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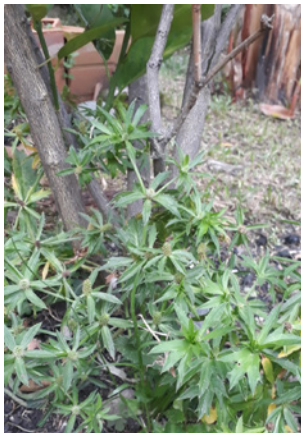
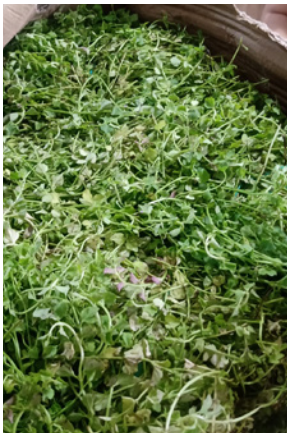
In vitro antibacterial activity of essential oils from *Eryngium foetidum* L. and *Clinopodium brownei* (Sw.) Kuntze

Actividad antibacteriana *in vitro* de aceites esenciales de *Eryngium foetidum* L. y *Clinopodium brownei* (Sw.) Kuntze





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***Eryngium foetidum* L. (left).**
Photo: B.E. Jaramillo-Colorado

***Clinopodium brownie* (Sw.) Kuntze (right).**
Photo: M. Ortiz-Ruiz

ABSTRACT

In the present work, the volatile chemical composition of the essential oils was determinated from Colombian *Eryngium foetidum* L. and *Clinopodium brownei* (Sw.) Kuntze, extracted by hydrodistillation, using gas chromatography coupled with mass spectrometry (GC-MS) technique. The essential oil of *E. foetidum* leaves is composed mostly of aliphatic aldehydes, mainly 2-dodecenal (43.0%), while in the essential oil of the aerial parts of *C. brownei* were menthone (54.3%), pulegone (17.7%), and neomenthol (16.1%). The susceptibility of the bacterial strains *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), and *Pseudomonas aeruginosa* (ATCC 27853) to both essential oils was tested with agar diffusion assays. It was found that *E. foetidum* essential oil inhibits the growth of *S. aureus* (90% at 150 μg mL^{-1}). Broth microdilution tests determined that the MIC and MBC of the *E. foetidum* essential oil against *S. aureus* were 105 and 150 μg mL^{-1}, respectively. 2-dodecenal had strong antibacterial activity against *S. aureus* with MIC and MBC of 105 μg mL^{-1} (98%). Binary combinations of 2-dodecenal and S-limonene or 2,4,6-trimethoxybenzaldehyde had an indifferent effect in checkerboard tests, so it could be stated that the antibacterial activity of the essential oil of *Eryngium foetidum* is mainly due to the action of the 2-dodecenal.

Additional key words: antimicrobial activity; *Staphylococcus aureus;* essential oil; hydrodistillation; bioprospecting.

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RESUMEN

En el presente trabajo, se determinó la composición química volátil de los aceites esenciales colombianos de *Eryngium foetidum* L. y *Clinopodium brownei* **(**Sw.) Kuntze*,* extraídos por hidrodestilación, usando la técnica de cromatografía de gases acoplada a espectrometría de masas (CG-EM). El aceite esencial de hojas de *E. foetidum* está compuesto en su mayoría de aldehídos alifáticos, principalmente 2-dodecenal (43,0%), mientras que en el aceite esencial de las partes aéreas de *C. brownei* fueron la mentona (54,3%), la pulegona (17,7%), y el neomentol (16,1%). La susceptibilidad de las cepas bacterianas *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), y *Pseudomonas aeruginosa* (ATCC 27853) a los aceites esenciales se probó con los ensayos de difusión en agar y se encontró que el aceite esencial de *E. foetidum* inhibe el crecimiento de *S. aureus* (90% a 150 μg mL^{-1}). Ensayos de microdilución en caldo determinaron que la MIC y MBC de *E. foetidum* contra *S. aureus* fue de 105 y 150 μg mL^{-1}, respectivamente. El 2-dodecenal tuvo actividad antibacteriana fuerte contra *S. aureus* con MIC y MBC de 105 μg mL^{-1} (98%). Combinaciones binarias de 2-dodecenal y S-limoneno o 2,4,6-trimetoxibenzaldehido tuvieron efecto indiferente en ensayos de tablero de ajedrez, por lo que se podría afirmar que la actividad antibacteriana del aceite esencial de *Eryngium foetidum* se debe principalmente a la acción del 2-dodecenal.

