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Antimicrobial and Antioxidant Activities of Four Essential Oils

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Abstract. Various opportunistic microorganisms, such as bacteria and fungi, are responsible for multiple infectious diseases, which represent a threat to global health. Essential oils (EOs) have shown antimicrobial and antioxidant properties, making them an excellent alternative to control multi-resistant bacteria. In this work, for the first time, the antimicrobial and antioxidant activities of four EOs were evaluated, namely *Trixis angustifolia* DC (EOTA), *Dalea bicolor* Humb & Bonpl. Ex Willd (EODB), *Tagetes parryi A.Gray* (EOTP) and *Eupatorium glabratum* Kunth (EOEG). They were obtained by hydrodistillation, and their chemical composition was determined by GC-MS (Gas chromatography-mass spectroscopy) using HP5-MS column. Their antimicrobial and antioxidant activities were determined by the microdilution method and the DPPH and ABTS techniques, respectively. The main compounds of the EOs were piperitone (36.67 %) for EOTA, β-pinene (27.25) for EODB, verbenone (31.13 %) for EOTP and α-cadinol (7.78 %) and bornyl acetate (6.45 %) for EOEG. The EOs EOTA, EODB, EOTP and EOEG inhibited the development of *Candida* at a concentration of 62.5–500 µg/mL, whereas the antibacterial activities of these oils were observed at concentrations from 125–500 µg/mL. The antioxidant activity of EOTA and EODB were IC_{50} = 0.641, 1.195 mg/mL, whereas those of EOTP and EOEG was lower. These results show that four EOs have antimicrobial activity.
Keywords: Essential oils; composition; antimicrobial activity; antioxidant capacity.

Resumen. Diversos microorganismos oportunistas, como bacterias y hongos, son responsables de múltiples enfermedades infecciosas, que representan una amenaza para la salud mundial. Los aceites esenciales (EOs) han demostrado propiedades antimicrobianas y antioxidantes, lo que los convierte en una excelente alternativa para el control de bacterias multirresistentes. En este trabajo, por primera vez, se evaluaron las actividades antimicrobianas y antioxidantes de cuatro EOs: *Trixis angustifolia* DC (EOTA), *Dalea bicolor* Humb & *Bonpl. Ex Willd (EODB), Tagetes parryi* A.Gray (EOTP) y *Eupatorium glabratum* Kunth (EOEG). Los aceites se obtuvieron por hidrodestilación y se determinó su composición química por GC-MS (cromatografía de gases-espectrometría de masas) utilizando una columna HP5-MS. Sus actividades antimicrobiana y antioxidante se determinaron por el método de microdilución y las técnicas DPPH y ABTS, respectivamente. Los principales compuestos de los aceites esenciales fueron piperitona (36,67 %) para EOTA, β-pineno (27,25 %) para EODB, verbenona (31,13 %) para EOTP y α-cadinol (7,78 %) y acetato de bornilo (6,45 %) para EOEG. Los aceites esenciales EOTA, EODB, EOTP y EOEG inhibieron el desarrollo de *Candida* a una concentración de 62,5–500 µg/mL, mientras que las actividades antibacterianas de estos aceites se determinaron a concentraciones de 125–500 µg/mL. La actividad antioxidante de EOTA y EODB fue de IC_{50} = 0,641, y 1,195 mg/mL

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respectivamente, mientras que las de EOTP y EOEG fueron menores. Estos resultados muestran que los cuatro EOs tienen actividad antimicrobiana.
Palabras clave: Aceites esenciales; composición; actividad antimicrobiana; capacidad antioxidante.

Introduction

Multi-resistant bacteria (ESKAPE) represent an inherent problem for the world population. In the United States, the estimated number of annual infections is higher than 2 million, whereas in developing countries, communicable diseases are the main cause of mortality, and emerging and re-emerging infectious diseases represent a major issue [1]. Antibiotic resistance jeopardises the achievements of modern medicine by impeding the treatment and prevention of infections. Some ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli*) can tolerate transient exposure to high doses of antibiotics without changes in their minimum inhibitory concentration (MIC). This tolerance is associated with the irreversible destruction of the active site of the antibiotic, modification of the bacterial target site, reduction of antibiotic accumulation by mutation or loss of membrane channels and persistence through cells embedded in biofilms [2,3].

The World Health Organization (WHO) and Pan American Health Organization (PAHO) have drawn special attention to multidrug-resistant bacteria, generating a critical priority list that includes dangerous multidrug-resistant bacteria that may be of nosocomial origin or acquired in the community. They are classified by their degree of lethality, treatment and hospitalization time; the ease with which they are transmitted between animals, from animals to people and between people. The list is divided into critical, high, and medium priority levels, which include *S. aureus, E. coli, E. faecalis* and *P. aeruginosa* [4].

