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Mexican oregano (*Lippia graveolens*) essential oil-in-water emulsions: impact of emulsifier type on the antifungal activity of *Candida albicans*

S. E. Herrera-Rodríguez[1] · R. J. López-Rivera[2] · E. García-Márquez[3] · M. Estarrón-Espinosa[2] · H. Espinosa-Andrews[2]

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Abstract This study examined the impact of emulsifier type on the physicochemical characteristics and antifungal capacity of oregano oil-in-water emulsions: Tween 80, hydroxylated soy lecithin, and gum arabic. GC/MS analysis showed that the major components of the *Lippia graveolens* essential oils were thymol (31.7%), *p*-cymene (18.7%), and carvacrol (14.6%). The oil-in-water emulsions were made using ultrasonic technology in which thymol and carvacrol quantities were 12.26–13.67 g/L and 5.6–6.2 g/L, respectively. The droplet size of the emulsions followed the next descendent order: gum arabic > lecithin > T80. The zeta potential of the emulsions favored the stability against coalescence. Finally, the antifungal activity of the emulsions was evaluated, in which, 30 µL/mL of gum arabic or hydroxylated soy lecithin emulsions inhibited the growth of *Candida albicans*. The result suggests that Mexican oregano essential oil emulsions can be used as an antifungal against of *C. albicans.*

Keywords Mexican oregano essential oil · Phenolic compounds · Emulsions · Emulsifier agent · *Candida albicans*

✉ H. Espinosa-Andrews
hespinosa@ciatej.mx

[1] Biotecnología Médica y Farmacéutica, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., 44270 Guadalajara, Jalisco, Mexico

[2] Tecnología Alimentaria, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., CIATEJ, 45019 Zapopan, Jalisco, Mexico

[3] Unidad noreste, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., Apodaca, Nuevo León, Mexico

Introduction

Candida albicans is one of the most common opportunistic pathogens in humans that causes significant morbidity and mortality around the world (Soliman et al., 2015). This microorganism is often responsible for recurrent infections, starting from oral or skin diseases to life-threatening systemic infections in immunocompromised hosts (Costa et al., 2010). Triazole derivatives such as fluconazole and ketoconazole have been some of the most prescribed antifungal agents for candidiasis in the last decades. However, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) indicates that ketoconazole can cause a hepatic dysfunction (EMA, 2013; FDA, 2013). In addition to the above, the low susceptibility of *C. albicans* to some pharmaceutical drugs has led to the study of antimicrobial agents from natural resources (Costa et al., 2010; Ganbarov and Mammadova, 2012). Among the most well-known spices that have high antimicrobial activity are clove, cinnamon, thyme, and oregano (Escobar et al., 2010; Hammer et al., 1999). Their antimicrobial effect is attributed to several volatiles compounds such as thymol, eugenol, linalool, α-terpineol, and carvacrol. *Lippia graveolens* is a plant of the *Verbenaceae* family found in North and Central America, commonly known as Mexican oregano, generally consumed as a food seasoning and a folk remedy. The antimicrobial effects of the oregano essential oils are mainly attributed to its monoterpenes thymol and carvacrol, against microorganisms such as *C. albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, among others (Arana-Sánchez et al., 2010; Bhargava et al., 2015; Rodriguez-Garcia et al., 2016). Due to the lipophilic properties of monoterpenes, the cell membrane is expanded; causing an increase in the fluidity and permeability, disturbance of embedded

proteins, inhibition of respiration, and altered ion transport (Bhargava et al., 2015; Rodriguez-Garcia et al., 2016). However, the volatile nature of the bioactive compounds in Mexican oregano essential oils and its hydrophobicity makes it challenging to use it directly in foods or pharmaceuticals products.

Oil-in-water emulsions are one of the best technological solutions to incorporate essential oils in those products (McClements, 2007; McClements, 2010). Nowadays, small emulsions are produced using high energy technologies, like high-pressure homogenization, microfluidization or ultrasonication (Ghosh et al., 2013). The last one, the dispersed and continuous phases are subjected to ultrasound waves above 20 kHz. The high frequency generates mechanical vibrations causing the collapse of the droplets, thus decreasing the emulsion droplet size (Ghosh et al., 2013; Hashtjin and Abbasi, 2015). The emulsion formation, stability, and functional properties are principally influenced by the nature of the oil phase, type of emulsifier, oil-to-water ratio, droplet size, charge, and technique used (McClements, 2016; Tadros et al., 2004; Zhang and McClements, 2018). However, a few scientifically papers are published to investigate the effect of the ultrasound process on the antimicrobial potential of essential oils incorporated in emulsions. Black pepper (*Piper nigrum*) and cinnamon (*Cinnamomum zeylanicum*) essential oil emulsions were used as inhibitors against pathogens and food spoilage bacteria (Jiménez et al., 2018). Ermis et al. (2017) found enhanced antifungal activity in nanoemulsion containing oregano essential oils against *Aspergillus niger* as compared to that of free oregano essential oils. However, Donsì et al. (2012) found that the emulsifier agent used to produce nanoemulsion has a fundamental role in the antimicrobial activity of different essential oil components than the droplet size of the emulsion. This work aimed (1) to evaluate the composition of Mexican oregano essential oils using GC/MS; (2) to produce and characterize the Mexican oregano essential oil emulsions using three emulsifier agents; and (3) to evaluate the antifungal activity against *C. albicans* of the Mexican oregano essential oil emulsions.