Palabras clave adicionales: actividad antimicrobiana; *Staphylococcus aureus*; aceite esencial; hidrodestilación; bioprospección.

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INTRODUCTION

Plants are of great importance in the lives of people around the world. Humans use them to satisfy basic needs such as food, clothing, shelter, and medical care. The plants serve as a food source directly and often feed livestock which are then consumed (Wali *et al*., 2022). In addition, they are an invaluable source of secondary metabolites that are traditionally used to treat all types of diseases, including bacterial infections. The wide chemical diversity of these compounds and their long history of medicinal uses make plants very important natural reservoirs for the research of new antimicrobial compounds (Álvarez-Martínez *et al*., 2021).

Low concentrations of antibiotics present in the environment have significant biological effects. There is an ongoing search for new antimicrobials to combat infections caused by pathogens and alternative solutions using natural products and therapies. Additionally, it is vital to keep the environment free of antibiotic residues and their active metabolites, which are known to be responsible for the incidence of drug resistance (Serwecinska, 2020).

The growing endurance of microorganisms to conventional chemicals and drugs is a serious and evident problem worldwide that has driven research into the identification of new biocides with broad activity. Plants and their derivatives, such as essential oils, are often used in folk medicine, they play an important role in plant protection, as they contain a wide variety of secondary metabolites that are capable of inhibiting or slowing the growth of bacteria, yeasts, and molds. Essential oils and their components have activity against a variety of targets, particularly the membrane and cytoplasm, and in some cases, completely change the morphology of cells (Nazzaro *et al*., 2013; Calo *et al*., 2015). For example, essential oils (EO) from the genera *Thymus*, *Origanum* and *Lippia* contain phenolic compounds such as thymol and carvacrol, to which antiseptic and bactericidal properties have been attributed (Baptista-Silva *et al*., 2020) Therefore, there is interest in contributing to current knowledge on the antimicrobial properties of essential oils and their mechanisms of action, components, and synergistic combinations of essential oils to find areas of research that can facilitate applications of essential oils to overcome the problem of multiresistant microorganisms (Chouhan *et al*., 2017).

Eryngium foetidum L., commonly known as *"culantro"* is an edible herb of the Apiaceae family native to Central America but grown in regions with a tropical climate. It is recognized for its traditional culinary uses as a condiment for typical dishes in several Latin American countries. It is also used in ethnomedicine

for its applications to diseases and ailments related to the digestive tract and acts as an antibacterial, analgesic, and anti-inflammatory agent, among others (Rodrigues *et al*., 2022). Spiny coriander, as it is called by indigenous Mexican communities, is one of the species of herbs most used by them to flavor traditional dishes. The herb grows wild during rainy and dry seasons and is harvested or cultivated for consumption (Pascual-Mendoza *et al*., 2022).

Clinopodium brownei, commonly called *"poleo"*, is an aromatic herb from the Lamiaceae family native to Central America that is used in traditional medicine in infusions to treat respiratory and digestive problems such as diarrhea, nausea, asthma, sinusitis, and the common cold, among others (Vandebroek and Picking, 2020). It is the most valuable medicinal plant for the Saraguro and Shuar indigenous people of Ecuador because it is the one with the greatest knowledge and the most used for medicinal purposes (Andrade *et al*., 2017; Herrera-Feijoo *et al*., 2022). It is used as a digestive and to relieve discomfort from menstrual cramps. It is also considered an effective expectorant agent and a remedy to cure colds, flu, coughs, bronchitis and asthma (Armijos *et al*., 2021).

