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Antifungal activity of *Verbenaceae* and *Labiatae* families essential oils

V. Tangarife-Castaño[1,3], V. Roa-Linares[1,3], L.A. Betancur-Galvis[1], D.C. Durán García[2,3], E. Stashenko[2,3], A.C. Mesa-Arango[1*]

[1] Grupo de Investigación Dermatológica. Facultad de Medicina. Departamento de Medicina Interna. Universidad de Antioquia, Medellín, Colombia.
[2]Centro de Investigación de Biomoléculas, CIBIMOL/ Universidad Industrial de Santander. Bucaramanga, Colombia.
[3]Centro Nacional de Investigaciones para la Agroindustralización de Especies Vegetales Aromáticas y Medicinales Tropicales denominada Unión Temporal "CENIVAM" / Universidad Industrial de Santander. Bucaramanga, Colombia.

*Prof. Ana Cecilia Mesa-Arango, amesa@medicina.udea.edu.co - phone: +57 4 2196064 - fax: +57 4 2196066

Abstract

Plant derivates as essential oils and extracts have shown important antifungal activity. *Fusarium oxysporum* is an important emergent fungus causing opportunistic infections as fungemia with high mortality rates. Also is known as onychomycosis agent. Currently, there are limited options for treatment of this fungus due to its relative resistance to most antifungal agents. In addition, *Trichophyton rubrum* and *T. mentagrophytes* are the most common etiologic agents of superficial fungal infection known as dermatophytosis that affects skin, hair and nails. These mycoses, although normally not are lethal, represent a cosmetic problem unpleasant with difficult to be cured, causing considerable financial losses. In this study, the antifungal activity of seventeen essential oils and three extracts belonging to the families *Verbenaceae* and *Labiatae*, was evaluate against this fungus according to the Clinical and Laboratory Standards Institute (CLSI-M38A). The MIC (Minimal Inhibitory Concentrations) was defined as the lowest essential oil and extract dilution that resulted in 80% of inhibition of visible growth after 48 h of incubation for *F. oxysporum* and after six days for dermatophytes. Also, the cytotoxicity assay for 18 samples was carried out using tetrazolium-dye MTT technique. IC_{50} and selective index values were calculated. The compositions and compounds quantification chemical of the most potent antifungal oils were determinate using GC-MS and GC-FID analysis, respectively. The results showed strong activity among 70% at 80% of samples evaluated against dermatophytes and from 20% against *F. oxysporum*. The lowest MIC values were obtained with citral chemotype *Lippia alba* oil (BC2) at concentrations of 31.25 and 125 µg/mL on *T. rubrum* and *T. mentagrophytes,* respectively, but not against *F. oxysporum*. Moreover, the oil from *Minthostachys mollis* (Kunth) Griseb (MEO2) showed strong activity against all fungi evaluated. Active samples against dermatophytes and *F. oxysporum* were not cytotoxic on Vero cells ATCC CCL-81; excluding *Lippia origanoides* Kunth (5E), carvone chemotype *Lippia alba* (TS) and *Mintostachys mollis* oil (MEO2). The essential oils with the highest selectivity index (SI) values were *Aloysia triphylla* (AEO1) and *L. alba* oil (BC2) on dermatophytes. The main component of most active *L. alba* oils was characterized by carvone (TS, CC1) and citral (BC2). To *L. origanoides* oils was found carvacrol (1A, 5E) and thymol (6F) as main component. Pulegone and *cis*-piperitone epoxide were the main constituents of *Minthostachys mollis* MEO1 and MEO2 oils, respectively. The presence of these main components in essential oils may be the responsible of the antifungal activity. These findings is very important because confirm the potential of essential oils as a source of new anti-dermatophytes.

Keywords: Essential oils, extracts, *Verbenaceae, Labiatae*, dermatophytes, *Fusarium oxysporum,* antifungal activity, cytotoxicity

Introduction

In folk medicine, medicinal plants are used in treating a wide spectrum of infectious disease. Many researchers have contributed in the search of new antifungal compounds from natural sources based on ethnobotanical approach, which were effective and nontoxic (1). Colombia is the second richest country in the world in biodiversity, and its floral diversity is estimated at 40,000 species of vascular plants. Out of these 5000 plants are used with medicinal purpose. This information makes our country a potential source of active compounds, as therapeutic options for infectious diseases (2).