Materials and methods

Chemical reagents

Lippia graveolens (Mexican oregano) essential oils were acquired in Nextipac, Jalisco, Mexico. Medium chain triglycerides (MCTs) oil was acquired from Gomas Naturales S.A. de C.V. (Mexico City, Mexico). Gum arabic (GA) Instant-gum was purchased from Nexira (Mexico City, Mexico), Tween 80 (T80) was purchased from Sigma-Aldrich (State of Mexico, Mexico). Hydroxylated soy lecithin Emulfluid HL 66 (HSL) was obtained from Cargill (Mexico City, Mexico). *C. albicans* (ATCC 10231) was used to evaluate the antifungal activity. Thymol and carvacrol standards (95% of purity) were bought from Sigma-Aldrich (State of Mexico, Mexico).

Chromatographic analysis of the Mexican oregano essential oil

The Mexican oregano essential oil was analyzed using a Hewlett Packard GC 5890 Series II gas chromatograph coupled with a Mass Selective Detector HP 5972 (Hewlett Packard, Santa Clara, CA, USA) (Castillo-Herrera et al., 2007). The sample (0.2 µL) was injected in the split mode. Compounds were separated in a polyethylene glycol HP-FFAP capillary column (50 m × 0.22 mm i.d., film thickness 0.33 µm; Agilent Technologies, Inc., Santa Clara, CA, USA). Helium was the carrier gas at 0.8 mL/min. The injector and detector temperatures were operated at 250 °C and 280 °C, respectively. The oven temperature was programmed as follows: 80 °C raised to 185 °C (5 °C/min) and held for 9 min; then increased to 220 °C (10 °C/min) and kept for 5 min. The data acquisition was made in SCAN mode and EI of 70 eV. Identification of the essential oil components was made by comparing their mass spectra with those provided by the Wiley library 275L, as well as from the retention times of some pure compounds (Sigma-Aldrich > 95%) injected under identical analytical conditions. The quantification was based on the percentage of the area from the automatic integration of the peaks detected. Furthermore, identical chromatographic conditions were also applied for thymol and carvacrol quantification in the Mexican oregano essential oil previously diluting in ethanol (1:20 v/v), analyzing in SIM mode. Calibration curves based on external standardization with concentration ranges of 2.4–12 g/L for thymol and carvacrol were used. The measurements were performed in duplicate.

Emulsions preparation

The Mexican oregano essential oil was diluted with MCTs oil at a mass ratio of 1:1. Two groups of emulsions were developed, one group with the Mexican oregano essential oil plus MCTs oil and the other group using only the MCTs oil, i.e., negative control. Each group included three emulsions with the different emulsifier agents (GA, HSL, & T80). All the emulsions were manufactured using a dispersed-phase volume fraction 0.10 (Essential oil plus MCTs oil) with an emulsifier/oil mass ratio of 0.5. The primary emulsions were obtained at 9500 rpm for five min using a high shear disperser (Ultra-Turrax T25, IKA

Works, Inc., Wilmington, NC, USA). Then, the fine emulsion was produced using an ultrasonic processor FB50 (FisherBrand, Pittsburgh, PA, USA) with a 3 mm stainless steel probe applying a 50% of amplitude for 15 min. The homogenization temperature was maintained lower than 40 °C using a circulation water bath FP50 (Julabo GmbH, Seelbach, Germany).

Quantification of thymol and carvacrol in the emulsions

Identical conditions used in the Mexican oregano essential oil chromatographic measurements were applied to determine the concentration of thymol and carvacrol in the emulsions stabilized whit the different emulsifier agents.

Droplet size distribution

The droplet size (*DS*) of the emulsions was measured using a Zetasizer Nano-ZS90 (Malvern Instruments, Malvern, UK) (García-Márquez et al., 2017). The Stokes–Einstein equation calculated the droplet size: $DS = kBT/3\pi\eta_s D$; where kB is the Boltzmann constant, T is the temperature (K), η_s is the viscosity of the solvent and D is the Z-average translational diffusion coefficient. Each emulsion was diluted 1:100 with distilled water. Measurements were made using glass cuvettes type ZEN0118 (Malvern Instruments). A refraction index of 1.334 and an absorption index of 0.001 were used (Bhattacharjee, 2016). The change of the droplet size of the emulsions was evaluated for 28 days storing the samples at two temperatures (25 and 40 °C). Results were reported as the mean and the standard deviation of two different samples with three replicates per sample.

