Antifungal, cytotoxic and chemical analyses of essential oils of *Lippia origanoides H.B.K* grown in Colombia

Actividad antifúngica, citotóxica y composición química de aceites esenciales de *Lippia origanoides H.B.K* recolectadas en Colombia

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ABSTRACT

Introduction: *Aspergillus fumigatus* is most commonly associated to invasive aspergillosis. Strong antifungal activity against *A. fumigatus* of *L. origanoides* essential oil gives a new added value to this natural product from Boyacá-Colombia. **Aims**: The increase in fungal infections, the development of resistance and toxicity of wide-spectrum antifungals have led to a constant search for therapeutic alternatives. The chemical composition, antifungal and cytotoxic activity of nine essential oils obtained from *L. origanoides* were evaluated and the relationship between the antifungal activities of the oil and of its major components were explored. **Methods and Results:** Antifungal activity was determined following the protocols AFST-EUCAST for *Candida krusei* and *C. parapsilosis,* and CLSI-M38A for *Aspergillus fumigatus* and *A. flavus*. The GC-MS analysis identified three chemotypes: thymol, carvacrol and *p*-cymene/*trans*-beta-caryophyllene. The essential oil of the thymol chemotype was the most active in antifungal assays with MIC values of 157.5, 198.4, 125 and 31 µg ml-1 against *C. parapsilosis, C. krusei*, *A. flavus* and *A. fumigatus,* respectively. The major components carvacrol and thymol were not active against *A. fumigatus* at concentrations below 157.5 µg ml-1. In general, the oils were not cytotoxic. **Conclusions:** The essential oil of the thymol chemotype of *L. origanoides* from the region of Boyacá-Colombia showed the highest antifungal activity against *A. fumigatus* among all the oils and major components tested. *Salud UIS* **2011; 43 (2): 141-148**

Keywords: *Lippia origanoides,* essential oil, antifungal activity, cytotoxicity, monoterpenes

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RESUMEN

Introducción: La infección por el hongo *Aspergillus fumigatus* está más comúnmente asociada a la aspergilosis invasiva. La fuerte actividad antimicótica del aceite esencial de *L. origanoides* contra *A. fumigatus* ha dado un nuevo valor agregado a este producto natural de Boyacá-Colombia. **Objetivos**: El aumento de las infecciones por hongos, el desarrollo de la resistencia y la toxicidad de los antifúngicos de amplio espectro han llevado a una constante búsqueda de alternativas terapéuticas. En este estudio fueron evaluados la composición química, la actividad antifúngica y citotóxica de nueve aceites esenciales obtenidos de *L. origanoides*; y la relación entre la actividad antifúngica de los aceites con respecto a la presencia de sus principales componentes. **Métodos y Resultados**: La actividad antifúngica se determinó siguiendo los protocolos AFST-EUCAST para *Candida krusei* y C. *parapsilosis*; y CLSI M38A para *Aspergillus fumigatus* y *A. flavus*. El análisis por GC-MS identificó tres quimiotipos: carvacrol timol y *p*-cymene/trans-beta-caryophyllene. El aceite esencial del quimiotipo timol fue el más activo en los ensayos antifúngicos con valores de MIC de 157,5, 198,4, 125 y 31 mg ml-1 frente a *C. parapsilosis, C. krusei, A. flavus* y *A. fumigatus*, respectivamente. El carvacrol y el timol, los principales componentes, no fueron activos frente a *A. fumigatus* en concentraciones inferiores a 157,5 g / ml-1. En general, los aceites no fueron citotóxicos. **Conclusiones:** El aceite esencial de *L origanoides*, quimiotipo timol, de la región de Boyacá-Colombia presentó la mayor actividad antifúngica frente a *A. fumigatus* entre todos los aceites evaluados; igualmente, sus principales componentes fueron los más activos en comparación a los otros quimiotipos. *Salud UIS* **2011; 43 (2): 141-148**