Additionally, yeasts of the genus *Candida* are opportunistic human pathogens [5] that affect mucous membranes. More than 90 % of clinical infections are caused by species of the genus *Candida,* such as *C. glabrata, C. albicans, C. krusei* and *C. tropicalis*, highlighting their virulence factors such as membrane and cell wall barriers, dimorphism, biofilm formation, signal transduction pathways, proteins related to stress tolerance, hydrolytic enzymes and toxin production [6]. Therefore, the study of these yeasts, whose incidence has increased in the last three decades, is imperative, due to the increase in the Acquired Immune Deficiency Syndrome (AIDS) epidemic, an increasingly aging population, a greater number of immunocompromised patients and the more widespread use of medical devices permanent [4]. Resistance to antifungals has increased in many *Candida* species, contributing to treatment failure and amplifying intra-hospital issues [7].

Free radicals are chemical species present in the body that can cause oxidative stress, damaging cells and body functions, which can result in various diseases such as cancer, arthritis and respiratory diseases, among others. Antioxidants have the ability to scavenge free radicals, playing an important role in defending the body against different chronic diseases [8]. It is therefore essential to develop new compounds with antimicrobial and antioxidant activity. In this context, plants are a source of secondary metabolites, many of which have these two effects, and one of these constituents is EOs, which are complex mixtures containing between 20 and 60 components, mainly monoterpenes, sesquiterpenes, aliphatic and aromatic compounds [9].

The composition of essential oils (EOs) varies with temperature, climate, plant maturity and season, among others, and this variability could influence the properties of the EOs [10]. They play an important role in protecting plants from pathogens and predators [11] and are applied in the production of food, flavours, cosmetics and pharmaceuticals [12]. The bioactive compounds of EOs present various biological activities such as anti-inflammatory, analgesic, anti-cancer [13], antimicrobial and antioxidant activities [14,15]. Different EOs from plants of the family Asteraceae have antioxidant and antimicrobial activities [16], such as those from *Achillea millefolium* subsp. *millefolium* Afan [17] and *Pulicaria inuloides* [18]. Some EOs of plants of the Fabaceae family also possess these activities, such as those from *Myrocarpus frondosus* [19].

Recent studies found that some extracts of aerial parts of *Trixis angustifolia, Dalea bicolor, Eupatoriun glabratum* and some species of *Tagetes* have antimicrobial activity against different bacteria [20-22]. However, there are no reports about antimicrobial effects of the EOs of these plants.

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In this study, we determined the composition of four EOs from plants of the family Asteraceae, namely essential oil of *Trixis angustifolia* (EOTA), essential oil of *Tagetes parryi* (EOTP) and essential oil of *Eupatorium glabratum* (EOEG), and of one EO from a plant *Dalea bicolor* of the family Fabaceae, namely EODB. For the first time, the antioxidant capacities of these EOs were evaluated, as well as their antimicrobial activities toward two Gram (+) bacteria and two Gram (-) bacteria and their antifungal activities toward four *Candida* species.

Materials and methods

General

The aerial parts of *T. angustifolia, D. bicolor, T. parryi and E. glabratum*, were collected in San Luis Potosí State, México. The plants were identified by the taxonomist José García Pérez, and a voucher specimen of each plant was deposited in the Herbarium Isidro Palacios of the Universidad Autónoma de San Luis Potosí (Table 1).

Table 1. Data about plant species, and yield of EOs.

Plant Species	Date and place	Coordinates	Plant part	Yield (w/w)	Voucher number
Trixis angustifolia	February 2008, 1 km from the junction to Guadalcázar, SLP	22°38'23.7"N 100°30'49.0"W	Aerial parts	0.64	SLPM44557
Dalea bicolor	February 2014, at the Cañada del Lobo dam, San Luis Potosí, SLP	22°05'44.0"N 100°57'56.9"W	Aerial parts	0.45	SLPM57550
**Tagetes parryi*	November 2013, Agua Blanca, Municipality of Villa de Zaragoza, SLP	22°03'35.7"N 100°37'11.5"W	Aerial parts	0.54	SLPM31975
Eupatorium glabratum	February 2008, in the Realejo, community of Guadalcázar, SLP	22°39'57.4"N 100°25'04.4"W	Aerial parts	0.19	SLPM44553

*Previosly reported by González-Velasco [23].

Essential oil extraction

The EOs were obtained by hydrodistillation from the aerial parts of the fresh plants. They were extracted with diethyl ether, and this solvent was eliminated under reduced pressure at 20 °C. The EOs were then stored at 5 °C.

