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Antimicrobial activities of plant species collected from the Northeastern region of Colombia

Actividades antimicrobianas de especies vegetales recolectadas en la región Noreste de Colombia

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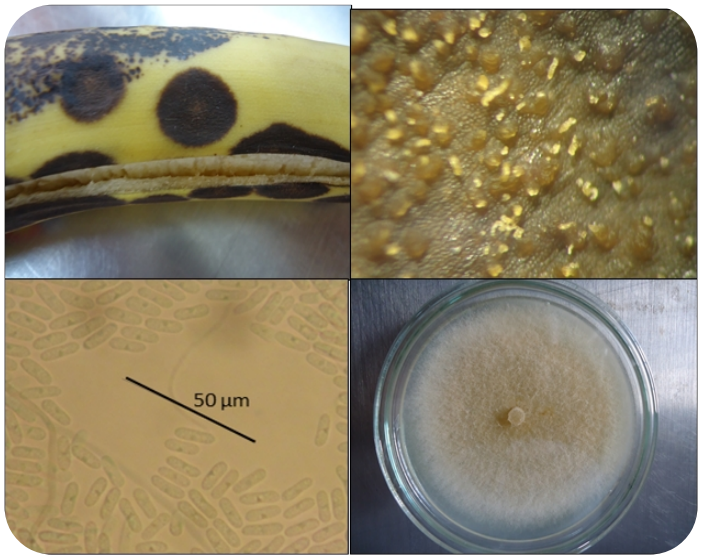

Growth of *Colletotrichum musae* from diseased plant tissue of banana fruits.
Photo: F.A. Ortiz-Meneses

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ABSTRACT

Several studies have been carried out on the antimicrobial potential of natural flora, on various bacteria (Gram-negative and Gram-positive) and fungi. Among the pathogens of great importance are those of the genus *Colletotrichum*, as they cause anthracnose in a wide variety of plants. The aim of this work was to determine the antimicrobial activities (AMA) of plant extracts (PE) and essential oils (EO) of plant material collected in the northeastern region of Colombia. For EO obtained from *Piper tenue* Kunth, *Piper eriopodon* (Miq.) C. DC., *Piper marginatum* Jacq., *Hyptis suaveolens* (L.) Poit, *Eriope crassipes* Benth. and *Lippia origanoides* Kunth., were evaluated the occurrence of inhibition halos against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Klebsiella* spp., *Escherichia coli*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus* spp. and *Candida* spp. On the other hand, the growth inhibition of *Colletotrichum musae* Berk. & Curt. and *Colletotrichum asianum* was observed for the PE obtained from *Ocotea aff. Ocotea caparrapi* (Sand.-Groot ex Nates) Dugand, *Trattinnickia rhoifolia* Willd., *Tetragastris panamensis* (Engl.) Kuntze and *Siparuna guianensis* Aubl.. *L. origanoides* EO and *E. crassipes* EO, inhibited the growth of *S. typhimurium*, *A. terreus* and *Candida* spp. For *C. musae*: *O. aff. O. caparrapi* and *T. rhoifolia* (1.0% w/v), showed a percentage inhibition of mycelial growth (IMG), and a percentage reduction of conidial production (RCP), greater than 85%; *T. panamensis* and *S. guianensis* showed IMG and RCP greater than 71%. For *C. asianum*: *O. aff. O. caparrapi* (1.0% w/v), achieved inhibition greater than 83% (IMG and RCP), followed by *S. guianensis*, *T. panamensis* and *T. rhoifolia*. AMA showed a high correlation with phenolic content. The EO of *L. origanoides* and *E. crassipes*, and the PE of *O. aff. O. caparrapi* exhibited high AMA, comparable to that of the controls.

Additional key words: Piperaceae; Lamiaceae; Verbenaceae; antifungal; antibacterial.

RESUMEN

Se han realizado estudios del potencial antimicrobiano de la flora natural, sobre múltiples bacterias (Gram-negativas y Gram-positivas) y hongos. Entre los patógenos de gran importancia se encuentran los del género *Colletotrichum*, que causan la antracnosis de una gran variedad de plantas. El objetivo de este trabajo fue determinar las actividades antimicrobianas (AAM), de extractos vegetales (EV) y aceites esenciales (AE) de material vegetal recolectado en la región