Zeta potential

The zeta potential was determined using an electrophoretic light scattering equipment Zetasizer Nano ZS90. The measurements were carried out using the DTS1061 cell (ZEN1002, Malvern Instruments) at 25 °C. The zeta potential was correlated with the emulsion droplets velocity into an electric field using the Smoluchowsky model (García-Márquez et al., 2015). Results were reported as the mean of duplicate measurements and the standard deviation.

Microorganism

Candida albicans used in the antifungal assay were obtained from the ATCC 10231. Stock inoculators (suspensions) of *C. albicans* were prepared from YPD agar, the cultures growth at 28 °C temperature. Fungal colonies were conserved by 2 weeks for further assays at 4 °C.

Preparation of inoculum and antifungal assay

The inoculum was prepared from a new colony of *C. albicans* in 5 mL of YPD culture media, was growth at 37 °C for 16 h using a rotary shaker (250 revolutions per min). From the inoculum, a volume of 250 µL was added in 5 mL of fresh YPD media, was incubated (same conditions), until reach an optical density (OD_{600} = 0.5) using microplate absorbance spectrophotometer (xMark, BioRad Laboratories, Inc. Hercules, CA, USA), in which the concentration of *C. albicans* (log phase) suspension was approximately 10^6 colony forming units per mL (CFU/mL). Based on previous results (López-Rivera et al., 2018), different concentrations of thymol and carvacrol were adding from the emulsions for antifungal assays. Aliquots of the emulsions in the range of 0.5–30 µL were added into one mL of *C. albicans* (Log phase) and were incubated at 37 °C for 16 h using a rotary shaker (KS 3000 i control, IKA Works, Inc., Wilmington, NC, USA). Then, the surviving organism's number was determined after incubation into agar YPD (37 °C for 24 h) using dilutions since 10^3–10^7. Control emulsion without essential oils was prepared with MCTs alone, and Ketoconazole (0.15 µg/mL) was used as the positive control for growth inhibition. All experiments were made in triplicate.

Statistical analysis

Data were analyzed using the program Statgraphics Centurion XVII (Version 17.0.16 Statpoint Technologies, Inc., Warrenton, VA, USA). Results were expressed as the mean ± standard deviation and statistical significance was determined by the Tukey test ($p > 0.05$ was considered not significant).

Results and discussion

GC/MS analysis

The Mexican oregano essential oils are complex mixtures of the volatile compounds, commonly applied to inhibit bacterial and fungal infections, parasites, inflammation, and candidiasis. Thirty major compounds were identified in the Mexican oregano essential oils by GC/MS analysis (Table 1). These compounds represented the 95.7% of the total oil composition. Monoterpenes hydrocarbons, oxygenated monoterpenes, sesquiterpenes hydrocarbons, and phenols were some of the compounds groups founded. Thymol (31.7%) was the major compound identified in the

Table 1 Major compounds in Mexican oregano essential oils determined by gas chromatography–mass spectrometry analysis

Compound	Retention time (min)	Mexican oregano essential oils % Peak area
Monoterpene hydrocarbons		
α-Thujene	4.30	2.41
Camphene	4.69	0.63
β-Pinene	5.09	0.16
β-Myrcene	5.48	1.68
α-Terpinene	5.87	1.68
Limonene	6.11	1.35
γ-Terpinene	6.82	2.42
p-Cymene	7.29	18.72
Oxygenated monoterpenes		
1,8-Cineole	6.35	3.44
trans-Sabinene hydrate	10.91	0.22
Linalool	12.49	0.70
cis-Sabinene hydrate	12.80	0.21
Terpinen-4-ol	13.99	1.55
p-Menth-2-en-1-ol	14.54	0.14
Borneol	16.11	1.25
Caryophyllene oxide	22.25	0.42
Sesquiterpene hydrocarbons		
trans-α-Bergamotene	13.28	0.49
β-Caryophyllene	13.83	5.62
α-Humulene	15.40	2.97
β-Bisabolene	16.43	0.41
Phenolic monoterpenes		
Thymol methyl ether	13.59	1.28
Thymol acetate	19.26	0.44
Carvacrol acetate	19.73	0.15
Thymol	27.06	31.66
Carvacrol	28.08	14.57
Carbonyl compounds		
1-Octen-3-ol	10.36	0.53
Others		
p-Cymenene	10.52	0.14
Benzoic acid	18.24	0.18
trans-Carveol	18.95	0.11
Benzaldehyde	21.07	0.19