Composition of the EOs

The composition of EOs was determined by GC-MS using a chromatograph (Agilet Technology, model 6890N) connected to a selective mass detector model 5973 Network (MSD, Agilent Technologies, Wilmington, DE, USA). An HP-5MS capillary column (30 m length, 0.25 mm internal diameter, and 0.25 µm film width) (J&W, Folsom, CA, USA) was used for the separation. The EOs samples (10 µL) were diluted with acetone (1 mL) and the injector temperature was 240 °C, operated in the splitless mode, and the carrier gas was helium at 1mL/min. The oven temperature was programmed at 50 °C/3 min, with a heating rate of 3 °C/min up to 240 °C/2 min. The MSD was operated at 70 eV, the ion source was set a 150 °C, and the transfer line was at 240 °C and the mass range was analyzed 15-600 *m/z*. The software MSD ChemStation (Agilent B.04.02) was

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used for data recording and the compounds were identified based on their mass spectra by comparison with the spectra reported in the Wiley 09 and NIST11 libraries. In addition, the Kovak index was calculated for each peak, with reference to the n-alkane standards (C6-C26) running under the same conditions.

Microorganisms

We used four yeast and four bacterial species. The yeasts, *Candida albicans* ATCC 10231, *C. glabrata* ATCC 32554, *C. krusei* ATCC 90878 and *C. tropicalis* ATCC 750, were inoculated in sterile Sabouraud dextrose broth and incubated at 37 °C/24–48 h. The bacteria, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027, were inoculated in sterile tryptocasein soy broth and incubated at 37 °C/24 h.

Inoculum preparation

First, 100 µL of bacterial and yeast suspensions were individually inoculated in 8 mL of sterile tryptocasein soy broth and sterile Sabouraud dextrose broth and incubated at 37 °C for 24–48 h. The microorganisms were then adjusted to a density of 10^5 colony-forming units (CFU)/ mL (corresponding to 0.5 McFarland standards). Finally, the suspensions were diluted to 1:1,000 with saline solution [24].

Determination of the minimum inhibitory concentration (MIC)

The antimicrobial activity of the EOs was evaluated by the microdilution technique in 96-well plates to determine the MIC. First, 50 µL of sterile tryptocasein soy broth (for bacteria) [24] and sterile Sabouraud dextrose broth (for yeasts) [25] were pipetted into 96-well plates. Then, 50 µL of EOTA, EODB, EOTP and EOEG were added, and a serial dilution of each extract was subsequently carried out to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.95 and 0.97 µg/mL. Finally, 50 µL of the 1:1,000 dilution of bacterial or yeast inoculate was added and incubated at 37 °C/24 h. As positive inhibition controls, we used fluconazole and itraconazole (250 to 0.12 µg/mL) for yeasts and ciprofloxacin (100 to 0.95 µg/mL) for bacteria. The MIC was determined at an absorbance of 625 nm. The activity of the EOs was compared with those of the respective controls; all tests were carried out six times.

Antioxidant activity 2,2-Diphenyl-1-picrylhydrazyl DPPH assay

The DPPH test was performed according to the method of Williams [26], with modifications. The reaction mixture contained 100 µL of 0.208 mM DPPH and 100 µL of the EOs dissolved in methanol [400–12.5 µg/mL]. The negative control consisted of 100 µL of 0.208 mM DPPH with 100 µL methanol. We used TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; 0–40 µg/mL) as a positive control. Absorbance was determined at a wavelength of 517 nm after 20 min in the dark. The reductive capacity of the EOs was determined using the following equation:

$$\text{RSA \%} = (A_{control}\text{-AEO}/A_{control}) \times 100$$

where $A_{control}$ is the absorbance of the negative control, and AEO is the absorbance of the EO. The concentrations of the samples responsible for a 50 % decrease in the initial activity of the DPPH free radical (IC_{50}) were calculated by linear regression.

Antioxidant activity ABTS assay

The radical scavenging capacity of the EOs was determined with ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) as described elsewhere [27]. An ABTS+radical solution was prepared by mixing 7 mM ABTS solution and 2.45 mM potassium persulphate ($K_2S_2O_8$) in a 1:1 (v/v) ratio. The solution was incubated at room temperature in the dark for 12 h and subsequently diluted with water to obtain an emerald-green solution with an absorbance close to 1,000. The negative control consisted of 20 µL methanol and 180 µL ABTS+; TROLOX was used as a positive control (0–40 µg/mL). The assay was performed in a 96-well plate, where 20 µL of EO dissolved in methanol in a range of 500–100 µg/mL was mixed with 180 µL

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ABTS+ solution, incubated for 20 min at room temperature in the dark and read at a wavelength of 734 nm. The RSA % was determined according to the following equation:

$$RSA\ \% = (Ac\text{-}As)/Ac) \times 100$$

where Ac is the control absorbance, and As is the sample absorbance. The concentrations of the samples responsible for a 50 % decrease in the initial activity of the ABTS free radical (IC_{50}) were calculated by linear regression.