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nororiental de Colombia. Para los AE obtenidos de *Piper tenue* Kunth, *Piper eriopodon* (Miq.) C. DC., *Piper marginatum* Jacq., *Hyptis suaveolens* (L.) Poit, *Eriope crassipes* Benth. y *Lippia origanoides* Kunth., se evaluó la aparición de halos de inhibición contra: *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Klebsiella* spp., *Escherichia coli*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus* spp. y *Candida* spp. Por otra parte, se estimaron las inhibiciones del crecimiento de *Colletotrichum musae* Berk. & Curt. y *Colletotrichum asianum* por EV obtenidos de *Ocotea aff. Ocotea caparrapi* (Sand.-Groot ex Nates) Dugand, *Trattinnickia rhoifolia* Willd., *Tetragastris panamensis* (Engl.) Kuntze y *Siparuna guianensis* Aubl. Los AE de *L. origanoides* y *E. crassipes*, inhibieron el crecimiento de *S. typhimurium*, *A. terreus* y *Candida* spp. Para *C. musae*: *O. aff. O. caparrapi* y *T. rhoifolia* (1,0% p/v), mostraron un porcentaje de inhibición del crecimiento micelial (ICM), y un porcentaje de reducción de la producción de conidios (PRPC) superiores al 85%; *T. panamensis* y *S. guianensis* mostraron ICM y PRPC superiores al 71%. Para *C. asianum*: *O. aff. O. caparrapi* (1,0% p/v), alcanzó una inhibición superior al 83% (ICM y PRPC), seguido de *S. guianensis*, *T. panamensis* y *T. rhoifolia*. Las AAM tuvieron una alta correlación con el contenido de fenoles. Los AE de *L. origanoides* y *E. crassipes*, y el EV de *O. aff. O. caparrapi* presentaron AAM altas, comparables con las sustancias de control.

Palabras clave adicionales: Piperaceae; Lamiaceae; Verbenaceae; antifungal; antibacterial

INTRODUCTION

Since ancient times, the plants have been used for medicinal and culinary uses. Currently, the use of medicinal plants is increasing due to the perceived safety for consumers (Osungunna, 2020; Vaou *et al*., 2021; Ahad *et al*., 2021). In particular, plants are increasingly recognized as valuable sources of natural products that promote sustainable agriculture (Mendoza *et al*., 2014; Ahad *et al*., 2021), and as effective antimicrobial agents, especially as pathogenic microorganisms continue to develop resistance to antibiotics (Mendoza *et al*., 2014; Chibane *et al*., 2019; García-Gutiérrez *et al*., 2019; Osungunna, 2020; Tariq *et al*., 2021; Vaou *et al*., 2021; Ahad *et al*., 2021). Plants are used directly or as a source of bioactive compounds, *e.g*. *Eucalyptus globulus*, *Azadirachta indica*, *Origanum vulgare*, *Rosmarinus officinalis*, among others, have been used directly for the prevention and treatment of various diseases and/or as food additives (Vaou *et al*., 2021; Chibane *et al*., 2019; Castronovo *et al*., 2021).

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The secondary metabolites (terpenes, alkaloids, phenols, lectins, lactones, naphthoquinones, sulphur compounds) are bioactive compounds with antimicrobial activities (AMA). Although they are not essential for plants, they perform various functions, including protection against microbial infections, pests, ultraviolet radiation, and support ecological relationships between different species. They are also associated with characteristics such as pigmentation, aroma, and flavor in plant species (Yang, 2018; Osungunna, 2020; Vaou *et al*., 2021; Mahomoodally, 2022). Bioactive substances isolated from plants include artemisinin, taxol, atropine, morphine, indoquinolines, caffeine, quinine, and berberine.

Terpenes, terpenoids and aromatic compounds are some of the components of essential oils (EO) and plant extracts (PE) (Yang *et al*., 2018). EO and PE are complex mixtures of organic compounds (volatile and non-volatile, respectively), some of which have been shown to inhibit the growth of microorganisms and insects (Chouhan *et al*., 2017; Sharifi-Rad *et al*., 2017). They can be isolated from leaves, seeds, barks, roots, flowers and other parts of plants (Butnariu and Sarac, 2018; Tongnuanchan and Benjakul, 2014). The chemical composition of EO and PE is related to several factors such as: location of the plant, part of the plant, time of harvest and method of extraction (Sarkic and Stappen, 2018).

Specifically, EO are a great demand in the market due to their applications as antioxidants, antimicrobials, anti-inflammatories and insecticides (Can Baer and Buchbauer, 2015; Pudziuvelyte *et al*., 2017; Ahad *et al*., 2021). E.g. EO from thyme, oregano, mint, cinnamon, sage, garlic and cloves are among the most active natural antimicrobials (Mahomoodally *et al*., 2022).

The pathogenic microorganisms tested include Gram-negative and Gram-positive bacteria and fungi, such as *Helicobacter pylori*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Cryptococcus neoformans*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis* (Chassagne *et al*., 2021; Mahomoodally *et al*., 2022). However, among the most important pathogens are those of the genus *Colletotrichum*, which cause anthracnose on a wide range of plants (e.g. strawberry, mango, avocado, coffee, maize, banana), resulting in symptoms such as deep necrotic lesions (Crouch *et al*., 2014; Yasmeen *et al*., 2014; Balendres *et al*., 2019; Da Silva *et al*., 2020).