sample, followed by *p*-cymene (18.7%) and carvacrol (14.6%). Castillo-Herrera et al. (2007) reported similar phenolic monoterpenes in Mexican oregano essential oils, in which thymol (27.3%) was the main constituent followed by *p*-cymene (14.2%) and carvacrol (12.3%). Rodriguez-Garcia et al. (2016) described that the major components in *L. graveolens* essential oils were carvacrol (47.4%), *p*-cymene (26.4%), and thymol (3.0%). However, Pradebon Brondani et al. (2018) found that the main compounds present in the essential oils from *Origanum vulgare* were 4-terpineol (41.2%), thymol (21.9%), γ-terpinene (5.9%), and carvacrol (4.7%). The variations in the composition of the essential oils are expected, due to several factors can change their composition as the harvest season, geographical sources, part of the plant, growth conditions, drying method, and extraction method (Arana-Sánchez et al., 2010; Castillo-Herrera et al., 2007; Rodriguez-Garcia et al., 2016).

Physicochemical properties of the emulsions

Three emulsions with different emulsifier agents were prepared using ultrasonication. The droplet size and zeta potential of emulsions are shown in Table 2. Based on their droplet size, emulsions are commonly classified as macroemulsion (diameters between 100 and 100 μm), and nanoemulsion (diameters between 20 and 200 nm) (Tadros et al., 2004; Zhang and McClements, 2018). The droplet size of the emulsions followed the next order: GA > HSL > T80. The use of T80 enhances the formation of nanoemulsion (< 200 nm), due to their higher HLB value and compatibility of the system (Bhargava et al., 2015; Ghosh et al., 2013; Hashtjin and Abbasi, 2015). Similarly, the HSL produced droplets size around of 200 nm, while the GA showed the highest droplet size (> 1 μm). The highest interfacial affinity, smaller size, and lower molecular weight of the T80 and HSL allowed them to adsorb at the interface very quickly, contributing to the production of small droplets in comparison with GA (Bouyer et al., 2012). The highly branched structure and viscoelasticity of the carbohydrate chains of GA can dissipate the energy supplied at the emulsion diminishing their efficacy to reduce the particle size compared to low molecular weight emulsifiers, e.g., T80 (Walstra, 1993). For T80 and HSL nanoemulsions, significant smallest droplet sizes were obtained for the emulsion group made with the oregano essential oil, compared with those emulsions produced with MCTs oil.

Significant zeta potential values can be related to the emulsion stability assessment. The zeta potential of the emulsions depends on the nature of the emulsifier agent used, e.g., anionic or cationic. Even though T80 is a non-ionic emulsifier (Ghosh et al., 2013), its emulsions showed a negative charge due to the anionic charge of free fatty acids and polar components in the oil phase (Bhargava et al., 2015). The emulsions prepared with the hydroxylated lecithin had the highest zeta potential values, a characteristic that promoted good physical stability of the emulsion due to strong electrostatic repulsion forces (McClements, 2016).

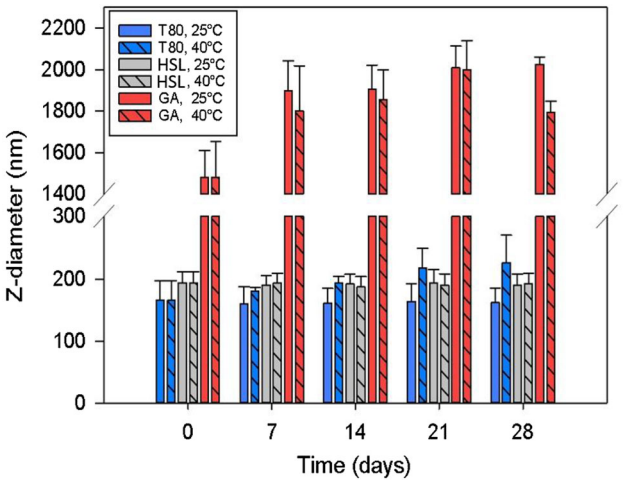

Fig. 1 Particle size trend of emulsions storage at three temperatures (25 and 40 °C): **A** Tween 80 (Blue bars), **B** Hydroxylated soy lecithin (gray bars), and **C** Gum arabic (Red bars). (Color figure online)

The physical stability of the emulsions can be affected significantly by the storage temperature. An emulsion is physically stable if no significant change of the droplet size occurs independently of the time and temperature of storage (Bodolato et al., 2008). The droplet size of the emulsions under different storage conditions are shown in Fig. 1. The T80 emulsion showed no significant differences ($p > 0.05$) in mean droplet size over 28 days of storage at 25 °C. This increase was more evident at 40 °C because the rise in temperature causes dehydration of the oxyethylene groups of the T80 and as a consequence, their interfacial activity decrease (Rosen, 2004). The HSL emulsion showed excellent stability in all temperatures tested. On the other hand, the emulsion prepared using gum arabic as emulsifier was unstable, creaming since day seven. Finally, no significant differences were found in the zeta potential values for each group of emulsifier agent at the different temperatures tested (data not shown).