Furthermore, there has been an increase in fungal infections. Currently, the mycotic agents constitute an important cause of cutaneous, mucosal and systemic infections, especially in immunosuppressed people (3). Among fungi of medical importance in humans, the most common are those that colonize the skin, causing infections known as dermatomycosis, that include dermatophytes infections (tineas or dermatophytosis) and dermatomycosis by environmental fungi (4).

Tineas are caused by a group of keratinophilic fungi known as dermatophytes; these fungi affect primarily skin, nails and hair. *Trichophyton rubrum* and *T. mentagrophytes* are the commonest etiological agents of dermatophytosis (5). These mycoses are distributed worldwide and have an easy transmission among infected people and animals or fomites (6). Although normally are not lethals, represent a cosmetic problem unpleasant and difficult to cure, that cause considerable financial losses, even in certain cases, cause complications as cellulitis and commitment of the limbs in patients with diabetes or peripheral vascular disease (7). Moreover, *Fusarium* spp. is an important environmental fungus that emerged as agent causative of opportunistic infections. *F. oxysporum* is the most frequently isolated non dermatophyte filamentous fungi causing onychomycosis (8). It is associated with systemic diseases, with high mortality rates and strong resistance to most antifungal agents (9).

The election treatment for dermatophytes infections with anti-mycotic agents as terbinafine has showed a failure rate of 20–30% (7, 10). Moreover, anti-mycotics as amphotericin B, nystatin and voriconazol have been used against fusariosis with relative successful (11). Besides, the resistance and toxicity to drugs and insufficient bioavailability (12) have encouraged the search for new alternatives among natural products. Previous reports have suggested that several essential oils and extracts, show important antifungal activity (13). The aim of this study was to evaluate the *in vitro* activity against the dermatophytes *Trichophyton rubrum* and *T. mentagrophytes* and the filamentous fungus *Fusarium oxysporum*, as well as the cytotoxic effect of essential oils and extracts of Colombian plants, belonging to the *Verbenaceae* and *Labiatae* families.

Material and Methods

Plant Materials and extracts and essential oils extraction

Stems and leaves of 20 plants of *Verbenaceae* and *Labiatae* families were collected in different regions of Colombia (Table 1), as part of a survey conducted by CENIVAM, a Research Centre devoted to the study of aromatic plants and essential oils in Colombia. The taxonomic identification of the botanical samples was performed by Dr. Jose Luis Fernandez at the "National Herbarium from Colombia (COL)", Institute of Natural Sciences, Faculty of Sciences, "Universidad Nacional de Colombia" (Bogotá); where exsiccata of each plant remain as permanent samples. The voucher numbers and the codes assigned to the oils obtained are presented in Table 1. Essential oils (17) and extracts (3) were extracted. The essential oils were isolated from dried stems and leaves (300 g) by microwave-assisted hydrodistillation as described Rodriguez-Quintanilla et al (2012) (14). The extracts were obtained from 40 g of dried leaves of each plant, macerated with 200 mL ethanol and left in suspension for 7 days at ambient temperature (28 °C). The mixture was filtered and concentrated using a Buchi rotavapor. Stock solutions of both, oils and extracts, were prepared in DMSO (≤ 1% v/v)

and were conserve at -70 °C until subsequent bioassays [15].

Essential oil composition analysis

Compound identification was determinate by GC – MS and GC–FID as described Rodriguez-Quintanilla et al. (2012) (14). To GC–MS, the essential oils was analyzed 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 MSD 5973), split/splitless injector (250 °C, 1:30 split ratio), and a data system (HP ChemStation 1.05), with WILEY 138K, NIST 2002 and QUADLIB 2004 mass spectra libraries. A DB-5MS fused-silica capillary column (J&W Scientific, Folsom, CA, USA) of 60 m (L) x 0.25 mm x 0.25 µm (df) was employed. The GC oven temperature was programmed to go from 45°C (5 min) at 4°C/ min to 150°C (2 min) at 5°C/min to 250°C (5 min) and finally 10°C/min to 275°C (15 min). Mass spectra and reconstructed ion currents (chromatograms) were obtained by automatic scanning at 5.19 scan/s within the mass range m/z 30–300. Chromatographic peaks were checked for their homogeneity with the aid of the mass chromatograms for the characteristic fragment ions.