Palabras clave: *Lippia origanoides*, aceite esencial, actividad antifúngica, citotoxicidad, monoterpenos

INTRODUCTION

The AIDS epidemic, chemotherapy in cancer patients, neutropenia and immunosuppressant therapies in recipients with transplants have increased the number of cases of mycotic infections[1,2]. Species of the genera *Candida, Cryptococcus, Aspergillus, Histoplasma*, *Rhizopus, Mucor*, *Acremonium,* and *Fusarium* are the most frequent causes of these infections[1]. Although *C. albicans* is the most frequent species, the prevalence of other non-*albican*s species has been increasing recently[3]. *Aspergillus* infections have also grown in importance in the last years[4,1]. *A. fumigatus* and *A. flavus* are the most widespread causes of invasive aspergillosis[4].

The development of resistance of some species to the antifungal drugs available and the toxicity of wide-spectrum antifungals, such as amphotericin B, have led to a constant search for therapeutic alternatives[2]. Many aromatic plants are frequently used in traditional medicine as antimicrobial agents, and the essential oils recovered from some of them have demonstrated antibacterial and antifungal effects[5,6]. In addition, some oils have shown activity against fluconazole and itraconazole resistant *Candida* spp.[5]

Lippia origanoides H.B.K. (*Verbenaceae*) is a shrub up to 3 m tall that grows wild in Central America (Mexico, Guatemala, Cuba) and northern South America (Guiana, Venezuela, Brazil, Colombia)[7,8]. In Colombia, it is found at altitudes between 500 and 800 m in several Andean states and in the northern peninsula of Guajira. Its green oval leaves are employed as a condiment (due to their thymol and carvacrol content) and as a traditional medicine (flower and leaf infusions)[9,8]. In Mexico, *L. origanoides* is called "oregano", and the Mexican Pharmacopoeia recognizes it as a substitute for common oregano – *L. graveolens* Kunth[8]. Essential oils with high thymol content have showed MIC values of 3.3 mg ml-1 against *C. albicans*[10]. Oliveira *et al.*[8] studied the antifungal activity of the essential oil of *L. origanoides* which presented high carvacrol content and showed active against human pathogenic fungi of genera *Candida.*

The composition and biological activity of essential oils from *L. origanoides* H.B.K. grown in Colombia have not been studied. In the present work, the chemical composition, antifungal and cytotoxic activity of nine essential oils obtained from *L. origanoides*, collected from four Colombian regions were evaluated and the relationship between the antifungal activities of the oil and of its major components were explored.

MATERIALS AND METHODS

Plant material and essential oil extraction

Nine samples (1 kg each) of *L. origanoides* stems and leaves were collected from four regions in Colombia, as part of a survey conducted by CENIVAM, a Research Center devoted to the study of aromatic plants and

essential oils in Colombia. The taxonomic identification of the botanical samples was performed by Dr. José Luís Fernández at "Herbario Nacional de Colombia (COL)", Institute of Natural Sciences, School of Sciences, "Universidad Nacional de Colombia" (Bogotá), where exsiccate of each plant remain as permanent samples. The essential oils were extracted from dried *L. origanoides* stems and leaves (300 g) by microwave-assisted hydrodistillation as described previously[11]. Anhydrous sodium sulfate (Merck, Darmstadt, Germany) was added as a drying agent to the decanted essential oil. The voucher numbers, the region of plant collection, date of collection and the codes assigned to the essential oils obtained are presented in **(Table 1)**.

Analysis of essential oils -Compound identification was based on mass spectra (EI, 70 eV), obtained with a gas chromatograph (Agilent Technologies 6890 Plus, Palo Alto, CA, USA), equipped with a mass selective detector (Agilent Technologies 5973), a split/splitless injector (split ratio 1:50), and a data system (HP ChemStation 1.05) with WILEY 138K, NIST 2002 and QUADLIB 2004 mass spectra libraries, as described previously[12]. Individual components were identified by comparing their retention indices[11] determined using a linear scale on the DB-5MS (60m x 0.25mm x 0.25µL, J and W Scientific, Folsom, CA, USA) column, and the mass spectra of each GC component to those of standard substances (Sigma-Aldrich, USA; Table 2). The hydrodistillation process ensured endotoxin-free material, since such a technique is unable to remove high-molecular mass molecules such as endotoxins (10 kDa) from plant material, and the molecular weight of essential oil components does not surpass 0.3 kDa. Stock solutions of oils were prepared in DMSO and frozen at -70ºC until required.

