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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF FIVE SELECTED ESSENTIAL OILS

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ABSTRACT

Essential oils, also known as volatile oils, are aromatic compounds extracted from medicinal and aromatic plants through various methods, including steam distillation, cold or hot pressing, aqueous infusion, and solvent extraction. Composed of a complex mixture of terpenes, terpenoids, hydrocarbons, oxides, alcohols, aldehydes, ketones, phenols, and esters, these oils exhibit a wide range of biological properties such as antibacterial, antioxidant, anti-inflammatory, analgesic, relaxing, allelopathic, repellent, and insecticidal effects. Their bioactivity, combined with the growing preference for natural, low toxicity compounds, supports their application across multiple industries, including pharmaceuticals, food, agriculture, and textiles. This study investigated the antimicrobial activity of five commercial essential oils, lavender, lavandin, lemongrass, peppermint, and oregano, against four microbial strains: Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, and Candida albicans. Essential oils (1, 5, 10, and 15 µL) were diluted in selective media (1:100, 1:20, 1:10, 1:7), and microbial growth was measured by optical density at 600 nm before and after 22 hours of incubation at 30°C and 500 rpm. Results showed that lemongrass, lavender, and oregano oils exhibited the strongest inhibitory effects at the lowest tested volume (1 µL of essential oil in 100 µL total culture volume), while lavender and peppermint oils showed the weakest inhibition at the same concentration. These findings highlight the antibacterial potential of certain essential oils as promising alternatives to conventional antimicrobial agents.

KEY WORDS*: essential oils, volatile compounds, plant extracts, bacterial strains, fungal strains, bacteriostatic effect, bactericidal effect*

INTRODUCTION

Essential oils, also termed volatile or ethereal oils, are concentrated hydrophobic liquids obtained from specialized secretory structures of aromatic and medicinal plants by methods such as steam distillation, cold or hot pressing, aqueous infusion, solvent extraction, and supercritical fluid extraction (Dhifi et al. 2016). First coined in the sixteenth century by Paracelsus to translate the medieval concept of "Quinta essentia," the term now denotes a complex mixture of monoterpenes, sesquiterpenes, diterpenes, aromatic hydrocarbons, alcohols, aldehydes, ketones, esters, lactones, and phenols, each contributing to the characteristic aroma and bioactivity of the oil (Dhifi et al. 2016).

Chemical composition of essential oils varies greatly with plant species, organ of harvest, developmental stage, harvest time, soil composition, and extraction technique (Russo et al. 2015). For example, oils extracted from *Lavandula angustifolia* flowers display high linalool and linalyl acetate content, whereas *Cinnamomum verum* bark yields cinnamaldehyde-rich fractions. Such variability modulates biological efficacy and necessitates thorough phytochemical profiling prior to application (Jugreet et al. 2020).

The alarming rise of antibiotic resistant pathogens has driven research into plant-derived antimicrobials as viable alternatives (Vasantha et al. 1970). Essential oils rich in phenolic constituents, such as oregano's carvacrol and thymol, exhibit potent *in vitro* inhibition of Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli* (Piccardi and Manissier 2009). In contrast, oils dominated by hydrocarbons or esters tend to show weaker antibacterial effects (Dhifi et al. 2016).

Quantitative assays reveal multiple mechanisms by which essential oil components disrupt microbial viability: cell wall degradation, cytoplasmic membrane damage, increased permeability with consequent leakage of cellular contents, inhibition of membrane bound protein functions, collapse of proton motive force, and ATP depletion (Pankey and Sabath 2004; Dmitriev et al. 2004). These multifaceted actions reduce the likelihood of rapid resistance development (Pankey and Sabath 2004).

Beyond antimicrobial properties, essential oils possess significant antioxidant activity, neutralizing reactive oxygen species and inhibiting lipid peroxidation (Tit et al. 2023). Their rich content of phenolic and carotenoid derivatives enhances endogenous enzymatic defences, upregulating catalase, superoxide dismutase, and glutathione peroxidase, thereby protecting cellular components from oxidative damage (Tit et al. 2023).

Anti-inflammatory effects of essential oils derive from suppression of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), inhibition of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), and upregulation of

anti-inflammatory mediators (IL-10) (Shen et al. 2017). Such modulation of MAPK, JNK, p38, and NF-κB signaling pathways underlies their efficacy in reducing tissue edema and inflammatory pain (Shen et al. 2017).