Banana (*Musa* sp.) and mango (*Mangifera indica*) fruit anthracnoses caused by *C. musae* and

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C. asianum, respectively, are considered to be among the most important crop and post-harvest diseases, increasing production costs (Khan and Haque, 2022). Several *in vitro* studies have been conducted on the effects of PE on the growth of *Colletotrichum* spp. According to Da Silva *et al*. (2024), PE of *Ipomoea alba*, *Alternanthera dentate*, *Allamanda blanchetii*, *Lippia alba*, *Solanum cordifolium* and *Solanum torvum* showed inhibitions of *C. musae*. Kwodaga *et al*. (2019) found significant inhibition of *C. gloesporioides* growth, with the PE of *Azadiratcha indica*, *Balanites aegyptiaca*, *Jatropha curcas*, *Khaya senegalensis*, *Icacina oliviformis* and *Capsicum annuum*. According to Masangwa *et al*. (2013), PE of *Carica papaya*, *Allium sativum*, *Syzygium cordatum* and *Agapanthus caulescens* were active against *C. lindemuthianum* and *C. dematium*.

In general, the antimicrobial action of plant material (PE and EO) is based on a combination of mechanisms due to the complex composition of the plant mixture (metabolites), which also prevents the acquisition of microbial resistance (Yang *et al*., 2018). Therefore, the aim of this work was to evaluate the antibacterial and antifungal activities (AMA) of plant material collected in the northeastern region of Colombia. Specifically, for the EO obtained from *Piper marginatum*, *Hyptis suaveolens*, *Lippia origanoides*, *Eriope crassipes*, *Piper tenue* and *Piper eriopodon*, the growth inhibition of *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Klebsiella* spp., *Escherichia coli*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus* spp. and *Candida* spp.; was evaluated. While the growth inhibition of *Colletotrichum musae* and *Colletotrichum asianum* was evaluated for the PE obtained from *Ocotea aff. Ocotea caparrapi*, *Trattinnickia rhoifolia*, *Tetragastris panamensis* and *Siparuna guianensis*.

MATERIALS AND METHODS

Chemicals

Mueller-Hinton agar, gentamicin and sodium chloride were bought from Merck; dimethyl sulfoxide (DMSO, 99%), Trolox, gallic acid (98%), Folin-Ciocalteu reagent (2 N), ethanol (99.8%), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, and quaternary ammonium were purchased from Sigma - Aldrich; potato dextrose agar (PDA) was purchased from Oxoid; clotrimazole was bought from Laproff; and benomyl was obtained from a commercial product (Benlate 50WP, Helm, Andina, LTDA).

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Genetic resources access

This work was authorized by the Autoridad Nacional de Licencias Ambientales (ANLA), under the Research Permit No. 8 de 2010 (Resolution 324 of 2014), and the Ministerio de Ambiente y Desarrollo Sostenible (Colombia), with the agreement No. 96 of 2014 to Access to Genetic Resources.

Plant material

Plant species were registered in the Herbario Nacional Colombiano (HNC), Instituto de Ciencias Naturales (ICN), Universidad Nacional de Colombia (UNAL) (Fig. 1): *Piper tenue* (Col 566663, 2013), was collected in Arauca, Arauca (70°47'54.5" W, 07°04'48.6" N); *Piper eriopodon* (Col 570495, 2013), was collected in Troya-Toledo, Norte de Santander (7°2'18.7" N, 72°3'1.8" W); *Piper marginatum* (Col 570497, 2013), was collected in Fortul, Arauca (6°47'36.2" N, 71°47'30.5" W); *Hyptis suaveolens* (Col 553357, 2011), was collected in Mata de Gallina-Arauca, Arauca (6°58'25,45" N, 70°42'24.69" W); *Eriope crassipes* Benth (Col 563484, 2012), was collected in Sabanas de La Vieja-Tame, Arauca (6°18'19.20" N, 71°52'54.78" W); *Lippia origanoides* (Col 580474, 2014), was collected in Sabana de la Vieja-Tame, Arauca (6°22'56" N; 71°53'48" W); *Ocotea aff. Ocotea caparrapi* (41), was collected in El Porvenir and Gibraltar-Toledo; Norte de Santander (7°3'51" N, 72°4'14" W); *Siparuna guianensis* (Col 557315, 2011), was collected at the Puerto San Salvador way-Tame, Arauca (6˚27' N, 71˚44' W); *Trattinnickia rhoifolia* (Col 563483, 2012), and *Tetragastris panamensis* (2013, A. Jara) were collected at the La Reforma farm, Rincón Hondo-Tame, Arauca (6°28'50.03" N, 71°41'15.09" W).

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A

B

C

D

E

F

Figure 1. Plant species studied. A: *E. crassipes*, B: *L. origanoides*, C: *P. tenue*, D: *P. eriopodon*, E: *P. marginatum*, F: *O. aff. Ocotea caparrapi*, G and H: *T. rhoifolia*, I and J: *T panamensis*, K: *H. suaveolens*, L: *S. guianensis*. Photos: G. Tafurt-García (continued on next page).

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G

H

I

J

K

L

Figure 1. Plant species studied. A: *E. crassipes*, B: *L. origanoides*, C: *P. tenue*, D: *P. eriopodon*, E: *P. marginatum*, F: *O. aff. Ocotea caparrapi*, G and H: *T. rhoifolia*, I and J: *T panamensis*, K: *H. suaveolens*, L: *S. guianensis.* Photos: G. Tafurt-García (figure ends here).