To GC – FID, a gas chromatograph (HP 5890 A Series II), equipped with flame ionisation detection (FID), split/splitless injector (250°C, 1:30 split ratio), and a data system (HP ChemStation HP Rev. A.06.03 [509]) was used for GC/FID analysis of the oils and quantification of their components. The detector and injector temperatures were set at 250°C. The same capillary column, as for the GC/MS analysis, was used for GC/FID separation and detection. The oven temperature was programmed from 40°C (15 min) to 250°C (20 min) at 5°C/min. Helium was used as the carrier gas, with 152 kPa column head pressure and 35.7 cm/s linear velocity. Hydrogen and air at 30 and 300 mL/min, respectively, were utilized in the FID, with N_2 (30 mL/min) as a make-up gas. The various compounds were identified by comparison of their Kovàts retention indices (10), determined utilizing a linear scale on the DB-5MS (60 m) column, and of the mass spectra of each GC component with those of standard substances.

Antifungal susceptibility testing

The antifungal *in vitro* activity of *Labiatae* and *Verbenaceae* families samples, were evaluated following the Clinical and Laboratory Standards Institute M38-A protocol for filamentous fungi with modifications (17). The filamentous fungus *Fusarium oxysporum* (ATCC 48112) and the dermatophytes, *Trichophyton rubrum* (ATCC 28188) and *T. mentagrophytes* (ATCC 24198) were used to evaluate antifungal activity at inoculum size of 0.2 – 2.5 x 10^5 CFU/mL. The oils and extracts were evaluated at five concentrations of 31.25 - 500 µg/mL dispensed into 96-well flat-bottom microdilution plates. Oils and extracts were considered active when they exhibited MIC values ≤ 500 µg/mL. The MICs were defined as the lowest dilution that resulted in an 80% of inhibition of visible growth after incubation at 28 °C to 48 hours to *F. oxysporum* and six days to dermatophytes. Amphotericine B (Sigma-Aldrich, Co, MO, USA) was evaluated with the strains *Aspergillus fumigatus* ATCC 204305 and *A. flavus* ATCC 204304, and Terbinafine (Recalcine Laboratories, Santiago de Chile, Chile) was used as positive controls at a range of 0.031 - 16 µg/mL on both dermatophytes. A negative control (inoculum without treatment) was also included. MIC values were expressed as geometric mean (GM-MIC) of tests performed in duplicate in three different assays against each strain.

Cytotoxicity assay

Cercopithecus aethiops african green monkey kidney cells (Vero cell line ATCC CCL-81) were used. The cells were grown in Eagle's Minimum Essential Medium (MEM) supplemented and maintained at 37°C in humidified 5% CO_2 atmosphere. The cytotoxicity of the essential oils and their components was examined *in vitro* using an MTT (dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Sigma, New Jersey, USA) assay, as described Betancur-Galvis et

al (2002) (19). Vero cell monolayers were trypsinized and washed with culture medium and then plated at 1.25 X 10^4 cells per well in a 96-well flat-bottomed plate. After 24 h of incubation, each diluted essential oils and extract were added to the appropriate wells and the plates were incubated for further 48 h at 37 °C in a humidified incubator with 5% CO_2. Essential oils were dissolved initially in DMSO and they were further diluted in medium for cell culture experiments resulting in a finally concentration of 0.05% DMSO in biological assays. Cell controls with DMSO at 0.05% were used. The minimal dilution of the essential oil that induced 50% growth inhibition of the cells was expressed as Inhibitory Concentration 50% (IC_{50}). The IC_{50} values were obtained by linear 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 were expressed as the Mean ± Standard Deviation (M ± SD) of two independent experiments done in quadruplicate. The cytotoxicity to Vero cells and the activity against fungi were compared using the selectivity indices (SI = IC_{50} of Vero cells /MIC of fungal).

Results

The *in vitro* antifungal activity against *Fusarium oxysporum* and the dermatophytes *Trichophyton rubrum* and *T. mentagrophytes,* as well as cytotoxic activity on Vero cell line of essential oils and extracts derivates of plants of *Verbenaceae* and *Labiatae* families, were evaluated in this study. The MIC values of samples tested are showed in Table 2. Three of five samples of *Labiatae* family were active against dermatophytes (GM-MIC range 78.75–396.85 µg/mL). The two *Mintostachys mollis* essential oils evaluated, MEO1 and MEO2, showed anti-dermatophytes activity. The MEO2 oil showed the highest anti- dermatophytes activity with GM-MIC values of 99.21 and 78.75 µg/mL for *T. rubrum* and *T. mentagrophytes*, respectively (Table 2). About *Verbenaceae* family, fifteen samples were evaluated and fourteen shown activity against dermatophytes (GM-MIC range 31.25 – 500 µg/mL). The most active sample was *Lippia alba* oil (BC2) with GM-MIC values of 31.25 and 125 µg/mL for *T. rubrum* and *T. mentagrophytes*, respectively (Table 2).