Table 2 Droplet size and zeta potential values of the emulsions produced with and without Mexican oregano essential oils and different type of emulsifier agent

Emulsions	Z-diameter (nm)		Zeta potential (mV)	
	With EO	Without EO	With EO	Without EO
T80	137.3 ± 4.2^{Bc}	193.4 ± 8.4^{Ab}	-27.3 ± 1.3^{Ac}	-26.1 ± 0.4^{Ac}
HSL	179 ± 5.5^{Bb}	208.5 ± 8.8^{Ab}	-70.3 ± 1.8^{Aa}	-64.7 ± 1.9^{Ba}
GA	1597 ± 11.1^{Aa}	1365 ± 21.4^{Ba}	-40.3 ± 0.5^{Ab}	-39.3 ± 1.0^{Ab}

Common capital letters are significantly different ($p < 0.05$) between columns. Common lower-case letters are significantly different ($p < 0.05$) between rows. Mean ± SD (n = 3). Tween 80 (T80), Hydroxylated lecithin (HSL), gum arabic (GA), and Mexican oregano essential oils (EO)

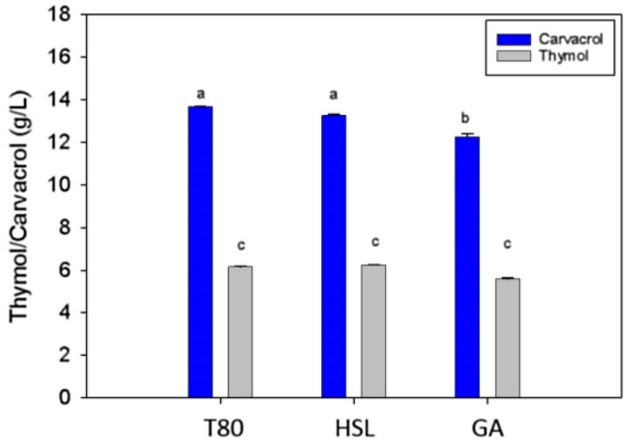

Fig. 2 Thymol and carvacrol concentration contained in the emulsions: **A** T80 (Tween 80), **B** HSL (Hydroxylated soy lecithin), and **C** GA (Gum arabic)

Antifungal activity of oregano oil emulsions against *C. albicans*

Antifungal activity of the oregano oil emulsions was evaluated measuring the inhibition of the growth of *C. albicans*. The biological properties of the essential oils are attributed to terpenes, terpenoids, aromatic, and aliphatic constituents. Several works in the literature have shown that essential oils with significant amounts of thymol and carvacrol have antibacterial effects in the different strain. For example, Botelho et al. (2007) reported that *Lippia sidoides* essential oils, thymol, and carvacrol have antibacterial activity against *S. mutans*. Also, Szczepanik et al. (2018) identified the antifungal activity of Greek oregano essential oils rich in carvacrol (84.4%) against *C. albicans*. In this sense, the thymol and carvacrol monoterpenes were analyzed because both monoterpenes are recognized as the most potent antifungal compounds of the Mexican oregano essential oils (Hernández-Hernández et al., 2014; Rodriguez-Garcia et al., 2016). The amount of thymol and carvacrol in the emulsions were estimated by GC/MS (Fig. 2). The concentration of thymol and carvacrol in the Mexican oregano essential oils was 262.5 ± 0.6 g/L and 122.0 ± 2.1 g/L, respectively. The molar ratio of thymol and carvacrol was 2.15 (mol thymol/mol carvacrol). The concentration of carvacrol present in the emulsions did not show a significant difference between all the emulsions ($p > 0.05$). However, the thymol