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Essential oils (EO) and plant extract (PE)

EOs were obtained from fresh plant material (aerial parts, 1 kg), by microwave-assisted hydrodistillation. A flask (2 L) was connected to a Clevenger distillation apparatus with a Dean-Stark distillation vessel. The EO was separated by decantation (after 2 h of extraction). Residual water was removed with anhydrous Na_2SO_4. Samples were stored in amber vials at 4°C, protected from light (Tafurt-García *et al*., 2014).

EO dilutions of 75, 50 and 25% v/v were prepared in DMSO 99% (only *L. origanoides* and *E. crassipes*). All EOs (pure and diluted) were stored in the dark at 4°C until use. PE were obtained from dried plant material, crushed, and homogenised by exhaustive extraction with ethanol as the solvent. The extracts were dried by vacuum distillation (Tafurt-García *et al*., 2015).

Microbial strains

S. aureus ATCC 25923, *B. cereus*, *S. typhimurium* ATCC 14028, *Klebsiella* spp., *Escherichia coli* ATCC 8739 and *Rhizopus* spp. were donated by the Microbiology Laboratory of the Universidad Nacional de Colombia, at Medellín. *A. terreus*, *A. fumigatus*, *A. niger* and *Candida* spp. were obtained from the microorganism collection of the Probiom research group (Copete-Pertuz *et al*., 2018). Bacteria and fungi were maintained in potato dextrose agar (PDA) nutrient at 4°C until use.

Isolation and identification of *C. musae* and *C. asianum*

Sections of *Musa* sp. and *Mangifera indica* fruit sections showing symptoms of anthracnose were washed with liquid soap, disinfected with quaternary ammonium (10%), rinsed with sterile distilled water, and placed in a humid chamber. As reported by Chaurasia and Bharati (2020), the conidia, which were produced on the lesions, were seeded on the surface of agar-agar culture medium (agar-agar: 15 g, potato extract: 1000 mL), and subsequently seeded and maintained in PDA culture medium (potato extract: 1000 mL, agar-agar: 15 g, dextrose: 10 g). Conidia, from infected tissue and PDA culture medium, were stained with lactophenol blue, for light microscopic visualisation. Microphotographs were taken using a digital camera (Sony Cyber-Shot DSC-W530, 14.1 megapixels, 2.4 zoom), and subsequently measured using imageJ photographic software (2023). Benomyl (Benlate 50WP) was used as a positive control.

The isolates were typed according to the following procedure (Corpogen, 2013): DNA

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extraction, PCR amplification of ITS1 - ITS2, purification of PCR fragments, sequencing with primers IT1 and ITS4, manual cleaning of each of the sequences of the fragments obtained, assembly of the sequences, obtaining the problem sequence, taxonomic analysis of the assembled problem sequence by comparison with National Center for Biotechnology Information (NCBI, 2013) databases, alignment and construction of a distance tree, using the sequences with the highest similarity to the problem sequence, and taxonomic classification of the sequence.

In vitro antimicrobial activities

Antimicrobial activity from EO

The antibacterial and antifungal activities of the EO of *P. marginatum*, *H. suaveolens*, *L. origanoides*, *E. crassipes*, *P. tenue* and *P. eriopodon*, were evaluated by the Kirby-Bauer disc susceptibility test (Jorgensen and Turnidge, 2015). The AMA were tested against Gram-positive (*S. aureus* ATCC 25923, and *B. cereus*), and Gram-negative bacterial strains (*S. typhimurium* ATCC 14028, *Klebsiella* spp., and *E. coli* ATCC 8739). Petri dishes with Mueller-Hinton agar were inoculated with bacterial suspension (100 µL, 10^6 CFU/mL); then, EO (10 µL) were added individually to sterile filter paper discs (Whatman No. 1; 6 mm in diameter), and placed on the surface of the inoculated plates (Cazella *et al*., 2019). Petri dishes were left at room temperature for 30 min to allow diffusion of EO and then incubated at 37°C for 24 h. The diameter of the growth inhibition halo (DGIH) was then measured around the filter paper discs. DMSO (99%) and gentamicin (10 mg mL^{-1}) were used as negative (C^-) and positive (C^+) controls, respectively. All assays were performed in triplicate.

Antifungal activities (AFA) against *A. terreus*, *A. fumigatus*, *A. niger*, *Rhizopus* spp. and *Candida* spp. were determined using a method similar to that used to determine antibacterial activities (ABA). Petri dishes containing PDA were inoculated with a spore suspension of each species (100 µL, 10^5 and 10^7 spores/mL or CFU/mL). Sterile filter paper discs were then placed on the plates and the EO (10 µL) was immediately added. Sterile distilled water (10 µL) and clotrimazole (10 µL, 2 mg mL^{-1}) were used as negative control (C^-), and positive control (C^+), respectively. Plates were kept at room temperature for 30 min and then incubated at 30°C for 5 d for *Aspergillus* sp. and *Rhizophus* spp., and 24-48 h for *Candida* spp. All assays were performed in triplicate.