For anti-*Fusarium* activity of *Labiatae* family samples only MEO2 oil shown activity against *F. oxysporum* with a GM-MIC value of 396.85 µg/mL. Three essential oils of *Verbenaceae* family belonging of *Lippia origanoides* specie (1A, 5E, 6F) showed activity (GM-MIC 500 µg/mL for three samples). The MIC values of positives control amphotericine B for *Aspergillus flavus* and *A. fumigatus*, were in the acceptable range (0.5 - 4 and 1 – 4 µg/mL, respectively), as well as terbinafine for both dermatophytes (<0.0062 µg/mL).

In addition, the Inhibitory Concentration 50% (IC_{50}) and selective indices (IC_{50} of Vero cells/MIC of each fungal) were calculated. According to American National Cancer Institute (USA) criteria (plant derivate extracts are cytotoxic when the IC_{50} value is < 30 µg/mL), all samples with anti - fungal activity evaluated were not cytotoxic on Vero cells, excluding TS (IC_{50} = 12.3 ± 2.9 µg/mL), 5E (IC_{50} = 29.3 ± 4.8 µg/mL) and MEO2 (IC_{50} ≤ 25 µg/Ml) oil (Table 3). The essential oils with the highest selectivity index (SI) values were *Aloysia triphylla* (AEO1) and *L. alba* oil (BC2). The AEO1 oil didn't show cytotoxicity on Vero cells up to the highest test concentration showing a selective indices ≥ 1.27 and ≥ 1.13 on *T. rubrum* and *T. mentagrophytes,* respectively. For *L. alba* oil (BC2) oil the highest selective index was found with *T. rubrum* (1.62) (Table 3).

The compositions and compounds quantification chemical of essential oils that showed a marked antifungal activity were determined by GC-MS and GC-FID analysis, respectively. The chromatographic analysis of the three essential oils of *Lippia alba* (BC2, TS, CC1), three *L. origanoides* (1A, 5E, 6F) and *Mintostachys mollis* oil (MEO2) that showed activity in this study have been previously report by us (17, 20-21). The constituents were identified by comparing their retention indices (RI) (22), and the mass spectrum of each GC component to those of standard substances. The GC analyses demonstrated the

presence of 40, 35 and 36 compounds by BC2, TS and CC1 oils, respectively, more of 50 compounds by 1A, 5E, 6F and 37 of MEO2 (data not shown). The list of major components of them is showed in Table 4. The compositions and quantification chemical of constituents of *Mintostachys mollis* essential oils MEO1 are reported for first time, in this study. Twenty components in MEO1 oil were detected respectively (Table 5).

The *L. alba* (Mill.) N.E. Brown essential oils studied were classified as citral and carvone chemotypes, which correspond to chemotypes I and III, respectively, according to the classification suggested by Hennebelle et al. (2008) [37].

In general, the chemical analysis of first four major components, among others of citral chemoype *Lippia alba* oil (BC2) identified were the geranial (28.9%), neral (21.5%) and β-caryophyllene (7.3%), and 6-methyl-5-hepten-2-one (4.1%). The major components of carvone chemotype *Lippia alba* (TS) and *Lippia alba* (CC1) oils were limonene (32.0% and 38.1%), carvone (26.2% and 26.6%) and bicyclosesquiphellandrene (16.4% and 12.2%), respectively (Table 4).

The *L. origanoides* Kunth oil (5E) showed the following major components: carvacrol (46.2%), *p*-cymene (12.0%),thymol (9.9%) and γ-terpinene (9.5%). The *L. origanoides* oil (1A) was characterizated by carvacrol (36.5%) *p*-cymene (13.9%), γ-terpinene (13.2%) and thymol (9.2%). The major components of *L. origanoides* oil (6F) were thymol (59.7%), carvacrol (12.2%), *p* - cymene (8.8%) and γ-terpinene (4.5%) (Table 4).

In addition, the monoterpenes cis-piperitone epoxide (29.9%), piperitenone oxide (25.6%), mentone (7.4%) and germacrene D (5.8%) were found as major components of *Minthostachys mollis* (Kunth) Griseb oil (MEO2) (Table 4). Meanwhile, pulegone (18.8%), trans- β - caryophyllene (17.9%), mentone (11.9%), biciclogermacrene (11.3%) and germacrene D (10.6%) were the main constituents of *Minthostachys mollis* essential oil (MEO1) (Table 5).