Table 1. Geographic origin and voucher numbers of the Lippia origanoides plants used for essential oil extraction.

Essential oil / code	Voucher number	Collection region	Date of collection	Chemotype
1A	512271	Santander, Jordán Sube	07/05/2005	Carvacrol
2B	512270	Santander, Jordán Sube	07/05/2005	*p*-Cymene/ *trans*-beta-Caryophyllene
3C	520285	Nariño, Pedregal	27/08/2006	Thymol
4D	516294	Santander, Los Santos	08/07/2006	*p*-Cymene/ *trans*-beta-Caryophyllene
5E	516290	Santander, Piedecuesta	09/07/2006	Carvacrol
6F[a]	517741	Boyacá, Soatá	03/10/2006	Thymol
7G	517741	Boyacá, Soatá	03/10/2006	Thymol
8H	512087	Cauca, Mercaderes	10/08/2005	Thymol
9I	512075	Santander, Bucaramanga	15/02/2006	Carvacrol

[a] The extraction time used in this oil was 15 min.

Monoterpenes and drug

Thymol, carvacrol, *p*-cymene, *trans*-beta-caryophyllene, gamma-terpinene and beta-myrcene and other standard substances for the analysis of essential oils were from Sigma (Chemical Company St Louis, MO, USA). Stock solutions of both oils and monoterpenes were prepared in DMSO for cytotoxicity and antifungal assays. Amphotericine B and itraconazole were from Sigma.

Antifungal activity assay

The antifungal activity of the oils and monoterpenes was evaluated following the Clinical and Laboratory Standards Institute M38-A protocol[13] for filamentous fungi, and the standard method proposed by the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing (EUCAST)[14] for fermentative yeasts, as described previously[12]. *Candida parapsilosis* (ATCC 22019), *C. krusei* (ATCC 6258), *Aspergillus flavus* (ATCC 204304) and *A. fumigatus* (ATCC 204305) served to evaluate antifungal activity. For the EUCAST method, MIC were determined after 24 hours of incubation, and defined as the lowest concentration that resulted in a 90% reduction of growth. For the CLSI M38-A method, the MICs were determined after 48 hours of incubation, and defined as the lowest dilution of essential oils that resulted in total inhibition of visible growth. Susceptibility testing was performed in duplicate in three different assays. Essential oils and monoterpenes were considered active when they presented MIC values below 500 µg ml-1.

Cytotoxicity assay

Cercopithecus aethiops African green monkey kidney cells (Vero cell line ATCC CCL-81) were used. The cytotoxicity of the essential oils and their major components were examined *in vitro* with an MTT (dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Sigma, New Jersey, USA) assay as described in Mesa-Arango *et al*.[12]. The concentration of compounds that induces 50% growth inhibition in 48h was expressed as IC_{50}. Vincristine was used as positive control (Sigma-Aldrich, Co, MO, USA).

Data analysis - The IC_{50} values for each compound were obtained by lineal regression analysis of the dose–response curves generated from the absorbance data with the statistical package R (Development Core Team, Vienna, Austria, 2008). IC_{50} values are expressed as the Mean ± S.E.M. of at least four dilutions performed in quadruplicates in two different assays. MIC values are expressed as geometric means (GM-MIC) of tests performed in duplicate in three different assays against each of the fungal species.

To test the association of oil activity with chemical composition of the major components, the Cox model with the determination of the hazard ratio was applied; a 95% confidence interval was adopted. Statistical analyses were conducted using the statistical package R (Development Core Team, Vienna, Austria, 2008).