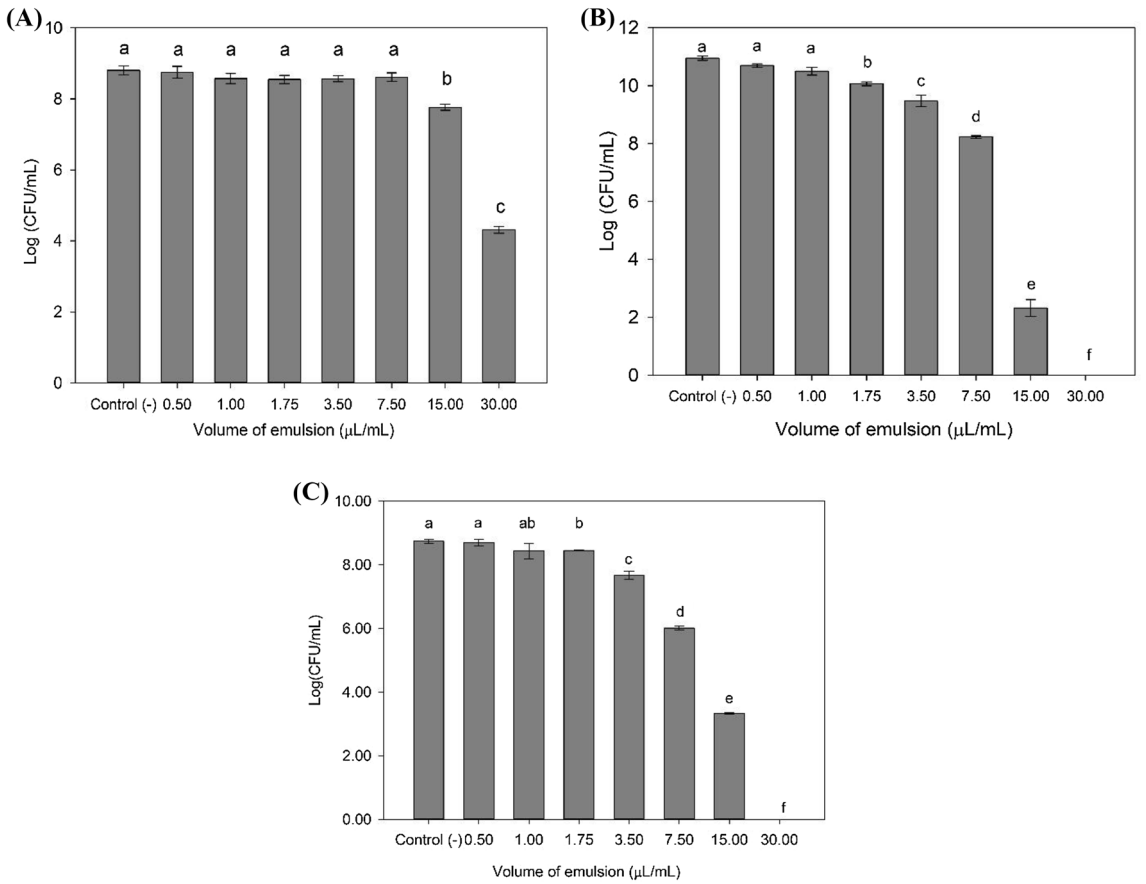

Fig. 3 Antifungal activity of major monoterpenes concentration (thymol and carvacrol) against *C. albicans*. **A** Tween 80, **B** Hydroxylated soy lecithin, and **C** Gum arabic. Columns having common letters are not significantly different ($p > 0.05$)

concentration was estimated 10% lower for the GA emulsion presumably due to the high branched structure of GA not allowed a complete separation procedure of thymol from the GA emulsion by the GC/MS.

Figure 3A–C show the antifungal activity of the Mexican oregano essential oil emulsions against *C. albicans.* Our results showed that the nature of the emulsions influenced its ability to inhibit the growth of *C. albic*ans during the in vitro test. As expected, the MCTs emulsions (30 µL/mL) did not show inhibitory effect on the *C. albicans* growth when were compared with those produced with the essential oil at the same concentration. For example, the addition of volumes lower than 7.5 µL/mL of the T80 emulsion did not show antifungal effect. The minimum lethal concentration (MLC_{99}) was estimated with a concentration of 21.5 µL/mL for the T80 emulsion, equivalent to a 293 µg/mL and 132 µg/mL of thymol and carvacrol, respectively (Fig. 3A). A similar antimicrobial effect for HSL or GA emulsion was found, where complete fungal inhibition was achieved at 30 µL/mL. Figure 3B shows that the addition of 7.5 µL/mL and 15 µL/mL of the HSL emulsion for an initial fungal population of 10.5 log CFU/mL reduced 3 log CFU/mL and 8 log CFU/mL of *C. albicans*, respectively. The MLC for the HSL emulsion was estimated at a concentration of 6.8 µL/mL, equivalent to an 83 µg of thymol/mL and 38 µg of carvacrol/mL. Figure 3C shows that the addition of 7.5 µL/mL and 15 µL/mL of the GA emulsion reduced 3-log and 5-log of *C. albicans*, respectively. The MLC for the GA emulsion was estimated at a concentration of 6.4 µL/mL, equivalent to an 85 µg of thymol/mL and 40 µg of carvacrol/mL. The addition of 0.15 µg/mL of ketoconazole as a positive control inhibited the *C. albicans* growth effectively, as we expected.