Discussion

Plants are usually used in traditional medicine as antimicrobial agents, and their essential oils and extracts, have been known to possess antibacterial and antifungal proprieties. The essential oils have been considered useful as a topical antifungal agents because to the lipophilicity of their components, and for their good distribution in the stratum corneum of the skin (22). *Verbenaceae* and *Labiatae* families constitute an important group of trees, shrubs and herbs, widely distributed worldwide and as a substantial part of Colombian flora (23). In this study, twenty oils and extracts from plants of these families were tested against two dermatophytes and *F. oxysporum*. Aligiannis et al (2001) (24) suggested that plant derivates with antifungal activity are strong inhibitors when have a MIC value up to 0.5 mg/mL. According to these criteria, we found strong anti-dermatophytes activity in 70-80 % of the samples evaluated and 20% anti – *F. oxysporum* activity. The most actives species were Minthostachys mollis, Aloysia triphiylla and Lippia spp.

Previous studies carried out in our laboratory, have shown the antifungal activity on *Candida* and *Aspergillus* species of extracts and oils of plants belonging to *Verbenaceae* and *Labiatae* families, at GM-MIC range of 7.82 to 500 µg/mL for *C. krusei*, 157.5 to 500 µg/mL on *C. albicans,* 280 to 500 µg/mL to *C. parapsilosis,* 180 to 500 µg/mL to *A. flavus* and 125 to 500 µg/mL against *A. fumigatus* (16, 19-20, 15-26). In addition, other studies with oils and extracts of the *Verbenaceae* and *Labiatae* families have showed activity *in vitro* against dermatophytes as *Microsporum canis*, *M. gypseum*, *Trichophyton mentagrophytes, T. rubrum, T. tonsurans, T. verrucosum and Epidermophyton floccosum* (27-30).

The specie *Minthostachys mollis* (*Labiatae*) is distributed in several countries in South America including Colombia. In folk medicine, has been used for anti-mycotic and antiparasitic purposes, among other applications (31). Zapata et al. (2009) (19), previously shown moderated activity of *Mintostachys mollis* oil against *C. krusei, C. parapsilo-*

sis, A. flavus and *A. fumigates* with MIC values of 250, 375, 314.9 and 314.9 µg/mL, respectively. In present study, strong activity of MEO2 oil against dermatophytes *T. rubrum* and *T. mentagrophytes* and *F. oxysporum* were found (GM-MIC activity 99.21, 78.75 and 396.85 µg/mL, respectively). This activity may be attributed to presence of major components cis-piperitone epoxide and piperitenone oxide, both which have previously demonstrated strong antibacterial and anti- *Candida* activity (32). In contrast, anti-dermatophyte activity against *T. rubrum and T. mentagrophytes* of MEO2 could be attributed to presence of pulegone, trans- β - caryophyllene mentone and germacrene D, some of monoterpenes found in plants of the same specie with anti-dermatophytes activity (28).

Furthermore, *Aloysia triphiylla* previously showed anti- *Candida* spp. activity and anti – *Fusarium verticiloides* (33-34). However, until our knowledge this is the first time that anti – dermatophytes activity of this oil is report. In plants of this genus has been found as major compounds citral (33), which one previously have shown antifungal activity against *C. krusei, C. parapsilopsis*, *A. fumigatus* and *A. flavus*, showing a high activity in a range of MIC (250- 39.72 µg/ml) (20).

In many countries, plants of *Lippia* spp. has been used with medicinal purposes, oils and extracts of this genus have shown biological activity (35). Our laboratory previously reports the antifungal activity against *Candida parapsilosis, C. krusei, A. flavus* and *A. fumigatus*, of eight oils of *Lippia origanoides* (including 1A, 5E and 6F oils) and two of their major components (carvacrol and thymol). The results suggest that biological activity of these essentials oils depends of the content of both substances (16). In present study, two samples showed high content of carvacrol monoterpen (1A and 5E) and one sample of thymol (6F) show activity against dermatophytes and important activity against the multiresistant fungi *F. oxysporum*.

Oliveira et al (2007) (36) using an agar diffusion method found important anti-fungal properties of *Lippia origanoides* oil against *T. rubrum,* however we cannot compare our results with theirs, because they used a technique that determined diameter of inhibition zones (mm) on solid agar at a single concentration but not broth microdilution method to determine the minimal inhibitory concentration (µg/mL) as we did. In addition, they evaluated the activity of thymol (18.5%), carvacrol (38.6%) chemotype of *L. origanoides* essential oil against *C. parapsilosis*, obtaining MIC values about two times higher than the Amphotericine B MIC. Both substances appear to make permeable the cell membrane and increasing the permeability of the cytoplasmic membrane to ATP (36).