RESULTS

The present work evaluated the chemical composition of nine essential oils obtained from *L. origanoides* plants, collected from four Colombian regions **(Table 1)**. The various components were identified by comparing their retention indices (RI)[11], and the mass spectrum of each GC component to those of standard substances. Table 2 lists the major components (>1%) found during the chromatographic analysis of essential oils. Kovàts retention indices (I_k), used as complementary identification criteria, were obtained at polar (DB-WAX) and non-polar (DB-5) stationary phases. The chromatographic analysis of the essential oils evaluated here detected ca. 50 compounds (data not shown). GC/MS analyses permitted to positively identify 40 oil constituents. The GC/MS analyses showed that the major constituents of the oils were monoterpene hydrocarbons and phenolic monoterpenes, but their concentration in the oils varied greatly. Quantitative oil differences were clear for seven compounds: *trans*-beta-caryophyllene, alpha-humulene, the two isomeric phenols, carvacrol and thymol, and their precursor gamma-terpinene and *p*-cymene, and beta-myrcene. GC/MS analysis of the oils revealed the presence of three chemotypes: thymol, carvacrol and *p*-cymene/*trans*-beta-caryophyllene **(Tables 1 and 2)**. Among the thymol-chemotype oils, the 6F oil obtained from plants collected in the Department of Boyacá showed the highest thymol concentration. The major components found in 6F oil were thymol (59.7%), followed by carvacrol (12.2%), *p*-cymene (8.8%), and gamma-terpinene (4.5%). In the carvacrol-chemotype oils, the 5E oil obtained from plants collected in the Department of Santander showed the highest carvacrol concentration. The major components found in 5E oil were carvacrol (46.2%), *p*-cymene (12.0%), thymol (9.5%), and gamma-terpinene (9.5%). The 9I oil showed carvacrol and thymol contents of 38.8% and 15.1%, respectively. In the *p*-cimene/*trans*-beta-caryophyllene chemotype oils, *p*-cymene was the main constituent (~13.5%), followed by *trans*-beta-caryophyllene (~10.4%), alpha-phellandrene (~9.3%), 1,8-cineol (~6.6%) and alpha-humulene (~5.9 %).

The results of the oils and the cytotoxic and antifungal activities of some of their major components are presented in **(Table 3)**. According to the significance threshold determined by the National Cancer Institute (USA) for crude extracts (inhibitory concentration 50 IC_{50} <30 µg ml-1)[5], the oils and monoterpenes evaluated were not cytotoxic on the Vero non-tumoural cell line. A dose-dependent inhibition on the growth of Vero cells, with R^2 determination coefficients of linear regression greater than 7.5, was observed for monoterpenes and oils **(Table 3)**. The results of the antifungal activity assays of *L. origanoides* oils against *Candida* and *Aspergillus* species are presented in **(Table 3)**. The MIC values for the reference antifungal drugs, amphotericine B and itraconazole used as positive controls were within the values established using the AFST-EUCAST and CLSI-M38-A protocols **(Table 3)**.

As can be observed in **(Table 3)**, only the 4D oil did not show activity for the fungi evaluated. The 3C oil showed the highest antifungal activity against *C. parapsilosis, C. krusei* and *A. flavus* but not against *A. fumigatus* with MG-MIC values of 157, 198, 125, 63 µg ml-1, respectively. The 7G oil showed the highest antifungal activity against *A. fumigatus,* with MG-MIC value of 31 µg ml-1. In general, *A. fumigatus* was more sensitive to essential oils than *A. flavus* and *Candida* species. The monoterpenoids, *trans*-beta-caryophyllene, gamma-terpinene, and beta-myrcene did not show activity at concentrations of 500 µg ml-1 for the fungi evaluated. Among the six compounds tested, carvacrol and thymol were the most active against the fungi evaluated.