It is well-known that active compounds of essential oils inhibit the growth of microorganisms by disturbing and damaging the cytoplasmic and protein membrane. The efficacy to inhibit the *C. albicans* growth was dependent on the type of emulsifier applied. Based on the droplet size of the emulsions, we expected that the T80 emulsion was the most effective against *C. albic*ans; conversely, with GA and HSL emulsions due to its particle size. However, our experiments did not show an interrelationship between the inhibition growth effect and the small droplet size, indicating that formulation of the emulsion influenced their ability to interact with the *C. albic*ans. This finding agrees with previous studies on nanoemulsion, which found that the antimicrobial activity of nanoemulsions was higher than large emulsions because they can diffuse and disrupt the membrane of microorganisms easily (Donsì et al., 2012; Donsì and Ferrari, 2016; Ghosh et al., 2013). Jiménez et al. (2018) reported that the antimicrobial activity depended on the droplet size and the composition of the essential oil emulsions. However, Salvia-Trujillo et al. (2013) observed that the antimicrobial activity of lemon myrtle oil emulsion did not depend on the droplet size (100–100 µm). Our result shows that the nature of the emulsifier agent determined the droplet size, charge, and antifungal activity of the emulsion.

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Compliance with ethical standards

Conflict of interest None of the authors has any financial interest or conflict with industries or parties.

Human and animal rights statement This article does not contain any studies with human or animal subjects.

References

Arana-Sánchez A, Estarrón-Espinosa M, Obledo-Vázquez EN, Padilla-Camberos E, Silva-Vázquez R, Lugo-Cervantes E. Antimicrobial and antioxidant activities of Mexican oregano essential oils (*Lippia graveolens H. B. K.*) with different composition when microencapsulated in β-cyclodextrin. Lett. Appl. Microbiol. 50(6): 585–590 (2010)

Bhargava K, Conti DS, da Rocha SRP, Zhang Y. Application of an oregano oil nanoemulsion to the control of foodborne bacteria on fresh lettuce. Food Microbiol. 47: 69–73 (2015)

Bhattacharjee S. DLS and zeta potential—What they are and what they are not? J. Control Release 235: 337–351 (2016)

Bodolato GG, Aguilar F, Schuchmann HP, Sobisch T, Lerche D. Evaluation of long term stability of model emulsions by multisample analytical centrifugation. Progr. Colloid Polym. Sci. 134: 66–73 (2008)

Botelho MA, Nogueira NAP, Bastos GM, Fonseca SGC, Lemos TLG, Matos FJA, Montenegro D, Heukelbach J, Rao VS, Brito GAC. Antimicrobial activity of the essential oil from Lippia sidoides, carvacrol and thymol against oral pathogens. Braz. J. Med. Biol. Res. 40: 349–356 (2007)

Bouyer E, Mekhloufi G, Rosilio V, Grossiord J-L, Agnely F. Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: Alternatives to synthetic surfactants in the pharmaceutical field? Int. J. Pharm. 436(1): 359–378 (2012)

Castillo-Herrera GA, García-Fajardo JA, Estarrón-Espinosa M. Extraction method that enriches phenolic content in oregano (*Lippia graveolens H.B.K.*) essential oil. J. Food Process Eng. 30(6): 661–669 (2007)

Costa CR, Jesuíno RSA, de Aquino Lemos J, de Fátima Lisboa Fernandes O, Hasimoto e Souza LK, Passos XS, do Rosário Rodrigues Silva M. Effects of Antifungal Agents in Sap Activity of *Candida albicans* Isolates. Mycopathologia 169(2): 91–98 (2010)

Donsì F, Annunziata M, Vincensi M, Ferrari G. Design of nanoemulsion-based delivery systems of natural antimicrobials: effect of the emulsifier. J. Biotechnol. 159(4): 342–350 (2012)

Donsì F, Ferrari G. Essential oil nanoemulsions as antimicrobial agents in food. J. Biotechnol. 233: 106–120 (2016)

EMA. European Medicines Agency recommends suspension of marketing authorisations for oral ketoconazole (2013). http://www.ema.europa.eu. (Accessed 25/01/2018)

Ermis E, Cicek PK, Meral R, Yilmaz A, Dertli E, Yilmaz MT. Nanoemulsion-based delivery systems to improve functionality