Statistical analysis

The data obtained between MIC and four EOs against four species of *Candida* and MIC of four EOs against Gram (+) and Gram (–), species were analyzed, by ANOVA test. The data obtained calculating the DPPH and ABTS indexes were analysed by Tukey's test. The data was analyzed using statistical program inerSTAT20-a v. 1.3. A p-value of less than 0.05 was considered statistically significant.

Results

Chemical composition of the EOs

The chemical composition of the EOs was determined by GC-MS [28]. We found the three EOs (EOTA, EOTP, EOEG) oxygenated compounds predominate 89.58 %, 69.14 %, 40.59 %, respectively. In the case of EODB the oxygenated compounds represent only 24.8 %. The table 2 is shown for the first time the composition of EOTA. Overall, 34 compounds were identified, accounting for 86.47 % of the oil; the main compounds were piperitone (38.67 %), 1,8-cineole (14.14 %) and α-terpineol (6.38 %).

Table 2. The chemical composition of EOTA.

Compound	**Rt (min)**	**Relative Abundance (%** ± SD**)**	**RI_R**	**RI_E**
Isovaleric acid	6.11	2.37 ± 0.37	816	808
2-Methylbutyric acid	6.76	2.45 ± 0.57	839	838
α-Phellandrene	10.38	0.53 ± 0.01	1007	1003
p-Cymene	11.30	0.47 ± 0.01	1011	1022
1,8-Cineole	11.62	14.14 ± 0.42	1023	1029
β-*cis*-Ocimene	12.45	0.19 ± 0.00	1024	1047
Linalool	14.83	1.07 ± 0.03	1082	1097
(*E*)-p-Menth-2-en-1-ol	15.73	0.44 ± 0.06	1123	1117
cis-p-Menth-2-en-1-ol	16.60	0.39 ± 0.02	1118	1136
4-Terpineol	18.34	0.18 ± 0.04	1175	1173
3,9-Epoxy-1-p-menthene	18.70	0.12 ± 0.03	1178	1181
α-Terpineol	19.03	6.38 ± 0.07	1172	1188

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Compound	**Rt (min)**	**Relative Abundance (%** ± SD**)**	**RI_R**	**RI_E**
trans-2-Hydroxy-1,8-cineole	20.68	0.11 ± 0.05	1228	1224
Piperitone	22.16	38.67 ± 0.48	1243	1257
β-Bourbonene	27.55	0.12 ± 0.00	1386	1378
β-Elemene	27.92	0.78 ± 0.02	1387	1387
α-Gurjunene	28.60	0.34 ± 0.00	1412	1402
Caryophyllene	29.00	1.34 ± 0.00	1421	1412
α-Bergamotene	29.77	0.23 ± 0.02	1427	1431
Humulene	30.40	0.25 ± 0.02	1454	1447
Aromandendrene	30.70	0.41 ± 0.01	1455	1454
α-Muurolene	32.39	0.13 ± 0.03	1494	1496
δ-Cadinene	33.33	4.11 ± 0.08	1514	1520
Elemol	34.36	0.86 ± 0.03	1535	1545
Palustrol	34.98	0.80 ± 0.03	1562	1561
Spathulenol	35.41	0.22 ± 0.02	1569	1571
Guaiol	36.23	1.95 ± 0.03	1588	1592
Ledol	36.37	0.43 ± 0.00	1597	1595
2-(4a,8-Diethyl-2,3,4,4a,5,6,7,8-octahydro-2-naphthalenyl)-2-propanol	36.56	1.14 ± 0.02	1598	1600
Agarospirol	37.24	0.55 ± 0.08	1631	1619
δ-Cadinol	38.06	0.28 ± 0.12	1646	1641
β-Eudesmol	38.15	0.18 ± 0.01	1644	1644
α-Cadinol	38.36	0.38 ± 0.03	1641	1650
Bisabolol	39.49	2.06 ± 0.05	1683	1681
Total identified		**86.47**		
Total unidentified		**13.53**		

Retention time (Rt), retention indexes in the literature (RI_R), and retention indexes calculated (RI_E), Standard Deviation (SD) duplicated analysis.

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For EOTP, 21 constituents were determined [23], according for 87.49 % of the EO (Table 3); the main compounds were dihydrotagetone (25.77 %) and verbenone (31.13 %).

Table 3. The chemical composition of EOTP.