To examine the correlation between oil activity and

the major components in situ, percentage composition of the major components of the oils were screened with oil antifungal activity in a Cox model. **(Table 4)** shows the results of these analyses that were adjusted for each fungus. Thymol and carvacrol were the major components that were associated with oil antifungal activity for *Candida* species. For *A. fumigatus* and *A. flavus,* thymol/beta-myrcene and thymol/carvacrol/*p*-cymene were the monoterpenoids associated with oil antifungal activity, respectively.

Table 2. Relative amounts and identification of the major secondary volatile metabolites isolated from L. origanoides grown in Colombia.

Compound	Ik		Relative amount percentages[a]								
	DB-5[b]	DB-WAX[c]	1A	2B	3C	4D	5E	6F	7G	8H	9I
beta-Myrcene[d]	990	1164	3.2	-	2.8	-	2.5	2.2	3.0	4.5	3.0
alpha-terpinene[d]	1021	1178	3.7	-	1.6	-	2.7	1.2	1.7	2.3	3.4
ρ-Cymene[d]	1031	1270	13.9	15.7	10.0	11.2	12.0	8.8	12.1	11.5	11.5
1,8-Cineol[d]	1035	1205	1.7	6.7	-	6.5	-	-	-	-	-
gamma-Terpinene[d]	1066	1224	13.2	-	5.0	-	9.5	4.5	6.2	6.3	12.6
Thymol[d]	1297	2181	9.2	-	54.5	-	9.9	59.7	43.8	53.6	15.1
Carvacrol[d]	1316	2212	36.5	-	1.7	-	46.2	12.2	17.3	-	38.8
alpha-Pinene[d]	934	1036	-	3.1	-	2.3	-	-	-	-	-
alpha-Phellandrene[d]	1011	1146	-	8.7	-	9.9	-	-	-	-	-
Limonene[d]	1034	1197	-	6.9	-	7.2	-	-	-	-	-
trans-beta-Caryophyllene[d]	1436	1610	-	9.4	2.4	11.3	2.0	1.8	2.2	3.2	2.6
alpha-Humulene	1471	1683	-	5.7	1.5	6.0	1.2	1.0	1.3	1.9	1.5
Caryophyllene oxide[d]	1602	2010	-	3.7	-	2.2	-	-	-	-	-
alpha-Eudesmol	1673	2240	-	3.2	-	-	-	-	-	-	-
Thymyl acetate[d]	1350	1866	-	-	4.8	-	-	-	-	2.2	-
Borneol[d]	1181	1710	-	-	-	3.1	-	-	-	-	-

[a]Relative amounts (percentages) were calculated from relative GC peak areas, obtained by GC/FID. [b]Retention indices calculated for the DB-5MS column. [c]Retention indices calculated for the DB-WAX column. [d]A standard compound was available for identification purposes.

Table 3. Cytotoxic and Minimal Inhibitory Concentration of essential oils isolated from L. origanoides chemotypes and some of their constituents.

Samples	Vero Cells		MIC[a] (µg ml-1)							
			C. parapsilosis		*C. krusei*		*A. flavus*		*A. fumigatus*	
	CC_{50}[b]	R^2[c]	GM[d]	Range	GM	Range	GM	Range	GM	Range
3C	118.6 ± 15.4	0.86	157.5	125.0 – 250.0	198.4	125.0 - 250.0	125.0	125.0 - 125.0	63.0	63.00 - 63.00
7G	31.4 ± 5.6	0.75	250.0	250.0 - 250.0	250.0	250.0 - 250.0	198.4	125.0 - 250.0	31.0	31.00 - 31.00
9I	74.6 ± 16.9	0.75	250.0	250.0 - 250.0	250.0	250.0 - 250.0	198.4	125.0 - 250.0	63.0	63.00 - 63.00
1A	52.3 ± 11.5	0.8	253.8	142.0 - 285.0	253.8	142.0 - 285.0	225.9	142.0 - 285.0	112.7	71.00 - 142.0
5E	29.3 ± 4.8	0.79	250.0	250.0 - 250.0	250.0	250.0 - 250.0	250.0	250.0 - 250.0	125.0	125.0 - 125.0
8H	104.4 ± 5.9	0.98	250.0	250.0 - 250.0	500.0	500.0 - 500.0	125.0	125.0 - 125.0	63.0	63.00 - 63.00
6F	34.3 ± 6.5	0.71	250.0	250.0 - 250.0	250.0	250.0 - 250.0	250.0	250.0 - 250.0	250.0	250.0 - 250.0
2B	NC[e]		239.4	142.0 - 285.0	478.7	285.0 - 569.0	>500.0	---	142.0	142.0 - 142.0

