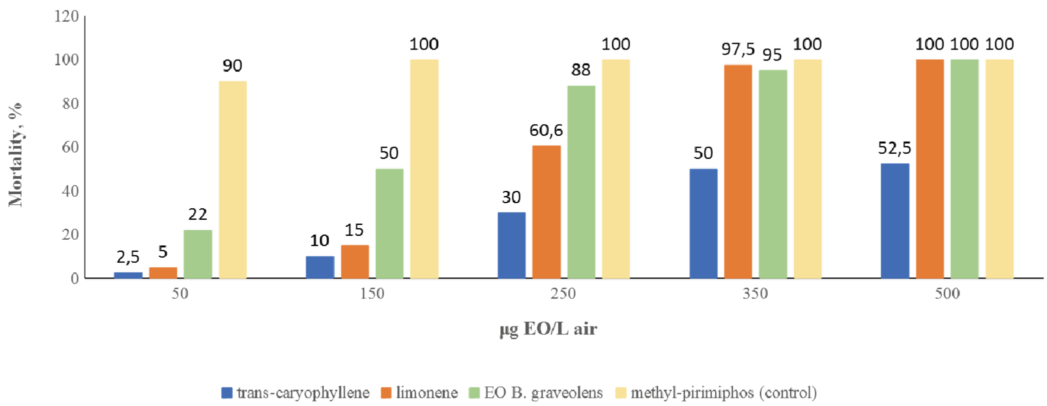

Figure 2. Fumigant activity of essential oils from *B. graveolens*, limonene, caryophyllene and methyl-pirimiphos (positive control) after 24 hours of exposure.

Table 3. LC_{50} of essential oil from *B. graveolens,* limonene, caryophyllene and methyl-pirimiphos on *T. castaneum*.

Essential oil or compound	Confidence level	LC_{50} (μg mL^{-1})	LC_{95} (μg mL^{-1})
B. graveolens	0.05	108.2	118.7
Limonene Caryophyllene	0.05	189.6	208.7
	0.05	472.6	565.4
Pirilan (methyl-pirimiphos)	0.05	87.4	440.6

Discussion

EO from *Bursera graveolens* has monoterpenes as its principal compounds: Limonene (42.2%), pulegone (20.9%), carvone (7.5%), caryophyllene (4.1%), and *trans*-carveol (3.8%). Figure 3 shows the structure of its major components. Other investigations carried out in Havana (Cuba) reported, for the EO of *B. graveolens,* compounds such as limonene (30.7%), (*E*)-β-ocimene (20.8%) and β-elemene (3.0%) (Carmona, Quijano-Celís, & Pino, 2009). Young, Chao, Casabianca, Bertrand, and Minga (2007) found limonene (58.6%) and α-terpineol (10.9%) in the essential oil from Ecuadorian *B. graveolens.* Muñoz-Acevedo et al. (2013) described germacrene D (20.7%), caryophyllene (18.0%), viridiflorol (8.0%), limonene (6.6%), linalool (6.5%) and dendrolasin (5.3%) in volatile fractions from leave extracts of *B. graveolens*. Luján-Hidalgo et al. (2012) reported limonene (42.90%), β-ocimene (17.39%), β-elemene (11.82%), menthofuran (6.79%) as major compounds for Mexican *B. graveolens.*

These divergences related to the composition of essential oils are attributed to different causes, including variations in ecological conditions (climate, type of soil, season of the year, geographical location) in which the plant develops; (extraction method, time, raw material) which can produce qualitative and quantitative changes in the oil (Figueiredo, Barroso, Pedro, & Scheffer, 2008; Andrade et al., 2008). Table 4 shows the major compounds present in *B. graveolens* EO's from Cuba, Ecuador, Mexico, Peru and Colombia (department of Atlántico).

This work showed that the essential oil of *B. graveolens* had repellent and fumigant activities. Many researchers explain the differences in repellent properties between the essential oil and the individual components, among which are the molecular structure, synergistic effect and different species reaction (Ju-Qin et al., 2018; Saad, El-Deeb, & Abdelgaleil, 2019). Malacrino, Campolo, Laudani, & Palmeri (2016) reports a higher repellent activity of R-(+)-limonene compared to the other enantiomer versus *Tribolium confusum*. Monoterpenes and phenylpropenes have exhibited significant repellent effects on adults of *T. castaneum*, among which (-)-menthone, trans-cinnamaldehyde, and α-terpinene are notable (Saad et al., 2019).