From a pharmacological point of view, *L. alba* is probably the most studied species in the *Lippia* genus (23). Different biological activities, such as cytotoxic, antifungal, antibacterial, antiviral and anti-inflammatory have been identified in essential oils or extracts from this specie (36, 38-40). In our Laboratory, Mesa-Arango et al. (2009) (41) , found important anti-fungal activity against *A. fumigatus* and *C. krusei* (GM-MIC of 78.7 and 270.8 µg/mL, respectively) of *L. alba* citral chemotype (SB1) as well as their major components geranial, neral, geraniol and trans- β - caryophyllene. In present study, all plants of *Lippia alba* specie shown high activity against dermatophytes.

The chemical analysis of BC2 oil identified it as a citral- chemotype. Among the oils evaluated, this oil showed the highest antifungal activity against dermatophytes (GM-MIC 31.25 and 125 µg/mL with *T. rubrum* and *T. mentagrophytes,* respectively). Previous studies realized by us, show a strong activity against *Candida* and *Aspergillus* species with a range of MIC of 140-280 µg/mL and 35-180 µg/mL, respectively. The high antifungal activity of this oil, may be explained by the high concentration of oxygenated monoterpenes, as it has been described by Oliveira et al. 2006 (36). *L. alba* TS and CC1 oils (carvone-chemotype) also showed a very strong activity (GM-MIC values below 500 µg/mL) against dermatophytes species tested. Mesa et al. (2009) (20) showed antifungal activity of both oils against *C. krusei, C. parapsilosis, A. fumigatus* and *A. flavus*. In addition, reported that relative amounts of limo-

nene, carvone, bicyclosesquiphellandrene in both essential oils could be associated with the antifungal activity of the *Lippia alba* (Miller) N.E Brown essential oil with a percentage-dependent effect on species of *A. fumigatus* (20).

Previous studies shown activity on *Fusarium* spp. of citral and eugenol *Lippia alba* major components (41), however we didn't find activity of *L. alba* oils against *F. oxysporum*. These compounds have been previously studied regarding their antimicrobial spectrum on some bacteria and fungi species; showing a high spectrum of activity against *Candida* spp., *Cryptococcus neoformans, Fonsecaea pedrosoi* and *T. rubrum* (23, 41).

The criteria of cytotoxic activity for the plant derivate, as established by the American National Cancer Institute (USA), is an IC_{50} < 30 µg/mL (42). According to this criteria, we consider that of eighteen samples evaluated on Vero cells were not cytotoxic excluding TS oil (IC_{50} = 12.3 ± 2.9 µg/mL), 5E oil (IC_{50} = 29.3 ± 4.8 µg/mL) and MEO2 (IC_{50} ≤ 25 µg/mL), being of special interest AEO1 that have not cytotoxic activity in any concentration tested (IC_{50} ≥ 200 µg/mL). Based on that described by Yamaguchi et al. (2011) (43), a value greater than 1 is considered more selective for activity against fungi which in Vero cell. In this sense, selective was found on two of most potent antifungal oils. AEO1 have the most favorable activity profile with selectivity indices of ≥ 1.27 and ≥ 1.13 for *T. rubrum* and *T. mentagrophytes*, respectively. In addition, a higher selective index (1.62) was found in BC2 oil with *T. rubrum*.

The minor compounds difference, between this essential oils, imposes a significant difference on their toxic activity against cell and fungal. The selectivity and specificity of a particular sample is apparently associated with the drug target at the cellular or molecular level, which is still unknown at this point. In conclusion, the results of this study confirm the anti-mycotic potential of essential oils and extracts of *Verbenaceae* and *Labiatae* families with activity against important dermatological fungi as *F. oxysporum* and dermatophytes as *T. rubrum* and *T. mentagrophytes.* Therefore, it is worthwhile to further study these antifungal essential oils to explore the therapeutic potential of this important class of natural products as antifungal leads for drug discovery.