The objectives of this study were (i) to characterize volatile chemical composition of essential oils from *Eryngium foetidum* L. and *Clinopodium brownei* (Sw.) Kuntze to determinate the major and minor compounds, and (ii) to evaluate the antibacterial activity of these essential oils and their main components against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*, to determine their individual minimum inhibitory (MIC) and bactericidal concentrations (MBC). Additionally, the combined effects according to fractional inhibitory concentrations (FIC) will be investigated.

MATERIALS AND METHODS

Reagents and standards

The individual substances used without further purification were trans-2-dodecenal, (S)-limonene, and 2,4,6-trimethoxybenzaldehyde from Sigma-Aldrich, USA. Mueller-Hinton culture media were purchased from Merck KGaA, Darmstadt, Germany. Trypticase Soy Broth culture medium was from Becton Dickinson GmbH, Heidelberg, Germany.

Plant material

5 kg of fresh *E. foetidum* leaves and 6 kg of *C. brownei* were purchased from a local market in Bogota (Colombia) and kept wrapped in kraft paper until use. The leaves were cleaned and cut into small pieces if necessary for essential oil extraction.

Taxonomic identification was performed in the Herbario Universidad de Antioquia (HUA) (Medellin-Colombia). The control leaves of each plant are archived as a permanent specimen in the Herbarium: *Eryngium foetidum* L. (No HUA 167357), and *Clinopodium brownei* (Sw.) Kuntze (Pending).

Essential oil extraction

The essential oil was extracted by hydrodistillation of the plant material using a Clevenger-type apparatus (Jaramillo-Colorado *et al*., 2019). Approximately 500 g of leaves were heated with 1 L of water for 3 h. The essential oil obtained was separated from the water by decantation and stored under refrigeration until use.

Chromatography analysis

The essential oils were analyzed using the gas chromatography coupled to mass spectrometry (GC-MS) technique in an Agilent Technologies System GC-MSD model 7890A gas chromatograph coupled to an Agilent Technologies model 5975C mass spectrometry detector. Helium (99.9%) was used as a carrier gas with a flow rate of 1 mL min^{-1} and a constant linear velocity of 36.8 cm s^{-1}. The injector temperature was 250°C and an HP-5MS capillary column (5% phenyl-95% polydimethylsiloxane, 30 m × 0.25 mm id × 0.25 μm df) was used. The oven temperature program had an initial temperature of 40°C, with a heating ramp of 3°C/min to 220°C followed by 6°C/min to 280°C, finally maintaining this temperature for 1 min. In the detector, the ionization chamber and transfer line temperatures were 150 and 300°C, respectively, with an energy of 70 eV and acquisition mass range of 30-700 m/z. 1 μL of each sample was injected in splitless injection mode. The identification of the volatile compounds was carried out by comparing the mass spectra obtained with those available in the database of the National Institute of Standards and Technology (NIST) and by comparing the theoretical retention indices (Adams, 2017).

Microorganisms

The reference bacterial strains analyzed were *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), and *Pseudomonas aeruginosa* (ATCC 27853) purchased from Microbiologics, Inc. Primary cultures were prepared and maintained according to the manufacturer's instructions.

Inoculum preparation

Bacterial strains were maintained in Tryptic soy broth – TSB/glycerol and stored at -80°C. Subcultures were prepared at the time of use by inoculating 10 μL of primary culture in 10 mL of TSB and incubated for 24 h at 37°C.