Studies show that the fumigant properties of essential oils are associated with the presence of mono and sesquiterpene compounds. Terpenes can be toxic due to their penetration of the insect cuticle (contact effect), respiratory system (fumigant effect) and via the digestive apparatus (ingestion effect) (Ibrahim, Kainulainen, & Aflatuni, 2001).

Plant-based pesticides are considered attractive alternatives to synthetic pesticides, as they usually are safer due to their rapid biodegradation and consequent short-term persistence in the environment (Isman, 2006; 2008). The action of some terpenes is similar to that of the organophosphorus and carbamate compounds present in some conventional insecticides, inhibitors of the enzyme acetylcholinesterase, which causes rapid death due to respiratory failure in certain insects from stored grain (López & Pascual-Villalobos, 2010; Abou-Taleb, Mohamed, Shawir, & Abdelgaleil, 2016). For example, limonene is a component of citrus essential oils recommended for controlling scale insects in ornamental plants and agricultural activities in the United States (Hollingsworth, 2005). Terpinen-4-ol, 1,8-cineol, linalool, R - (+) - limonene and geraniol were tested in vapor form against different stages of *Tribolium confusum* (Stamopoulos, Damos, & Karagianidou, 2007). Kim, Kang, & Park (2013) reported α-pinene as the most potent inhibitor of AchE activity followed by β-pinene and limonene, while Saad et al. (2019) reported in a fumigant toxicity assay claiming that (-)-terpinen-4-ol (LC_{50} = 20.47 μl l air^{-1}) and α-terpinene (LC_{50} = 23.70 μl l air^{-1}) exhibited the highest toxicity without any significant differences between them. Moreover, (-)-menthone and p-cymene showed strong toxicity. Chaubey (2012) found that 1-8-cineole was most effective against *Sitophilus oryzae* and *Tribolium castaneum.* Pulegone also displayed strong fumigant toxicity against adults of *S. zeamais and T. castaneum* (7 day $LC_{(50)}$ = 3.47 and 11.56 mg cm^{-3}, respectively) Liu, Chu, & Jiang (2011).

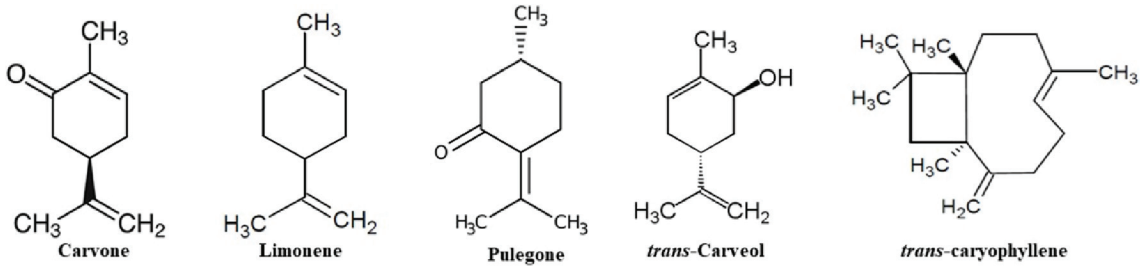

Figure 3. *Structure of main compounds present in B. graveolens essential oil.*

Table 4. Main compounds of essential oil from *B. graveolens* obtained in different countries.

Compounds	Cuba Carmona et al. (2009)	Ecuador Young et al. (2007)	Mexico Luján-Hidalgo et al. (2012)	Peru Sotelo-Mendez et al. (2017)	Colombia (department of Atlántico) Muñoz-Acevedo et al. (2013)
Limonene	26.5	58.6	42.9	-	6.6
β-Ocimene	13.0	-	17.3	4.93	1.5
Linalool	-	-	-	1.5	6.5
Pulegone	-	1.1	0.9	-	-
α-Terpinene	-	-	-	31.5	-
α-Terpineol	-	10.9	-	-	3.1
Carvone	-	2.0	-	-	-
Menthofuran	5.1	6.6	6.7	-	0.8
Piperotine	-	-	-	5.6	-
trans-Carveol	-	1.1	-	-	-
β-Elemene	14.1	-	11.8	-	-
Caryophyllene	-	-	-	-	18
Iso-caryophyllene	-	-	-	6.6	-
Germacrene A	3.9	-	-	-	-
Germacrene D	-	1.7	-	-	20.7

Conclusion

The repellent and fumigant activity of *B. graveolens* essential oil against *T. castaneum* (stored-product insect) was tested in this work. EO and limonene were more active than commercial repellent (Ethylbutylacetylaminopropionate). The results demonstrate that *B. graveolens* EO and limonene have the potential for the development of natural fumigants and repellents.

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