In oncology contexts, fermented nutraceuticals enriched with antioxidant oil extracts have demonstrated reduction in skin aging markers and may enhance chemotherapeutic outcomes by modulating detoxification enzymes, DNA repair pathways, anti-angiogenic, and anti-metastatic processes (Bertuccelli et al. 2016; Gautam et al. 2014). Lipophilic essential oil constituents further induce apoptosis and necrosis in cancer cells by disrupting mitochondrial membranes and triggering caspase cascades (Gautam et al. 2014).

Agricultural applications exploit essential oils' allelopathic and insecticidal actions. Oils rich in oxygenated monoterpenes inhibit weed seed germination and seedling growth by compromising membrane permeability and chlorophyll content (Benchaa et al. 2018). Insecticidal mechanisms target acetylcholinesterase, GABA-gated chloride channels, and proton pumps, offering environmentally benign pest control alternatives (Rattan 2010).

The multifunctional bioactivity of essential oils has led to their widespread incorporation across industries. In food packaging, thyme and oregano oil-infused edible films extend shelf life by suppressing foodborne pathogens (Dao et al. 2018). Cosmeceutical formulations leverage their antioxidant, anti-inflammatory, and antimicrobial properties to develop creams, serums, and masks that protect and rejuvenate skin (Michalak et al. 2021; Draelos 2010). As demand grows for natural, low toxicity ingredients, essential oils stand poised to meet needs in pharmaceuticals, agriculture, textiles, and beyond.

In particular, while many studies have focused on individual oil constituents or specialty applications, few have directly compared the antimicrobial potency of commercially available essential oils against both bacterial and fungal pathogens in parallel. Therefore, the present work was undertaken to (i) assess the antimicrobial activity of selected essential oils, lavender, lavandin, lemongrass, peppermint, and oregano, against representative Gram-positive, Gram-negative, and yeast strains, and (ii) quantify their effects on microbial viability. By providing a rigorous, side by side comparison of volatile oil efficacy, this study aims to identify promising natural alternatives for infection control and to inform the development of safer, plant based antimicrobial formulations.

MATERIALS AND METHODS

Reagents. All culture media components and reagents were of analytical grade and sourced from reputable suppliers. Luria-Bertani (LB) and yeast-peptone-dextrose (YPD) media were prepared using agar (Carl Roth, Germany), yeast extract (Difco, USA), peptone (Carl Roth, Germany), sodium chloride (Carl Roth, Germany), and D-glucose (Carl Roth, Germany). The pH of liquid LB medium was adjusted to 7.4 with sodium hydroxide (Carl Roth, Germany) using a Mettler Toledo pH meter (Mettler Toledo, USA). All solid and liquid media, glassware, and pipette tips were sterilized at 121°C for 20 minutes in a LABOCON autoclave (LABOCON, UK).

Microorganisms strains. The bacteria *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and the yeast *Candida albicans* ATCC 10231 were purchased from American Type Culture Collection, Manassas, Virginia, USA. *Enterobacter cloacae* DSM 106614 bacterium was acquired from Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany.

Essential oils. Four commercially available essential oils were selected for antimicrobial testing. Lavender oil (Fares) was steam distilled from the flowers of *Lavandula angustifolia* and is characterized primarily by linalool and linalyl acetate. Lavandin oil (Profissimo DM), obtained by steam distillation of the flowers, leaves, and stems of *Lavandula hybrida grosso*, contains linalool, limonene, geraniol, and coumarins. Lemongrass oil (Profissimo DM) was also produced via steam distillation of the aerial parts of *Cymbopogon spp*., yielding a chemotype rich in citral, along with geraniol, linalool, limonene, isoeugenol, and citronellol. Peppermint oil (Fares), distilled from the flowers, leaves, and stems of *Mentha piperita*, is dominated by menthol and menthone. Finally, oregano oil (Purarom) was steam distilled from the aerial parts of *Origanum compactum* and exhibits high levels of carvacrol, thymol, and γ terpinene. These oils were chosen to represent a range of chemistries, from monoterpene alcohols to phenolic monoterpenes, and to assess how their differing compositions influence antibacterial and antifungal efficacy.