Antibacterial activity

Determination of the inhibition zone diameter (IZD). The susceptibility of bacterial strains to the essential oils was tested using a modified agar diffusion method. For this test, 100 mm diameter petri dishes were filled with 25 mL of Mueller-Hinton agar (MHA), sterile swabs were used to inoculate the agar with the prepared bacterial suspension, and 5 wells of 6 mm diameter were bored in each plate. Then, 10 μL of essential oil dissolved in dimethyl sulfoxide (DMSO) at concentrations between 1,000 and 10,000 μg mL^{-1} was dispensed in the wells along with a positive control of 10 μL of 10,000 μg mL^{-1} kanamycin in sterile water. If the kanamycin inhibition zone diameters were within the acceptable quality control range of 19-26 mm for *S. aureus* (CLSI, 2020) this indicated that inoculum density, growth medium, and incubation conditions were optimal for test performance. If the kanamycin IZDs were outside acceptable quality control ranges the test was repeated. DMSO was tested as a negative control separately. The plates were immediately incubated at 37°C for 24 h, and after this time the inhibition zones were measured. This test was performed in triplicate and repeated at least twice (Orlanda and Nascimento, 2015).

Determination of minimum inhibitory (MIC) and bactericidal concentrations (MBC). To determine the MICs of the oils and their major components against the subject bacteria, broth microdilution tests were performed using flat-bottomed 96-well microtiter plates with lids. 3 μL aliquots of the essential oil and standard solutions dissolved in DMSO to final concentrations of 15, 60, 105, and 150 μg mL^{-1} were added to wells inoculated with 20 μL of bacterial suspension containing up to 200 μL of Mueller-Hinton broth (MHB), as well as 3 μL of kanamycin aqueous solution as a positive control, 3 μL of DMSO as a negative control, a viability inoculum or growth control without added treatment, and broth without inoculum as a sterility blank. The plates were incubated at 37°C for 24 h. The lowest dilution of the essential oil and each of the standards without visible bacterial growth was considered the MIC. If the kanamycin MICs were within acceptable quality control ranges of 1-4 μg mL^{-1} for *S. aureus* (CLSI, 2020) this indicated that inoculum density, growth medium, and incubation conditions were optimal for test performance. If the kanamycin MICs were outside acceptable quality control ranges the test was repeated. Next, an aliquot of the contents of each well with no visible growth was used to inoculate MHA plates, which were incubated at 37°C for 24 h. After this time, the plate corresponding to the minimum concentration of oil or standard without visible bacterial colonies was considered the MBC. These tests were performed in triplicate and repeated at least twice (Orlanda and Nascimento, 2015).

Inhibition percentage (%). In addition to MICs, percentage inhibition for essential oils and their major constituents were calculated using absorbance data from the microdilution assay. The absorbance of each well at 620 nm was measured at 0 and 24 h using a Varioskan Lux microplate reader (Thermo Fisher Scientific, Inc.). Net absorbance (A) values were obtained for each well by subtracting the absorbance at hour 0 (A_0) from the absorbance at hour 24 (A_{24}). The percentage of inhibition for each test substance was determined using the formula 1.

$$\text{Percent of inhibition} = 100 \times (1 - (\text{A of test well}/\text{A of growth control})) \quad (1)$$

Any resulting negative value was assigned a value of zero and any value greater than 100 was considered 100.

Determination of the fractional inhibitory concentration index (FICI). The fractional inhibitory concentrations of the standards were determined by the checkerboard assay. To do this, 100 μL of inoculum and up to 900 μL of MHB were added to each well of microtiter plates with lids. Each standard was added in combination at final concentrations

ranging from 1/8 to 8 MIC. To dissolve these standards in MHB, 1% Tween 20 (Sigma-Aldrich, USA) was used for trans-2-dodecenal and (S)-(-)-limonene, while for 2,4, 6-trimethoxybenzaldehyde DMSO was used. Likewise, some wells were reserved for 10% DMSO and 1% Tween 20 as negative controls. The plates were incubated at 37°C and the absorbance of each well was measured at 620 nm at 0 and 24 h to determine bacterial growth as before. Fractional inhibitory concentration indices (FICI) were calculated using the formula 2.

$$FICI = \begin{pmatrix}\text{MIC of A in combination with B/MIC of A alone}\end{pmatrix} + \begin{pmatrix}\text{MIC of B in combination with A/MIC of B alone}\end{pmatrix} \quad (2)$$

where, A and B are two different standards.

The results were interpreted according to the FIC indices as follows: FICI≤0.5: synergy; 0.5<FICI≤4: additive; and FICI>4: antagonist. The MBCs of each combination were determined as before. These tests were performed in triplicate and repeated twice.