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Plant Name	Plant Code	Place of Collection	Date of collection	Voucher specimen	Sample
***Verbenaceae* family**					
Aloysia triphylla	AEO1	Bolivar, Santander	March, 2007	517189	Oil
Lippia alba (Mill.) N.E.Brown	SB1*	Turbaco, Bolivar	May, 2005	516929	Oil
	BC1*	Cubará, Boyacá	March, 2005	512083	Oil
	TS*	San Jerónimo, Tolima	April, 2005	484650	Oil
	TF2*	Flandes, Tolima	April, 2005	484650a	Oil
	BC2*	Colorado, Bolivar	May, 2005	512272	Oil
	CC1*	Cachipai, Cundinamarca	January, 2005	484650a	Oil
	CA1*	Anolaima, Cundinamarca	April, 2005	484350	Oil
	CA2*	Anolaima, Cundinamarca	April, 2005	484350	Oil
	SB2*	Bucaramanga, Santander	October, 2005	512077a	Oil
Lippia micromera Schauer	LM1	Manaure, Cesar	March, 2006	516924	Oil
Lippia origanoides Kunth	5E**	Piedecuesta, Santander	July, 2006	516290	Oil
	1A**	Jordán Sube, Santander	May, 2005	512271	Oil
	6F**	Soatá, Boyacá	October, 2006	517741	Oil
Lantana fucata Lindl.	LF1	Sutamarchán, Boyacá	August, 2007	521031	Extract
***Labiatae* family**					
Minthostachys mollis (Kunth) Griseb.	MEO2	Aratoca, Santander	July, 2006	516286	Oil
	MEO1	Bogotá D.C., Cundinamarca	March, 2005	521089	Oil
Origanum vulgare	OV1	Armenia, Quindío	December, 2005	557889	Oil
Lepechinia conferta	LC1	Toca, Boyacá	August, 2007	521068	Extract
Salvia melaleuca subsp. *melaleuca*	SM1	Aquitanía, Boyacá	August, 2007	521076	Extract

Table 1. Geographic origin and voucher number of *Verbenaceae* and *Labiatae* plants from which essential oils and extracts were obtained.

Previously report by: *Agudelo-Gomez et al (2010) [15]. ** Betancur-Galvis et al (2011) [16]

Plant Code	GM – MIC (µg/mL)		
	Fusarium oxysporum ATCC48112	*Trichophyton rubrum* ATCC28188	*Trichophyton mentagrophytes* ATCC24198
	***Verbenaceae* family**		
AEO1	*	157.49	176.78
SB1	*	354.98	*
BC1	*	500	500
TS	*	125	198.43
TF2	*	*	353.55
BC2	*	31.25	125
CC1	*	157.49	250
CA1	*	250	396.85
CA2	*	176.78	198.43
SB2	*	250	250
LM1	*	*	*
5E	500	500	500
1A	500	*	396.85
6F	500	500	500
LF1	*	125	125
	***Labiatae* family**		
MEO2	396.85	99.21	78.75
MEO1	*	250	396.85
OV1	*	*	*
LC1	*	157.49	125
SM1	*	*	*

Table 2. Geometric Means of Minimal Inhibitory Concentration (GM – MIC, µg/mL) of essential oils and extracts of *Verbenaceae* and *Labiatae* families.

* MIC > 500 µg/mL

Plant Code	Vero ATCC CCL-81		*Fusarium oxysporum* ATCC48112	*Trichophyton rubrum* ATCC28188	*Trichophyton mentagrophytes* ATCC24198
	IC_{50} ± SD (µg/mL)	R^2	SI	SI	SI
***Verbenaceae* family**					
AEO1	≥200	N.A	N.D	≥1.27	≥1.13
SB1	74.1 ± 13.2	0.89	N.D	0.59	0.59
BC1	≥200	N.A	N.D	≥ 0.4	≥ 0.4
TS	12.3 ± 2.9	0.77	N.D	0.10	0.06
TF2	116.7 ± 12.9	0.9	N.D	N.D	0.33
BC2	50.5 ± 9.2	0.78	N.D	1.62	0.4
CC1	32.8 ± 3.6	0.89	N.D	0.21	0.13
CA1	99.9 ± 3.8	0.99	N.D	0.4	0.25
CA2	126.5 ± 12.6	0.91	N.D	0.72	0.64
SB2	110.6 ± 2.5	1	N.D	0.44	0.44
LM1	129.1 ± 20	0.8	N.D	N.D	N.D
5E	29.3 ± 4.8*	0.79*	0.06	0.06	0.06
1A	52.3 ± 11.5*	0.8	0.1	N.D	0.15
6F	34.3 ± 6.5*	0.71	0.07	0.07	0.07
LF1	Not evaluated				
***Labiatae* family**					
MEO2	≤25**	N.A	≤0.06	≤ 0.25	≤ 0.32
MEO1	≥200**	N.A	N.D	≥ 0.8	≥ 0.5
OV1	38.6 ± 10	0.8	N.D	N.D	N.D
LC1	64.3 ± 14.7**	0.74	N.D	0.89	0.51
SM1	Not evaluated				

Table 3. The 50% inhibitory concentration (IC_{50}) in µg/mL and selective index (SI) of essential oils of *Verbenaceae* and *Labiatae* on Vero cells.