of oregano essential oil: Molecular characterization and in vitro antifungal activity. J. Biotechnol. 256: S35 (2017)
Escobar P, Milena Leal S, Herrera LV, Martinez JR, Stashenko E. Chemical composition and antiprotozoal activities of Colombian Lippia spp essential oils and their major components. Mem. Inst. Oswaldo Cruz 105: 184–190 (2010)
FDA. FDA Drug Safety Communication: FDA limits usage of Nizoral (ketoconazole) oral tablets due to potentially fatal liver injury and risk of drug interactions and adrenal gland problems (2013). https://www.fda.gov. (Accessed 25/01/2018)
Ganbarov K, Mammadova R. Antibacterial activity of essential oils of aromatic plants. J. Biotechnol. 161: 27 (2012)
García-Márquez E, Higuera-Ciapara I, Espinosa-Andrews H. Design of fish oil-in-water nanoemulsion by microfluidization. Innov. Food Sci. Emerg. Technol. 40(Supplement C): 87–91 (2017)
García-Márquez E, Román-Guerrero A, Cruz-Sosa F, Lobato-Calleros C, Álvarez-Ramírez J, Vernon-Carter EJ, Espinosa-Andrews H. Effect of layer (calcium phosphate-chitosan)-by-layer (mesquite gum) matrix on carotenoids-in-water-emulsion properties. Food Hydrocoll. 43: 451–458 (2015)
Ghosh V, Mukherjee A, Chandrasekaran N. Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. Ultrason. Sonochem. 20: 338–344 (2013)
Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86: 985–990 (1999)
Hashtjin AM, Abbasi S. Optimization of ultrasonic emulsification conditions for the production of orange peel essential oil nanoemulsions. J. Food Sci. Technol. 52: 2679–2689 (2015)
Hernández-Hernández E, Regalado-González C, Vázquez-Landaverde P, Guerrero-Legarreta I, García-Almendárez BE. Microencapsulation, chemical characterization, and antimicrobial activity of Mexican (*Lippia graveolens H.B.K.*) and European (*Origanum vulgare L.*) oregano essential oils. The Scientific World Journal 1–12 (2014) https://doi.org/10.1155/2014/641814
Jiménez M, Domínguez JA, Pascual-Pineda LA, Azuara E, Beristain CI. Elaboration and characterization of O/W cinnamon (*Cinnamomum zeylanicum*) and black pepper (*Piper nigrum*) emulsions. Food Hydrocoll. 77: 902–910 (2018)
López-Rivera RJ, Espinosa-Andrews H, García-Márquez E, Herrera-Rodríguez SE. Efecto antifúngico de emulsiones a base de aceite esencial de orégano mexicano (*Lippia graveolens*) contra *Candida albicans*. Rev. Sal. Jal. 5(1): 42–45 (2018)
McClements DJ. Critical review of techniques and methodologies for characterization of emulsion stability. Crit. Rev. Food Sci. 47: 611–649 (2007)
McClements DJ. Emulsion Design to Improve the Delivery of Functional Lipophilic Components. Annu. Rev. Food Sci. T. 1: 241–269 (2010)
McClements DJ. Emulsion Ingredients. pp. 125–149. In: Food Emulsion Ingredients: Principles, Practices, and Techniques. McClements DJ (ed). Taylor & Francis Group, Boca Raton, FL, USA (2016)
Pradebon Brondani L, Alves da Silva Neto T, Antonio Freitag R, Guerra Lund R. Evaluation of anti-enzyme properties of Origanum vulgare essential oil against oral *Candida albicans*. J. Mycol. Med. 28: 94–100 (2018)
Rodriguez-Garcia I, Cruz-Valenzuela MR, Silva-Espinoza BA, Gonzalez-Aguilar GA, Moctezuma E, Gutierrez-Pacheco MM, Tapia-Rodriguez MR, Ortega-Ramirez LA, Ayala-Zavala JF. Oregano (*Lippia graveolens*) essential oil added within pectin edible coatings prevents fungal decay and increases the antioxidant capacity of treated tomatoes. J. Sci. Food Agric. 96: 3772–3778 (2016)
Rosen MJ. Adsorption of Surface-Active Agents at Interfaces: The Electrical Double Layer. pp. 34–104. In: Surfactants and Interfacial Phenomena, Rosen MJ (ed). John Wiley & Sons, Inc., Hoboken, NY, USA (2004)
Salvia-Trujillo L, Rojas-Graü MA, Soliva-Fortuny R, Martín-Belloso O. Effect of processing parameters on physicochemical characteristics of microfluidized lemongrass essential oil-alginate nanoemulsions. Food Hydrocoll. 30(1): 401–407 (2013)
Soliman AM, Fahmy SR, Mohamed WA. Therapeutic efficacy of chitosan against invasive candidiasis in mice. J. Basic Appl. Zool. 72: 163–172 (2015)
Szczepanik M, Walczak M, Zawitowska B, Michalska-Sionkowska M, Szumny A, Wawrzeńczyk C, Brzezinska MS. Chemical composition, antimicromicrobial activity and insecticidal activity against the lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) of *Origanum vulgare* L. ssp. hirtum (Link) and *Artemisia dracunculus* L. essential oils. J. Sci. Food Agricul. 98: 767–774 (2018)
Tadros T, Izquierdo P, Esquena J, Solans C. Formation and stability of nano-emulsions. Adv. Colloid Interface Sci. 108–109: 303–318 (2004)
Walstra P. Principles of emulsion formation. Chem. Eng. Sci. 48: 333–349 (1993)
Zhang Z, McClements DJ. Overview of Nanoemulsion Properties: Stability, Rheology, and Appearance. pp. 21–49. In: Nanoemulsions: Formulation, Applications, and Characterization. Jafari SM, McClements DJ (ed). Academic Press (2018)