Compound	**Rt (min)**	**Relative Abundance (%** ± SD**)**	**RI_R**	**RI_E**
3-Hexenol-1-ol	5.66	0.16 ± 0.00	838	806.3
β-Phellandrene	10.03	0.37 ± 0.04	964	957.0
β-Pinene	10.13	0.32 ± 0.02	961.7	960.3
β-Myrcene	10.86	0.21 ± 0.01	979	985.6
α-Phellandrene	11.40	0.23 ± 0.00	997	1000.0
1,8-Cineole	12.63	1.46 ± 0.02	1023	1028.2
trans-β-Ocimene	13.06	2.10 ± 0.12	1034	1037.1
Dihydrotagetone	13.87	25.77 ± 1.57	1055	1054.1
Chrysanthenone	17.20	0.31 ± 0.10	1099	1123
Neo-allo-ocimene	17.46	0.17 ± 0.09	1131	1128.4
Tagetone	18.70	19.76 ± 1.47	1124	1153
4-Terpineol	19.75	0.11 ± 0.02	1161	1188.5
α-Terpineol	20.42	0.55 ± 0.01	1172	1188.5
2-Ethylidene-6-methyl-3,5-heptadienal	21.22	0.37 ± 0.05	1182	1205
Verbenone	22.95	31.13 ± 3.19	1228	1242.4
Thymol	23.47	0.14 ± 0.06	1266	1253.6
Isopiperitenone	24.32	2.31 ± 0.34	1249	1271.9
Eugenol	29.96	1.46 ± 0.05	1392	1393.3
Caryophyllene	31.06	0.34 ± 0.04	1424	1418
p-Cresol	33.12	0.11 ± 0.07	1503.9	1474.1
Elemol	36.61	0.10 ± 0.01	1535	1551.2
Total identified		**87.49**		
Total unidentified		**12.51**		

Retention time (Rt), retention indexes in the literature (RI_R), and retention indexes calculated (RI_E).
Standard Deviation (SD) duplicated analysis. This composition was reported for González-Velasco [23].

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In EODB, we identified 46 compounds (Table 4), accounting for 65.98 % of the total EO; the main component was β-pinene (27.25 %), followed by *tau*-cadinol (6.73 %), β-myrcene (6.23 %) and camphene (3.85 %).

Table 4. The chemical composition of EODB.

Compound	**Rt (min)**	**Relative Abundance (%** ± SD**)**	**RI_R**	**RI_E**
(*E*)-2-Hexenal	7.35	0.12 ± 0.00	822.4	810.8
Camphene	11.25	3.85 ± 0.09	943	926.3
Benzaldehyde	11.79	0.36 ± 0.01	927.2	942.5
β-Pinene	12.59	27.25 ± 0.53	961	966.1
β-Myrcene	13.29	6.23 ± 0.04	981	986.9
α-Phellandrene	13.89	0.06 ± 0.01	997	1003.1
(3*E*)-3-Hexenyl acetate	14.07	0.05 ± 0.01	983	1006.8
3-methyl-3-vinylciclohexanone	14.23	0.03 ± 0.01	1115	1009.9
α-Terpinene	14.49	0.04 ± 0.01	1008	1015.2
p-Cymene	14.89	0.05 ± 0.00	1025	1023.2
Limonene	15.10	1.88 ± 0.01	1018	1027.3
1,8-Cineole	15.23	0.25 ± 0.01	1020	1030.0
β-Ocimene	16.11	1.15 ± 0.09	1024	1047.5
γ-Terpinene	16.62	0.06 ± 0.02	1047	1057.6
trans-Sabinene hydrate	17.05	0.08 ± 0.00	1050	1066.3
Terpinoleno	18.13	0.11 ± 0.00	1080	1087.7
Linalool	18.71	1.14 ± 0.06	1082	1099.3
Pinocarveol	20.66	0.10 ± 0.02	1143	1137.7
Camphor	20.97	0.14 ± 0.01	1146	1143.7
Endo-Borneol	22.04	0.15 ± 0.01	1148	1164.7
4-Terpinenol	22.62	0.25 ± 0.04	1162	1176.1
α-Terpineol	23.29	0.70 ± 0.01	1172	1189.1
Myrtenol	23.58	0.17 ± 0.02	1212.8	1195.0
cis-3-Hexenyl valerate	25.34	0.05 ± 0.01	1243	1232.0
Bornyl acetate	27.97	2.34 ± 0.04	1270	1287.9