RESULTS AND DISCUSSION

From 3 kg of *E. foetidum* plant material, 0.5 mL of essential oil was obtained, while 5.5 kg of *C. brownei* produced 3.5 mL. That is, the extraction of the essential oils had a yield of 0.01% and 0.13% (v/p) for *E. foetidum* and *C. brownei*, respectively.

The GC-MS analysis identified nine compounds with area percentage above 1% in the essential oil of *E. foetidum* which are shown in table 1. The major compounds found in this oil were (E)-2-dodecenal (eryngial) (43.0%) and 2,4,5-trimethylbenzaldehyde (duraldehyde) (14.8%). On the other hand, in *C. brownei* essential oil there were seven compounds with an area percentage greater than 1% (Tab. 2). The major components obtained in *C. brownei* oil were menthone (54.3%) and pulegone (17.7%).

Table 1. Main volatile components found in the essential oil from *E. foetidum*.

No.	Compound	Area (%)	RI HP-5 (theorical)*	RI HP-5 (experimental)
1	Octanal	0.2	998	994
2	*p*-Cymene	0.5	1,024	1,022
3	Limonene	3.9	1,029	1,031
4	γ-Terpinene	0.2	1,059	1,062
5	Nonanal	1.2	1,100	1,100
7	Decanal	3.2	1,201	1,201
6	Thymol	0.2	1,289	1,294
8	Tridecane	0.2	1,300	1,310
9	3,4,5-Trimethyl-phenol	1.2	-	1,330
10	Duraldehyde (Benzaldehyde, 2,4,5-trimethyl-)	14.8	1,364	1,358
11	*trans*-Caryophyllene	0.5	1,420	1,430
12	(E)-2-Dodecenal	43.0	1,466	1,470
13	*trans*-2-Dodecen-1-ol	3.0	1,469	1,472
14	2,4,6-Trimethoxybenzaldehyde	1.0	-	1,583
15	*trans*-2-Dodecenoic acid	3.9	-	1,692
16	Hexadecanal	0.5	-	1,818

I: Retention index. (*Adams, 2017).

Table 2. Main volatile components found in the essential oil from *C. brownei.*

No.	Compound	Area (%)	RI HP-5 (theorical)*	RI HP-5 (experimental)
1	*trans*-Thujene	0.5	924	929
2	α-Pinene	0.5	932	938
3	Camphene	0.3	946	952
4	Sabinene	0.3	965	960
5	*trans*-ρ-Menthane	0.3	979	982
6	β-Pinene	1.1	979	985
7	Octanone <3->	0.2	983	989
8	Myrcene	0.2	990	994
9	Limonene	1.9	1,029	1,036
10	γ-Terpinene	0.2	1,059	1,054
11	Menthone	54.3	1,152	1,158
12	Menthone <iso->	3.6	1,162	1,160
13	Neomenthol	16.1	1,165	1,170
14	Menthol	3.0	1,171	1,178
15	α-terpineol	1.0	1,188	1,186
16	Myrtenol	0.2	1,195	1,199
17	Pulegone	17.7	1,237	1,237
18	β-Caryophyllene	1.4	1,419	1,422
19	β-Selinene	0.2	1,490	1,498
20	δ–Cadinene	0.2	1,523	1,535
21	Caryophyllene oxide	0.3	1,583	1,599

RI: retention index. (*Adams, 2017).

Antibacterial activity

E. foetidum essential oil inhibited the growth of *S. aureus* (Tab. 3) in the range between 1,000 and 10,000 μg mL^{-1}. No concentration inhibited the growth of *E. coli*, *K. pneumoniae*, or *P. aeruginosa*. *C. brownei* essential oil did not show antibacterial activity against any of the strains studied in the same range of concentrations.