gamma-Terpinene	47 ± 6	0.98	>500	---	>500	---	>500	---	>500	---
p-Cymene	≥200		>500	---	500	500.0 -500.0	>500	---	>500	---
beta-Myrcene	≥200		>500	---	>500	---	>500	---	>500	---
trans-beta-Caryophyllene	39.7 ± 6.9	0.75	>500	---	>500	---	>500	---	>500	---
Carvacrol	105.5 ± 7.3	0.97	250.0	250.0 - 250.0	500.0	500.0 - 500.0	500.0	500.0 - 500.0	250.0	250.0 - 250.0
Thymol	126.6 ± 16.5	0.86	250.0	250.0 - 250.0	445.4	250.0 - 500.0	250.0	250.0 - 250.0	157.5	125.0 - 250.0
Itraconazole	NC			0.06 - 0.12		0.06 - 0.25	0.25	0.25 - 0.25		0.06 - 0.25
Amphotericin B	38.9 ± 4.3	0.9	1	1 - 1	0.5	0.5 - 0.5	1	1 - 1	2	2 - 2
Vincristine	1.1 ± 0.2	0.9								

[a]MIC: minimal inhibitory concentration; [b]$CC_{50:}$ minimal dilution of the compound that induces 50% killing of the cells; [c]R^2 : coefficient of determination of linear regression; [d]GM: geometric mean; [e]NC: not calculated

Table 4. Correlation of oil activity with the percentage of their major components in situ. Cox model analysis with determination of the hazard ratio adjusted for each fungus.

		Coefficient	Hazard[a]	P value
C. parapsilosis				
	Thymol	0.03	1.03	0.001
	Carvacrol	0.02	1.02	0.037
C. krusei				
	Thymol	0.05	1.05	0.000
	Carvacrol	0.07	1.07	0.000
A. flavus				
	Thymol	0.02	1.02	0.018
	beta-Myrcene	0.80	2.22	0.000
A. fumigatus				
	Thymol	0.08	1.08	0.000
	Carvacrol	0.04	1.04	0.002
	p-Cymene	0.71	2.03	0.000

[a]Hazard ratio

DISCUSSION

Lippia origanoides H.B.K. Fam. (*Verbenaceae*) is a slender, very aromatic shrub[7]. Oliveira *et al*.[8] reported the composition of *L. origanoides* essential oil from Oriximiná - Brazil, in which carvacrol was the main constituent (38%), followed by thymol (18%) and *p*-cymene (10%). Rojas *et al*.[15] compared the compositions of *L. origanoides* oils from leaves collected during the rainy and dry seasons in Venezuela; thymol (61.9 and 44.7%) and carvacrol (7.9 and 16.8%) were the main components in these oils, respectively. Oliveira *et al*.[8] suggests the existence of two different chemotypes for this species – thymol and carvacrol.

In the present study, the chemical composition of nine essential oils obtained of *L. origanoides* collected from four Colombian regions were evaluated. The GC/MS analysis of the oils revealed the presence of three chemotypes: thymol, carvacrol, and *p*-cymene/*trans*-beta-caryophyllene. The *p*-cymene/*trans*-beta-caryophyllene chemotype was reported previously[16], and it was observed that the composition of the oil was not caused by microclimate or geobotanical differences. The findings suggest that there are different chemotypes for this species, in a way similar to what happens with *Lippia alba*[5]. In spite of the existence of several *L.*

origanoides chemotypes in Colombia, there had been no evaluation of the antifungal and cytotoxic activity of their essential oils.