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Compound	**Rt (min)**	**Relative Abundance (%** ± SD**)**	**RI_R**	**RI_E**
Lavandulyl acetate	28.18	0.13 ± 0.00	1292	1292.2
Myrtenyl acetate	29.84	0.21 ± 0.08	1299	1327.6
δ-Elemene	30.43	0.62 ± 0.03	1334	1340.1
Eugenol	31.29	0.12 ± 0.01	1363	1358.4
Methyl cinnamate	32.46	0.54 ± 0.01	1380	1383.3
β-Elemene	32.94	0.12 ± 0.10	1387	1393.5
Caryophyllene	34.21	0.77 ± 0.00	1421	1422.8
Humulene	35.73	0.33 ± 0.05	1454	1459.0
γ-Muurolene	36.71	0.19 ± 0.01	1471	1481.8
δ-cadinene	38.03	0.20 ± 0.02	1514	1513.2
6-Epishyobunone	38.31	2.06 ± 0.16	1538	1519.8
6-Epi-shyobunol	38.57	0.68 ± 0.00	1555	1525.9
Elemol	39.76	1.09 ± 0.14	1535	1554.2
Elemicin	39.98	0.14 ± 0.06	1531	1559.2
Caryophyllene oxide	41.26	0.78 ±0.09	1575	1589.5
Viridiflorol	41.60	0.55 ± 0.08	1594	1597.6
Guaiol	41.80	1.48 ± 0.07	1588	1602.4
Dehydroxy-isocalamendiol	42.17	2.06 ± 0.24	1593	1612.2
tau-cadinol	43.54	6.73 ± 0.52	1628	1647.8
7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	46.59	0.40 ± 0.01	1754	1727.1
Isocalamendiol	47.55	0.18 ± 0.04	1725	1752.2
Total identified		**65.98**		
Total unidentified		**34.02**		

Retention time (Rt), retention indexes in the literature (RI_R), and retention indexes calculated (RI_E). Standard Deviation (SD) duplicated analysis.

Finally, 45 compounds were determined in EOEG, corresponding to a total of 54.00% (Table 5), the major compounds were α-cadinol (7.78 %), bornyl acetate (6.45 %), and caryophyllene oxide (5.96 %).

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Table 5. The chemical composition of EOEG.

Compound	**Rt (min)**	**Relative Abundance (%** ± SD**)**	**RI_R**	**RI_E**
β-Pinene	9.17	0.45 ± 0.01	961.7	949
Myrcene	9.90	0.23 ± 0.00	981	983
α-Phellandrene	10.38	0.12 ± 0.01	1007	1002
p-Cymene	11.27	1.16 ± 0.06	1025.4	1021
Limonene	11.44	0.19 ± 0.00	1018	1025
1,8-Cineole	11.55	0.02 ± 0.01	1023	1027
trans-β-Ocimene	12.46	0.03 ± 0.00	1034	1046
Linalool	14.83	0.25 ± 0.02	1081	1085
Fenchol	15.31	0.21 ± 0.03	1100	1107
Perillen	15.59	0.12 ± 0.01	1109	1113
(E)-*p*-2-Menthen-1-ol	15.72	0.25 ± 0.00	1123	1116
α-Campholenal	15.94	0.11 ± 0.01	1120	1121
cis-2-*p*-Menthen-1-ol	16.60	0.19 ± 0.02	1118	1135
(*Z*)-β-Terpineol	16.87	0.27 ± 0.00	1125	1141
Endo-Borneol	17.73	0.41 ± 0.03	1148	1159
Terpinen-4-ol	18.32	0.18 ± 0.01	1175	1172
α-Terpineol	18.95	2.03 ± 0.04	1172	1186
cis-Sabinol	19.47	0.74 ± 0.08	1179	1197
(*E*)-Carveol	19.81	0.37 ± 0.03	1206	1204
cis-Carveol	20.33	0.49 ± 0.08	1207	1216
Methylthymol	21.04	0.96 ± 0.02	1215	1232
Bornyl acetate	23.32	6.45 ± 0.14	1285	1283
Carvacrol	24.23	0.30 ± 0.01	1278	1303
Myrtenyl acetate	24.85	1.70 ± 0.21	1306	1317
α-Cubebene	26.07	0.09 ± 0.02	1350	1345
α-Copaene	27.17	0.17 ± 0.01	1376	1369
β-Bourbonene	27.54	0.19 ± 0.01	1386	1378