R^2: linear regression coefficient; NA: Not applicable; N.D: Not determined (MIC > 500 µg/mL). Previously report by: * Betancur-Galvis et al (2011) [16], ** Zapata et al (2010) [19]

Plant Code	Chemical composition (%)
TS*	Limonene (32.0), carvone (26.2), bicyclosesquiphellandrene (16.4), β-bourbonene (6.8), (E)- β-farnesene(2.5), β-caryophyllene (1.8), α-muurolene (1.6), piperitone (1.4), piperitenone (1.3), β-copaene (1.1), 9-epi-β-caryophyllene (1.1).
BC2*	Geranial (28.9), neral (21.5), β-caryophyllene (7.3), 6-methyl-5-hepten-2-one (4.1), geraniol (3.9), limonene(3.2), caryophyllene oxide(2.3), linalool (2.0), bicyclosesquiphellandrene (1.9), α-guaiene (1.8), α-humulene (1.8), nerol (1.8), methyl citronellate (1.7), geranyl acetate (1.6), α-bulnesene (1.2).
CC1*	Limonene (38.1), carvone (28.6), bicyclosesquiphellandrene (12.2), β-bourbonene (5.4), (E)- β-farnesen (1.7), piperitone (1.5), piperitenone (1.5), β-caryophyllene (1.4), α-muurolene (1.0).
5E**	Carvacrol (46.2), p-cymene (12.0), thymol (9.9), γ-terpinene (9.5), α-terpinene (2.7), β-myrcene (2.5), trans-β-caryophyllene (2.0), α-thujene (1.5), α-humulene(1.2), terpinen-4-ol (1.1).
1A**	Carvacrol (36.5), p-cymene (13.9), γ-terpinene (13.2), thymol (9.2), α-terpinene (3.7), β-myrcene (3.2), methyl thymil eter (2.8), α-thujene (2.2),carvacrol acetate (1.9), 1,8-cineol (1.7).
6F**	Thymol (59.7), carvacrol (12.2), p-cimene (8.8), γ-terpinene (4.5), β-mircene (2.2), methyl thymil eter (1.7), trans-β-caryophyllene (1.8), α-terpinene (1.2), β-bisabolene (1.1), α-humulene (1.0).
MEO2***	cis-piperitone epoxide (29.9), piperitenone oxide (25.6), mentone (7.4), germacrene D (5.8), pulegone (5.5), trans-β-caryophyllene (4.5), bicyclogermacrene (2.6) piperitenone (1.7), linalool (1.2), α-humulene (1.1).

Table 4. Chemical composition of major components of essential oils of *Lippia alba* (BC2, TS, CC1, *Verbenaceae*) and *L. origanoides* (1A, 5E, 6F, *Verbenaceae*) and *Mintostachys mollis* (MOE2, *Labiaceae*) with increased antifungal activity.

Previously report by: * Mesa-Arango et al (2010) [21] ** Betancur-Galvis et al (2011) [17]
*** Zapata et al (2010) [20]

Compound	RI- DB-5	Relative amount percentages
Mentone	1136	11.9
cis-Isomentone	1134	1.4
Mentol	1159	0.3
neo- mentol	1153	6.3
Pulegone	1218	18.8
β-Elemeno	1392	1.2
δ-Elemeno	1339	0.8
trans-β-caryophyllene	1426	17.9
α-humulene	1459	4.3
cis-Muuorola-4(14) dieno	1466	0.7
γ-Muurolene	1477	0.9
Germacrene D	1484	10.6
bicyclogermacrene	1499	11.3
δ-Cadinene	1520	1.2
γ-Cadinene	1513	0.6
Caryophyllene oxide	1581	0.6
cis-caryophyllen	1411	0.4
Aromadendrene	1446	2.0
Espatunelol	1573	0.7
epi-Cubenol	1628	0.4

Table 5. Essential oil composition (%) of Minthostachys mollis oils (Labiaceae) from different regions of Colombia with antifungal activity.

RI = retention indices