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AMA of the EO were estimated using the DGIH (width, mm) as: not sensitive (no AMA) for diameters equal to or less than 8 mm; sensitive (low AMA) for diameters between 8 and 14 mm; very sensitive (moderate AMA) for diameters between 14 and 20 mm; and extremely sensitive (high AMA) for diameters equal to or greater than 20 mm. (Jorgensen & Turnidge, 2015; Cazella *et al*., 2019).

***Antifungal activities from PE with* C. musae *and* C. asianum**

AFA of PE from *O. aff. Ocotea caparrapi*, *T. rhoifolia*, *T. panamensis* and *S. guianensis* (1.0% w/v) was assessed using the poisoned food technique (Liu *et al*., 2017). Plates were incubated at 25ºC, in the dark. Measurements were taken daily over a period of 7 d. Colony radius was measured until the control treatment completely covered the Petri dish (4 d). A completely randomised experimental design was used, with a repeated measures arrangement over time, consisting of three replicates and four subsampling units per treatment, and a Tukey's mean comparison test, with an $P<0.05$ (Statistix 10, 2013). The number of conidia was assessed in a Neubauer chamber. AFA was estimated as percentage inhibition of mycelial growth (*IMG, %*), and a percentage reduction of conidial production (*RCP, %*), for *C. musae* and *C asianum*, according to equations 1 and 2:

$$IMG(\%) = \frac{(MGt - MGo)\,x\,100}{MGo} \quad (1)$$

$$RCP(\%) = \frac{(CPt - CPo)\,x\,100}{CPo} \quad (2)$$

MGt and *MGo* are the mycelial growths, and *CPt* and *CPo* are the conidial production in treated (PE, and positive control) and untreated (negative control) samples, respectively. Water (type I) and benomyl (0.5 mg L^{-1}) were used as negative (C^-) and positive (C^+) controls, respectively. All assays were performed in triplicate.

Total phenolic content (TPC) and total antioxidant activity (TAA)

The TPC was calculated as gallic acid equivalents (mg GAE/g extract) following the procedure described by Tafurt-García *et al*. (2015) and Dastmalchi *et al*. (2007). The TAA was determined using the colorimetric assay with the pre-formed ABTS•+ radical monocation (Re, 1999; Tafurt-García, 2015). The TAA of ethanolic extracts was estimated with reference to trolox (mmol trolox/kg extract).

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Statistic

An entirely random experimental design was used with the Tukey mean comparison test, to verify significant differences in the response (DGIH, IMG, RCP), with an $P<0.05$, using Statistix 10 software (2013).

RESULTS AND DISCUSSION

In vitro antimicrobial activities of EO

Statistical differences were observed in the antibacterial (Tab. 1) and antifungal (Tab. 2) activity of the essential oils at a 100% concentration, as determined by measuring the diameter of growth inhibition halos in the culture medium.

Table 1. Antibacterial activities of essential oils (100%), from Colombian plants.

Bacterium	Diameters of growth inhibition halos DGIH (mm)							
	P. tenue	***H. suaveolens***	***L. origanoides***	***E. crassipes***	***P. marginatum***	***P. eriopodon***	**C^+**	**C^-**
Gram-positive								
S. aureus	15.0±0.7 b	12.5±0.35 c	35.0±0.1 a	ND	ND	ND	35.0±0.1 a	0 d
B. cereus	ND	ND	ND	31.0±0.6 b	25.0±1.4 d	27.5±1.1c	34.0±0.1 a	0 e
Gram-negative								
E. coli	8.0±0.1 c	7.5±0.1 d	16.0±0.1 b	ND	ND	ND	33.5±0.2 a	0 e
S. typhimurium	14.0±0.9 e	17.5±0.6	34.5±0.1 a	27.5±0.4 c	12.0±0.3 f	9.5±0.1 g	32.5±0.4 b	0 h
Klebsiella spp.	ND	ND	ND	15.0±0.7 b	7.5±1.1 c	6.5±0.1 c	31.0±0.1 a	0 d

DGIH: calculated as the mean of the replicates, C^+: positive control (gentamicin, 10 mg mL^{-1}), C^-: negative control (DMSO 99 %). ND: Not determined. The statistical analysis was performed for each row independently. Values with the same letter do not differ with Tukey test ($P<0.05$).

Table 2. Antifungal activities of essential oils (100%), from Colombian plants.