The major compound of *E. foetidum* essential oil, 2-dodecenal, had an activity very similar to that of the oil in the same range of concentrations in both the agar diffusion test (Tab. 4) and the microdilution test (Tab. 5). While the essential oil had MIC and

Table 3. Inhibition zone diameter (IZD) of the essential oil of *E. foetidum* against *S. aureus.*

Concentration (μg mL^{-1})	DIZ (±0.05 mm)
1,000	-
2,000	8.5±0.45
3,500	11.5±0.51
4,000	13±0.52
5,000	16±0.53
7,000	17.5±0.53
10,000	23±0.59

Positive control: Kanamycin 10,000 μg mL^{-1} (28 mm).

MBC of 105 and 150 μg mL^{-1}, respectively, 2-dodecenal had 105 μg mL^{-1} as inhibitory and bactericidal concentrations.

Table 4. Inhibition zone diameter (IZD) of 2-dodecenal against *S. aureus*.

Concentration (μg mL^{-1})	DIZ (±0.05 mm)
1,000	-
4,000	12±0
7,000	18±0.76
10,000	25±0.38

Positive control: Kanamycin 10,000 μg/mL (28 mm).

Table 5. Percentages of inhibition of the essential oil (EO) of *E. foetidum* and 2-dodecenal on *S. aureus*.

Substance	Concentration (μg mL^{-1})	Inhibition (%)
Kanamycin	150	79±4
E. foetidum EO	15	1±12
	60	16±6
	105	69±45
	150	90±10
2-dodecenal	15	13±1
	60	71±39
	105	98±11
	150	100±13

The checkerboard test results showed that the minimum inhibitory concentrations of the binary combinations correspond to those of 2-dodecenal tested individually in most cases, but bacterial growth (turbidity) is also observed in wells with concentrations higher than the minimum inhibitory ones.

Table 6 summarizes the antibacterial activity of 2-dodecenal in combination with two typical minor components of *E. foetidum* essential oil, limonene and 2,4,6-trimethoxybenzaldehyde, which do not individually inhibit the growth of *S. aureus* in the range of concentrations studied. For this reason and according to the calculated FICIs, both combinations had an indifferent effect.

DISCUSSION

In this study, the yield of essential oil extraction from fresh *E. foetidum* leaves was 0.01% (v/w), which is low compared to the values of previous works, summarized in table 7. Banout *et al*. (2010) investigated the yield of extraction of *E. foetidum* leaves dried by three different methods compared to fresh leaves. The average hydrodistillation yield for each method was 0.4, 0.42, 0.42 and 0.49% (w/w) for direct solar drying, indirect solar drying, laboratory oven, and fresh leaves, respectively. Thi *et al*. (2008) investigated the yield and composition of essential oil extracted by conventional hydrodistillation in contrast to the microwave-assisted method. Microwave-assisted extraction was found to be more energy efficient, reaching a maximum yield of 0.061% in 27 min, while the conventional method reached a maximum yield of 0.053% in 6 h.

Table 7. Yield of essential oil from *Eryngium foetidum* leaves.

Origin	Yield (%)	Reference
India	0.2	Paw *et al*., 2022
Nigeria	0.2	Thomas *et al*., 2017
India	0.15	Chandrika *et al*., 2015
Cameroon	0.066	Ngang *et al*., 2014
Colombia	0.2	Jaramillo *et al*., 2011
Peru	0.49	Banout *et al*., 2010
Vietnam	0.053	Thi *et al*., 2008
Nepal	0.5	Thakuri *et al*., 2006
Venezuela	0.082	Cardozo *et al*., 2004
Sao Tome and Principe	0.18	Martins *et al*., 2003
Cuba	0.13	Pino *et al*., 1997b
Malaysia	0.02	Wong *et al*., 1994
Vietnam	0.12	Leclercq *et al*., 1992

Table 6. Antibacterial activity of binary combinations against *S. aureus*.

2-dodecenal (A) + limonene (B)			2-dodecenal (A) + 2,4,6-trimethoxybenzaldehyde (B)		
FIC	FICI	Effect	FIC	FICI	Effect
1 (A) 0 (B)	1	Indifferent	1 (A) 0 (B)	1	Indifferent

FIC, fractional inhibitory concentration; FICI, fractional inhibitory concentration index.