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Compound	**Rt (min)**	**Relative Abundance (%** ± SD**)**	**RI_R**	**RI_E**
Alloaromadendrene	29.79	0.69 ± 0.03	1459	1431
Aristolene	30.02	0.22 ± 0.04	1423	1437
α-Curcumene	31.73	1.60 ± 0.03	1472	1479
Carvacryl propionate	31.91	0.43 ± 0.15		1484
β-Bisabolene	32.13	0.81 ± 0.07	1500	1489
α-Muurolene	32.39	0.96 ± 0.03	1494	1496
γ-Cadinene	32.91	1.70 ± 0.03	1505	1509
δ-Cadinene	33.34	3.93 ± 0.03	1514	1519
Nerolidol	34.99	0.90 ± 0.04	1545	1560
Spathulenol	35.42	2.18 ± 0.01	1577	1571
Caryophyllene oxide	35.61	5.96 ± 0.35	1576	1576
Ledol	35.94	1.77 ± 0.08	1597	1584
(4-*tert*-Butylphenoxy)methyl acetate	36.44	0.21 ± 0.09	1563	1597
Humulene-1,2-epoxide	36.59	0.47 ± 0.06	1601	1600
Cubenol	37.35	0.76 ± 0.09	1631	1621
tau-Muurolol	37.89	4.88 ± 0.24	1628	1636
α-Muurolol	38.05	1.08 ± 0.21	1646	1641
α-Cadinol	38.40	7.78 ± 0.33	1641	1650
Total identified		**54.00**		
Total unidentified		**46.00**		

Retention time (Rt), retention indexes in the literature (RI_R), and retention indexes calculated (RI_E). Standard Deviation (SD) duplicated analysis.

Minimum inhibitory concentration

The antimicrobial activity of the four oils was tested *in vitro* on four yeasts, two Gram (+) bacteria and two Gram (-) bacteria. As controls were used fluconazole and itraconazole for yeasts and ciprofloxacin for bacteria. The results (table 6) showed that any of the EOs inhibited the growth of *C. krusei*. However the other three yeasts were sensitive to all EOs, and the highest antimicrobial activity was found against *C. albicans*, with an inhibition concentration of 62.5 µg/mL. *C. tropicalis* was inhibited by EOTA, EOTP and EOEG and the oils had activity on *C. glabrata* at 250 µg/mL. It should be noted that the EOs inhibited the growth of three yeasts examined, with MIC values ranging from 62.5–250 µg/mL, highlighting the inhibitory activity against *C. albicans* and *C. tropicalis* (Table 6).

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Table 6. Minimum inhibitory concentration of four EOs against four species of Candida.

Essential oils	MIC of yeast [µg/mL]			
	C. krusei	***C. glabrata***	***C. tropicalis***	***C. albicans***
EOTA	500	250	125	62.5
EODB	500	250	250	62.5
EOTP	500	250	125	62.5
EOEG	500	250	125	62.5
Fluconazole	0.97	1.95	0.97	0.24
Itraconazole	0.48	0.12	0.12	0.12

p value using ANOVA test p=0.0607

Table 7 shows the antibacterial activities of the EOs, with MIC values ranging from 125–500 µg/mL. The four oils presented mean inhibition of *S. aureus* and *P. aeruginosa* at a concentration of 125 µg/mL, except for EOTA, which inhibited *P. aeruginosa* at 500 µg/mL. However, the activity of EOTA against *E. coli* was highest at a concentration of 250 µg/mL with respect to EODB, EOTP and EOEG. In contrast, any EOs inhibited the growth of *E. faecalis.*

Table 7. Minimum inhibitory concentration of four EOs against Gram (+) and Gram (–), species.

EOs	MIC of bacteria [µg/mL]			
	Gram (+)		**Gram (-)**	
	S. aureus	***E. faecalis***	***E. coli***	***P. aeruginosa***
EOTA	125	500	250	500
EODB	125	500	500	125
EOTP	125	500	500	125
EOEG	125	500	NA	125
Ciprofloxacin	0.19	0.19	0.095	0.19

NA (not activity). p value using ANOVA test p=0.1104

Antioxidant activity (DPPH and ABTS)

The antioxidant activities of the EOs were calculated in terms of the radical scavenging activity (RSA) %, which reflects the capacity of the EOs to reduce the concentrations of the radicals DPPH and ABTS. The EOs EOTA, EODB and EOTP showed antioxidant activity. With DPPH the IC_{50} values were 0.814, 1.195 and 1.050 mg/mL, respectively, and with ABTS IC_{50} values were 0.183, 0.252, 0.137. However, EOEG had a lower antioxidant activity DPPH (IC_{50} = 3.480 mg/mL) and ABTS (IC_{50}= 0.410 mg/mL) (Table 8).

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Table 8. DPPH and ABTs radical scavenging activity of EOs.

EOs	DPPH		ABTS	
	RSA ± SE %	**IC_{50} [mg/mL]**	**RSA ± SE %**	**IC_{50} [mg/mL]**
EOTA	21 ± 1.61[a]	0.814	14 ± 0.54[ab]	0.183
EODB	20 ± 0.46[a]	1.195	10 ± 0.44[b]	0.252
EOTP	21 ± 0.32 [a]	1.050	22 ± 0.14[a]	0.137
EOEG	6 ± 0.091 [b]	3.480	7 ± 0.05[b]	0.410
Trolox	86 ± 0.70	0.005	90 ± 2.25	0.002

DPPH (1,1'-diphenyl-2-picrylhydrazine), ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid, RSA (radical scavenging activity). p value between EOs using Tukey´s test DPPH p<0.003 and ABTS p<0.009. Means not joined by the same letter show significant differences.