Fungus	Diameters of growth inhibition halos, DGIH (mm)							
	P. tenue	***H. suaveolens***	***L. origanoides***	***E. crassipes***	***P. marginatum***	***P. eriopodon***	**C^+**	**C^-**
A. terreus	0 f	0 f	38.0±0.2 a	20.3±0.2 c	10.3±0.2 d	7.0±0.1 e	25.0±0.1 b	0 f
A. fumigatus	0 e	0 e	62.0±0.3 a	21.0±0.4 c	ND	14.5±0.4 d	25.5±0.1 b	0 e
A. niger	0 e	0 e	46.3±0.2 a	29.6±1.2 b	ND	7.0±0.1 d	17.0±0.1 c	0 e
Rhizopus spp.	0 e	0 e	43.0±0.1 a	15.5±0.1 c	ND	9.5±0.1 d	18.0±0.4 b	0 e

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	Diameters of growth inhibition halos, DGIH (mm)							
Fungus								
Candida spp.	0 g	12.0±0.4 f	38.3±0.3 a	33.3±0.4 b	15.0±0.3 e	19.6±0.2 d	24.0±0.1 c	0 g

DGIH calculated as the mean of the replicates. C^+: positive control (clotrimazole, 2 mg mL^{-1}), C^-: negative control (sterile distilled water). ND: Not determined. The statistical analysis was performed for each row independently. Values with the same letter do not differ with Tukey test (P<0.05).

P. tenue (100%), had moderate ABA against *S. aureus* and *S. typhimurium* (15.0±0.7 and 14.0±0.9 mm, respectively), whereas it had low ABA against *E. coli* (8.0±0.1 mm). *H. suaveolens* (100%), only had a moderate ABA against *S. typhimurium* (17.5±0.6 mm) (Tab. 1). *L. origanoides* (100%), had high ABA against *S. aureus* and *S. typhimurium* (35.0±0.1 and 34.5±0.1 mm, respectively), and moderate against *E. coli* (16.0±0.1 mm).

The DGIH obtained with *L. origanoides* (100%), on *S. aureus* and *S. typhimurium* were statistically similar with those obtained with gentamicin (10 mg mL^{-1}), which was used as positive control. *B. cereus* (Gram-positive bacterium) was also found to be extremely sensitive to *E. crassipes* (100 %), *P. marginatum* (100%), and *P. eriopodon* (100 %), with a DGIH of *B. cereus* greater than 25 mm. For *S. typhimurium*, a DGIH of 27.5±0.4 mm was observed in the presence of *E. crassipes*. *Klebsiella* spp. were not sensitive to the evaluated EO (*P. marginatum* and *P. eriopodon*) (Tab. 1). A behavior that may be related to the thick layer of murein present on the outer membrane of Gram-negative bacteria, which prevents the entry of inhibitory substances into the cell (Jackson, 2014; Irazoki *et al*., 2019).

With regard to AFA (Tab. 2), *P. tenue* and *H. suaveolens* showed no activity on the fungi evaluated. However, *Candida* spp. were sensitive to the presence of *H. suaveolens* EO (DGIH of 12±0.4 mm). The highest AFA were observed for *L. origanoides*, with DGIH of 38.0±0.2, 62.0±0.3, 46.3±0.2, 43.0±0.1 mm, and 38.3±0.3 mm, for *A. terreus*, *A. fumigatus*, *A. niger*, *Rhizopus* spp. and *Candida* spp., respectively. This shows a high AFA of *L. origanoides*, which significantly exceeded to the positive control (clotrimazole, 2 mg mL^{-1}) with DGIH values between 17.0±0.1 and 25.5±0.1 mm.

All the EO evaluated inhibited the growth of the yeast *Candida* spp. with the exception of *P. tenue* EO. *L. origanoides* EO had the highest AFA (DGIH 38.3±0.3 mm). In addition, *Aspergillus* spp. and *Candida* spp. were extremely sensitive to *E. crassipes*, with DGIH greater than 20 mm. *Rhizopus* spp. were sensitive to *E. crassipes*, with a DGIH of 15.5 ± 0.07 mm (Tab. 2).

In continuation of the evaluations, essential oils of *L. origanoides* and *E. crassipes* were tested

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at 75%, 50%, and 25% v/v concentrations for their antibacterial (Tab. 3) and antifungal (Tab. 4) activity, with statistically significant differences observed when compared to the controls. *L. origanoides* EO, completely inhibited the growth of *S. aureus*, and all fungal strains tested (Tab. 3).

Table 3. Antibacterial activities of essential oils from *L. origanoides* and *E. crassipes* at 75, 50 and 25% v/v.

Bacterium	Diameters of growth inhibition halos, DGIH (mm)									
	L. origanoides					*E. crassipes*				
	75 %v/v	50 %v/v	25 %v/v	C^+	C^-	75 %v/v	50 %v/v	25 %v/v	C^+	C^-
S. aureus ATCC 25923	>35 a	>35 a	>35 a	>35 a	0 d	16.5±3.5 b	13.5±0.7 bc	11.0±0.1 c	35.5±0.7 a	0 d
B. cereus	11.0±1.4 b	11.5±2.1 b	9.0±1.4 b	32.0±2.8 a	0 c	13.0±1.4 b	13.5±2.1 b	12.5±2.1 b	33.0±2.8 a	0 c
E. coli	13.0±1.4 c	12.5±0.7 c	10.5±0.7 c	22.5±3.5 b	0 d	11.1±2.1 c	9.5±2.1 c	9.0±1.4 c	28.0±2.8 a	0 d

DGIH calculated as the mean of the replicates, C^+: positive control (gentamicin, 10 mg mL^{-1}), C^-: negative control (DMSO 99%). The statistical analysis was performed for each row independently. Values with the same letter do not differ with Tukey test (P<0.05).