In this study, the yield of essential oil extraction from fresh *C. brownei* aerial parts was 0.13% (v/w), which is low compared to the values of previous works, summarized below in table 8.

Table 8. Yield of essential oil from *Clinopodium brownei* leaves.

Origin	Yield (%)	Reference
Ecuador	0.25	Noriega *et al*., 2023
Ecuador	0.44	Matailo *et al*., 2019
Colombia	0.35	Jaramillo *et al*., 2010
Venezuela	0.12	Rojas and Usubillaga, 2000
Cuba	1	Pino *et al*., 1997a

This study found that *E. foetidum* essential oil is mostly composed of 2-dodecenal. Thi *et al*. (2008) found that the composition of the essential oil of *E. foetidum* varies greatly depending on the geographical origin of the plant, but the aldehyde fraction is always the majority (Cardozo *et al*., 2004).

The composition of the essential oil of *C. brownei* also varies with the geographical origin of the plant. In this case, menthone and pulegone were the principal components. Rojas and Usubillaga (2000) state that it is not possible to attribute the differences in composition to climatic conditions since these are very similar in the sites studied. It is possible that the plant has evolved different phenotypes in the Caribbean and the Andes.

The biological activity of essential oils is often attributed to the most prevalent compounds, which show high antibacterial activity when tested independently (Guimarães *et al*., 2019). Among the phytochemicals with the highest biological activity are polyphenols and terpenes, which can be found in plant extracts and essential oils. In particular, low molecular weight phenolic compounds are the components extracted from plants that most commonly present antimicrobial activity, with greater effectiveness against Gram-positive than Gram-negative organisms (Gutiérrez-Larraínzar *et al*., 2012). The specific mechanisms of polyphenols and terpenes that produce membrane alterations seem to be related to the disruption of the plasma membrane potential by the transport of ions and bonds with other molecules such as membrane proteins. There are also compounds that can insert into the lipid bilayer or bind to it with high affinity, causing structural changes that lead to increased permeability, resulting in leaks or alterations in bacterial homeostasis. The presence of hydroxyl groups in certain positions of the phenolic rings, double bonds, delocalized electrons and conjugation with sugars in the case of flavonoids are common elements in compounds with greater antimicrobial capacity. One of the challenges for phytochemists is the need to determine the compounds responsible for antimicrobial activity in complex mixtures such as extracts and essential oils, and their potential drug interactions. For this, the use of modern technologies and antimicrobial tests with internationally recognized standardized protocols is essential (Álvarez-Martínez *et al*., 2021). The factors that determine the activity of essential oils are the composition, the functional groups present, the inactive components, and their synergistic interactions (Chouhan *et al*., 2017).

Limonene effectively inhibits the growth of *Staphylococcus aureus* at a MIC of 20 mL L^{-1}. Scanning electron microscopy, reduction of AKP activity and fluorescence microscope observation confirm that limonene causes destruction of cell morphology and cell wall integrity of *S. aureus*. The reduction of MFI in the fluorescein diacetate staining assay and the leakage of biological macromolecules (nucleic acids and proteins) indicate that limonene damages the cell membrane and increases its permeability. Furthermore, the reduction of membrane potential further confirms the damage to the membrane and the reduction of respiratory metabolic activity (Han *et al*., 2021).

Asaraldehyde (2,4,5-trimethoxybenzaldehyde) is an active component found in the rhizomes of some plants such as *Acorus gramineus, Mosla scabra,* and *Alpinia flabellata.* Its structural isomers, 2,3,4-trimethoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, and 2,4,6-trimethoxybenzaldehyde have anti-Candida activity with MIC and MFC (fungicide) of 0.25 and 0.5 mg mL^{-1}, respectively, and are non-toxic at MIC. Additionally, the last two inhibit the adhesion and formation of biofilm of *Candida albicans* (Rajput *et al*., 2013).