With respect to antifungal activity there is no agreement on the acceptance level of activity for plant material when compared to standard drugs[12]. Duarte *et al.*[17] indicated a strong activity of *L. alba* essential oils against *C. albicans* with a MIC value of 60 µg ml-1, when nistatin was used as positive control (MIC of 50 µg ml-1). According to these criteria the 7G oil was very active against *A. fumigatus* with a MIC value of 31 µg ml-1. The 3C oil showed a strong antifungal activity against the four fungi evaluated. Both oils were thymol chemotypes. In contrast the oils 2B and 4D, *p*-cymene-/*trans*-beta-caryophyllene chemotype, showed moderate and weak activity, respectively.

Studies of antifungal activity of essential oils with high thymol and carvacrol have been carried out on *Candida* species. Botelho *et al.*[18] evaluated the essential oil activity from *L. sidoides* of the thymol (56.7%)/carvacrol (16.7%) chemotype against *C. albicans.* The resulting value of the MIC was about a thousand times higher than the ketoconazole value. Oliveira *et al.*[8] showed the activity of the thymol (18.5%)/carvacrol (38.6%) chemotype of *L. origanoides* essential oil against *Candida* species. In contrast, in the present study *A. fumigatus* was more sensitive to essential oils of *L. origanoides* than *Candida* species. The most active oil with content of thymol (54.5%) and carvacrol (1.7%) showed a MIC value against *A. fumigatus* about ten times higher than the Amphotericin B value. Interestingly, the 6F oil obtained by extraction for 15 min and with the highest thymol concentration was less active than 7G oil obtained of the same plant by extraction for 30 min. This finding shows the importance of the percentage composition of components of oils.

To determine the contribution of each major component to *L. origanoides* oil activity, the antifungal activity of the thymol, carvacrol, *p*-cymene, *trans*-beta-caryophyllene, gamma-terpinene and beta-myrcene were evaluated. The lack of activity of *p*-cymene and gamma-terpinene is in agreement with previous studies[19]. However, Tampieri *et al.*[20] reported that monoterpenes as gamma-terpinene, *p*-cymene and carvacrol displayed good antifungal activity at 100 µg ml-1 against *C. albicans*. In our study, only thymol and carvacrol were active against the fungi evaluated, however, their MIC values were higher than those found for the 3C and 7G oils.

It is difficult to attribute the activity of a complex mixture as it is an essential oil to some particular constituent. Therefore, to confirm the correlation of oil activity with the percentage of their major components in situ, in a Cox model, percentage composition of the major components of the oil were screened to evaluate a possible relationship with oil antifungal activity. Thymol and carvacrol were the major components that were more closely associated with oil antifungal activity for *Candida* species. However, for species of *Aspergillus fumigatus* these analyses suggest that there is a concentration-dependent effect among the monoterpenes thymol, carvacrol, and *p*-cymene.

A. fumigatus is the most prevalent species in the genus and it is most commonly associated to invasive aspergillosis[21]. In our study, essential oils from Boyacá-Colombia *L. origanoides* have shown a marked antifungal activity against *A. fumigatus* and their activity could give a new added value to these natural products. Essential oil from Boyacá-Colombia *L. origanoides* is promising candidate to evaluate their gaseous contact activity against filamentous fungi and could be employed to sterilize hospital rooms of immunosuppressant patients.

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ETHICAL CONSIDERATIONS

Ethical compliance is not applicable to this study.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. Liliana Amparo Betancur Galvis certify that: The manuscript represents valid work and that neither this manuscript nor one with substantially similar content has been published under my responsibility or is being considered for publication elsewhere. There are no financial interests in relation to this manuscript. All material and financial support for this work is clearly expressed in the manuscript.

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