Table 4. Antifungal activities of essential oils from *L. origanoides* and *E. crassipes* (75, 50 and 25% v/v).

Fungus	Diameters of growth inhibition halos, DGIH (mm)									
	L. origanoides					*E. crassipes*				
	75	50	25	C^+	C^-	75	50	25	C^+	C^-
A. terreus	>35 a	>35 a	>35 a	>35 a	0 e	21.5±0.7 b	19.5±2.1 b	15.0±0.1 c	10.5±2.1 d	0 e
A. fumigatus	>35 a	>35 a	>35 a	>35 a	0 d	19.5±3.5 c	17.5±2.1 c	15.5±0.7 c	26.0±1.4 b	0 d
A. niger	>35 a	>35 a	>35 a	>35 a	0 c	19.5±3.5 b	16.0±5.6 b	16.0±5.6 b	17.5±0.7 b	0 c
Rhizopus spp.	>35 a	>35 a	>35 a	>35 a	0 c	16.5±4.9 b	17.0±0.1 b	15.5±0.7 b	17.0±1.4 b	0 c
Candida spp.	29.0±1.4 a	25.0±1.4 b	24.5±0.1 b	24.0±0.1 b	0 e	18.5±0.7 c	13.5±0.7 d	11.5±0.7 d	24.5±0.7 b	0 e

DGIH calculated as the mean of the replicates. C^+: positive control (clotrimazole, 2 mg mL^{-1}), C^-: negative control (sterile distilled water). The statistical analysis was performed for each row independently. Values with the same letter do not differ with Tukey test (P<0.05).

In vitro antifungal activities from Colombian plant extract

The results of the antifungal assays against *C. musae* and *C. asianum* revealed statistically significant differences among the various plant extracts tested, indicating variability in their inhibitory effects (Tab. 5).

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Table 5. Antifungal activities from plant extracts (1 % w/v).

Fungus	Inhibition of mycelial growth (%)					
	O. aff. Ocotea caparrapi	***T. rhoifolia***	***T. panamensis***	***S. guianensis***	**C^+**	**C^-**
C. musae	100.0 a	85.1 b	71.2 c	72.4 c	100 a	0 d
C. asianum	83.0 b	49.9 c	51.1 c	59.3 d	100 a	0 e
	Reduction of conidial production (%)					
C. musae	100.0 a	93.7 a	92.3 a	76.0 b	100 a	0 c
C. asianum	98.8 a	73.3 b	92.1 a	93.9 a	100 a	0 c

C^+: positive control (benomyl, 0,5 mg L^{-1}), C^-: negative control (water). The statistical analysis was performed for each row independently. Values with the same letter do not differ with Tukey test (P<0.05) (n=3).

Based on the analysis of the separation of the mean and standard deviation for IMG and RCP of the different PE, it was found that *O. aff. Ocotea caparrapi* had the highest IMG of *C. asianum* (83.0%), a result statistically different from the treatments with *S. guianensis* (59.3%), *T. rhoifolia* (49.9%), and *T. panamensis* (51.1%). The latter two species had statistically similar IMG values. Meanwhile, *O. aff. Ocotea caparrapi* (98.8%), *S. guianensis* (93.9%), and *T. panamensis* (92.1%) showed the highest RCP of *C. asianum* with statistically similar values, followed by *T. rhoifolia* (73.3%) with a statistically different RCP.

On the other hand, the highest IMG of *C. musae* was observed for *O. aff. Ocotea caparrapi* (100%), followed by *T. rhoifolia* (85.1%), *S. guianensis* (72.4%), and *T. panamensis* (71.2%), the last two results being statistically similar. The highest RCP *of C. musae* was obtained with *O. aff. Ocotea caparrapi* (100%), *T. rhoifolia* (93.7%), and *T. panamensis* (92.3%), with no statistically significant variation, followed by the treatment with *S. guianensis* (76.0%).

In general, the PE of *O. aff. Ocotea caparrapi* was the most effective treatment against *C. musae* and *C. asianum*; it was comparable to the positive control (benomyl, 0.5 mg L^{-1}), with IMG and RCP equal or greater than 100%.

AMA and AFA were related to the chemical composition of the plants studied (EO and PE). For the Lamiaceae studied, Tafurt-García *et al*. (2014) showed that α-phellandrene, limonene, 1,8-cineole, fenchone, E-caryophyllene and germacrene D, were the main compounds in *H. suaveolens* EO; and citronellic acid and methyl citronellate for *E. crassipes* EO. In addition, terpenoids, tannins, steroids, saponins, alkaloids, glycosides and abietane type endoperoxides were detected in *H. suaveolens* (Barbosa *et al*., 2013; Okonta *et al*., 2021).