(2E)-dodecenal showed inhibitory and bactericidal activity in macrodilution tests against *Salmonella choleraesuis* ssp. *choleraesuis* (ATCC 35640) with MIC and MBC of 6.25 μg mL^{-1}, while *E. coli*, *E. aerogenes*, *P. aeruginosa*, and *P. vulgaris* were resistant. The bactericidal effect of 2-dodecenal against *S. choleraesuis* was confirmed with thetime-kill method, which measures the kinetics of bacterial death by counting the colonies that remain viable after exposure to the

antibacterial agent. To do this, bacterial cultures were exposed to concentrations of 2-dodecenal equivalent to ¼ MIC, ½ MIC and the MIC. The number of viable colonies is determined after different incubation periods on agar plates. The results of this trial verified that the MIC and MBC in this case are the same and that lethality occurs rapidly in the first hour after the addition of the aldehyde, which suggests that the antibacterial activity of 2-dodecenal against *S. choleraesuis* is associated with alteration of the cell membrane due to its non-ionic surfactant capacity (Kubo *et al*., 2004).

The essential oil of *Clinopodium brownei* from the Ecuadorian Amazon had as its majority components ethyl cinnamate (21.4%), pulegone (20.76%), methyl cinnamate (16.68%), and caryophyllene (8.17%). The extraction yield was 0.25% w/w. In contrast to most research in which only pulegone and menthone are the majority, this oil has an atypical profile with a high content of esters derived from cinnamic acid: methyl cinnamate and ethyl cinnamate. In microdilution tests, this oil inhibited the growth of *Staphylococcus epidermidis* ATCC 14990 (MIC 13.57 mg mL^{-1}), E*scherichia coli* ATCC 25922 (MIC 6.22 mg mL^{-1}), *Proteus vulgaris* ATCC 6380 (MIC 4.62 mg mL^{-1}), *Klebsiella oxytoca* ATCC 8724 (MIC 7.19 mg mL^{-1}), *Pseudomonas aeruginosa* ATCC 9027 (MIC 8.38 mg mL^{-1}), and *Candida albicans* ATCC 10231 (MIC 3.11 mg mL^{-1}). Direct bioautography assays allowed caryophyllene to be identified as the compound responsible for the antibacterial activity of this oil. Evidence of the action of this molecule is the significant antimicrobial activity of other essential oils in which caryophyllene is found in high concentration (Noriega *et al*., 2023). The ethanolic extract of Colombian *C. brownei* inhibited the growth of *Staphylococcus epidermidis* and *Staphylococcus warneri* isolated from patients with conjunctivitis, but did not inhibit isolates from multiresistant *Staphylococcus aureus* (Pabón *et al*., 2023). Ben Akacha *et al*. (2024) reported that the monoterpenes α-pinene, α-terpineol, and 1,8-cineole, alone or in combination, were effective like antimicrobial when were tested on *S. aureus*, *L. monocytogenes*, *B. cereus*, *S. enterica*, and *E. coli.*

CONCLUSION

The essential oil of the aerial parts from Colombian *Clinopodium brownei* analyzed by GC-MS had as main components menthone, pulegone, and neomenthol. This oil had no antibacterial activity against bacterial strains *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), or *Pseudomonas aeruginosa* (ATCC 27853).

The essential oil from Colombian *Eryngium foetidum* leaves analyzed by GC-MS was composed mostly of aliphatic aldehydes, mainly 2-dodecenal. This oil showed antibacterial activity against *Staphylococcus aureus* subsp. *aureus* (ATCC 11632) in agar diffusion and broth microdilution tests, with MIC of 105 μg mL^{-1} and MBC of 150 μg mL^{-1}.

2-Dodecenal had antibacterial activity against *Staphylococcus aureus* subsp. *aureus* (ATCC 11632) with MIC and MBC of 105 μg mL^{-1}. Binary combinations of 2-dodecenal and S-limonene or 2,4,6-trimethoxybenzaldehyde had an indifferent effect in checkerboard tests, so it could be stated that the antibacterial activity of the essential oil of *Eryngium foetidum* is mainly due to the action of 2-dodecenal.

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