Discussion

Infectious diseases caused by microorganisms and their resistance to antimicrobials have increased the costs of hospital care as well as morbidity and mortality, making them some of the major public health problems [1,29]. Between 2016 and 2020, ESKAPE pathogens were the most isolated in hospitals [1], and candidiasis infections have increased in the last three decades [30]. The results of this study on ATCC microorganisms suggest need future research in clinical isolates.

In the present research, the inhibitory activity of EOTA against *Candida* strains and Gram (+) and Gram (-) bacteria was observed (Tables 6 and 7). This inhibition could be due to piperitone, whose antifungal effect has been described [31]. 1,8-cineol, a compound present in EOTA, inhibits the growth of different *Candida* species by blocking hyphal transition, the expression of genes that code for ergosterol biosynthesis (ERG11), and efflux pumps (CDR1 and CDR2) [32]. This suggests that in our study, these compounds are responsible for the inhibition of *C. glabrata, C. tropicalis* and *C. albicans*.

1,8-cineole also inhibits the growth of Gram (+) and Gram (-) bacteria by modifying the permeability of the bacterial membrane, an intracellular and morphological alteration of the cell, which could explain the inhibition observed for *S. aureus* and *E. coli* (125 and 250 µg/mL, respectively) [33].

Dihydrotagetone, the main bioactive component of EOTP, has antibacterial activity against Gram (+) and Gram (-) bacteria and also decreases the oxidative damage of food [34]. In our study, we observed antibacterial activity of EOTP on *S. aureus* and *P. aeruginosa* (125 µg/mL), as well as antifungal activity mainly on *C. albicans* and *C. tropicalis* (62.5 and 125 µg/mL, respectively).

Another bioactive compound is β-pinene, which is one of the main bioactive compounds identified in EODB, with antibacterial and antifungal activity. Rivas da Silva [35] documented its ability to inhibit the formation of biofilms in *C. albicans* and, consequently, the growth of this yeast. This effect is similar to the inhibition of growth observed for *C. albicans* at 62.5 µg/mL, and EODB was also able to inhibit *C. glabrata* and *C. tropicalis* (250 µg/mL). In contrast, the antibacterial activity against *S. aureus* and *P. aeruginosa* was 125 µg/mL. This may be related to the lipophilic nature of EO, which allows this oil to easily cross the cell wall, causing microbial death [36].

The main components of EOEG are α-cadinol, caryophyllene oxide and tau-Muurolol. This EO showed antifungal activity to the three yeasts studied, highlighting its activity to *C. albicans* and *C. tropicalis*; the antibacterial activity was the same as that presented by EODB to *S. aureus* and *P. aeruginosa*. Other authors also reported the antimicrobial activity of caryophyllene oxide to *S. aureus* [37].

The antimicrobial activities of the EOs tested in this study suggest that they can be used as alternatives in the treatment of nosocomial infections caused by multiresistant bacteria [38]. The EOs have antimicrobial

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activity, especially against different *Candida* strains. However, some *in vivo* studies about the toxicity of these oils will be done in the close future.

Oxidative stress is generated by an excess of free radicals and has been associated with different diseases such as atherosclerosis, cancer, hypertension [39] and infections [40]. The antioxidant capacity of the four oils was determined by scavenging-methods using DPPH and ABTS. These oils diminished stable radicals, but their antioxidant activity was low (table 8). Then, these results suggest that the antimicrobial and antioxidant effect are not related. In this study was determined the antioxidant capacity by two assays, because the DPPH assay determined radical dissolved in organic solvents then this assay is suitable to hydrophobic systems, whereas ABTS assay is useful to lipophilic and hydrophilic antioxidant systems [41]

Conclusions

The rise of multidrug resistant microbes has produced high rates of morbidity and mortality, therefore, one of the main challenges of researches is to find new efficient drugs to treat infectious diseases. Many EOs possess antimicrobial activity, which could be attributed to synergism between their components. In the future might explore the activity of the main compounds and the synergistic mechanism.

The results obtained of this study show that EODB, EOEG, EOTP have a low antioxidant activity, which might relate to their oxygenated components.

This study tested the antifungal activity of these EOs against the yeasts, *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis*, and against the bacteria, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The results show that EODB, EOEG, EOTP and EOEG inhibited the growth of bacteria Gram+ and Gram - also, they have antimicrobial activity against *C. glabrata*, *C. tropicalis* and *C. albicans*. The results of this study suggest need future research in clinical isolates.

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