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For *L. origanoides* EO (Verbenaceae), carvacrol and thymol were the major compounds, according to our GC-MS analysis. Three chemotypes of *L. origanoides* (trans-β-caryophyllene/ρ-cymene, thymol and carvacrol), were reported by Almeida *et al*. (2018). Barreto *et al*. (2014), pointed out that the AMA of phenolic compounds, such as carvacrol and thymol, is related to their lipophilicity. These compounds can intercalate into the phospholipid bilayer causing lysis and cell death (Jackson, 2014; Irazoki *et al*., 2019; Miranda-Cadena *et al*., 2021). In addition, carvacrol and thymol have been shown to be promising alternatives for the treatment of candidiasis due to their antibiofilm capacities (Miranda-Cadena *et al*., 2021).

Monoterpenes, sesquiterpenes, phenylpropanoids, flavonoids, lignans and alkaloids have been detected in *Ocotea* spp. (Lauraceae) (Salleh and Ahmad, 2017). According to Tafurt-García and Muñoz (2018), for *O. aff. Ocotea caparrapi*: linalool, terpinen-4-ol, α-terpineol, methyleugenol and bicyclogermacrene were identified in leaf EO; and α-terpineol, methyleugenol, elemicin and myristicin were found in branch bark EO. Although, the AFA of PE are produced by the synergistic effect between the components of the mixture, the AFA of *O. aff. Ocotea caparrapi*, could be attributed to the high content of phenolic compounds (methyl eugenol, elemicin and myristicin), of this species.

On the other hand, the Burseraceae family is characterized by the production of EO, with high sesquiterpene diversity (De Souza *et al*., 2021; Daly *et al*., 2022). For example, β-caryophyllene, α-humulene, β-selinene, β-bisabolene have been obtained from the EO of *T. rhoifolia* aerial parts (De Souza *et al*., 2021). α-Pinene, camphene, sabinene, β-pinene, α-phellandrene, α-terpinene, p-cymene, β-phellandrene, γ-terpinene, α-terpinolene, trans-α-dihydroterpineol, terpinen-4-ol, p-cymen-8-ol, α-terpineol were isolated from the resin of *T. rhoifolia* (De Souza *et al*., 2021). α- and β-Amyrin, α- and β-amyrenone were the major constituents in the hexane extract of *T. panamensis* resin (Rüdiger *et al*., 2013). Furthermore, according to Tafurt-García *et al*. (2015), the barks of *T. rhoifolia* and *T. panamensis* showed relatively high total antioxidant activities (TAA) and total phenolic content (TPC).

Hydrocarbonated sesquiterpenes were the main compounds detected by our GC-MS analysis in the EO of the studied *Piperaceae* spp. (*P. marginatum* and *P. eriopodon*). δ-Elemene, β-caryophyllene and E-nerolidol were determined as the major components in *P. marginatum*; β-copaene, β-eudesmol + (5-epi-7-epi)-α-eudesmol and caryophyllene were the main compounds in the *P. eriopodon* (Tafurt-Garcia *et al*., 2023). According to Ustáriz Fajardo, *et al*. (2020), 1,8-

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cineole, β-pinene, myristicin, α-pinene, trans-caryophyllene and β-selinene were the main compounds of *P. eriopodon* EO, with high activity against *S. aureus*.

Safrole (6.1%), germacrene D (12.0%), bicyclogermacrene (11.0%), and curzerenone (33.8%) were determined, as majority, in EO from *S. guianensis* leaves, by our GC-MS analysis. In general, a low content of monoterpenes was found (3.1%). α-Pinene and limonene were the monoterpenes that contributed most to this distribution. As for the sesquiterpenes, 83.1 % was determined. The content of germacrene type sesquiterpenes was 28.6 %. In addition, the TAA and TPC of *S. guianensis* were 602±18 mmol trolox/kg extract, and 0.12±0.01 g GAE/g extract, respectively.

According to Vaou *et al*. (2021), bioactive compounds affect the structure, integrity, permeability and functionality of the cell membrane, and their effect depends on the structure, number and position of the substituents and the nature of the functional groups. For example, the antimicrobial activity of terpenes is related to their lipophilic properties, which allow them to increase the fluidity and permeability of the cell membrane (Vaou *et al*., 2021). Phenolic compounds can also modify cell membrane permeability, promote granule formation and cytoplasmic membrane rupture (Chibane *et al*., 2019). For example, coumarins are phenolic compounds that can affect bacterial biofilm formation and virulence factor production (Vaou *et al*., 2021).

CONCLUSION

The essential oils of *Lippia origanoides* and *Eriope crassipes*, as well as the plant extract of *Ocotea aff. Ocotea caparrapi*, exhibited significant antimicrobial activity due to their chemical composition. Therefore, these plant materials could be used in the formulation of natural antimicrobials.

Conflict of interests: the authors declare that they have no competing financial interests.

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