Culture media. Liquid Luria-Bertani (LB) medium was prepared by dissolving 5 g yeast extract, 10 g peptone, and 10 g NaCl in 500 mL deionized water. The pH was adjusted to 7.40 with 1 M NaOH, and the volume was brought to 1 L with additional water. The medium was sterilized at 121°C for 30 min and allowed to cool to room temperature before use.

Liquid yeast extract-peptone-dextrose (YPD) medium was made by dissolving 10 g yeast extract and 20 g peptone in 500 mL deionized water, sterilizing at 121°C for

30 min, then adding 100 mL of sterile 20 % glucose solution. The final volume was adjusted to 1 L with deionized water.

Solid LB and YPD media were prepared in the same way, with the addition of 20 g agar per liter prior to sterilization to yield a 2 % (w/v) solid substrate for bacterial and fungal viability assays.

Precultivation conditions of microorganisms. A volume of 5 µL of each bacterial (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Enterobacter cloacae* DSM 106614) and fungal stock strain (*Candida albicans* ATCC 10231) was inoculated on 5 mL of specific medium, LB or YPD, then the cultures were incubated at 30°C, 250 rpm, for 24 hours. Precultures were used for determination of antimicrobial activity of plant extracts.

Minimal inhibitory concentration determination. The minimal inhibitory concentration (MIC) of each essential oil was assessed in sterile, flat bottom 96 well microplates. In each well, 50 µL of either LB (for bacteria) or YPD (for fungi) medium was combined with 1 µL of microbial preculture. Volumes of sterile water and essential oil were then added to achieve the concentration gradients. Immediately after inoculation, the optical density at 600 nm (OD_{600}) was recorded (t = 0) using a BioTek Synergy H1 microplate reader. Plates were incubated at 30°C with linear shaking at 500 rpm for 24 h, after which OD_{600} was measured again to determine growth inhibition. The MIC was defined as the lowest oil concentration that prevented any increase in OD_{600} over the incubation period.

Bacterial and fungal viability assay. Following the 24 h OD_{600} measurement, four 1 µL aliquots, one per microbial strain, were withdrawn from the wells containing the lowest essential oil concentration (1 µL, 1:100 dilution) and diluted into 100 µL sterile water. Each suspension was then spread onto the appropriate solid medium in Petri dishes. Plates were incubated at 30°C for 48 h, after which colony formation was assessed to determine the viability of each bacterial or fungal strain in the presence of the test essential oil.

RESULTS AND DISCUSSIONS

Microbial proliferation is influenced by environmental parameters such as pH, temperature, oxygen availability, nutrient composition, and the presence of inhibitory compounds. In this study, we evaluated the antimicrobial potential of five commercial essential oils, lavender, lavandin, lemongrass, peppermint, and oregano, against one Gram-positive (*Staphylococcus aureus*), two Gram-negative (*Escherichia coli*, *Enterobacter cloacae*) bacterial strains, and one yeast (*Candida albicans*). Each oil was

tested at four volumes (1, 5, 10, and 15 µL), corresponding to 1:100, 1:20, 1:10, and 1:7 dilutions, respectively, to define their dose-response profiles. Figure 1 displays five medicinal and aromatic plant species from which essential oils were extracted and analyzed for their chemical composition and biological activities. Oregano (*Origanum vulgare*) (A) is a perennial herb known for its aromatic, oval-shaped green leaves. It is widely used in culinary and medicinal applications due to its rich content of phenolic compounds such as carvacrol and thymol. Peppermint (*Mentha piperita*) (B) is a hybrid mint variety recognized by its sharply serrated, dark green leaves with a distinctive menthol aroma. Peppermint oil is commonly used in food, pharmaceuticals, and cosmetics for its antimicrobial and soothing properties. Lavender (*Lavandula angustifolia*) (C) is characterized by dense spikes of fragrant purple flowers, lavender is valued for its calming scent and therapeutic effects. Its essential oil contains linalool and linalyl acetate as major constituents. Lavandin (*Lavandula intermedia*) (D) is a hybrid between *Lavandula angustifolia* and *Lavandula latifolia*, lavandin has longer flower stalks and is more vigorous. It is often cultivated for its higher yield of essential oil, which has a slightly more camphorous aroma than true lavender. Lemongrass (*Cymbopogon citratus*) (E) is a tall tropical grass with long, slender, and fragrant leaves. It is a source of citral rich essential oil known for its lemon like aroma and potent antimicrobial activity.

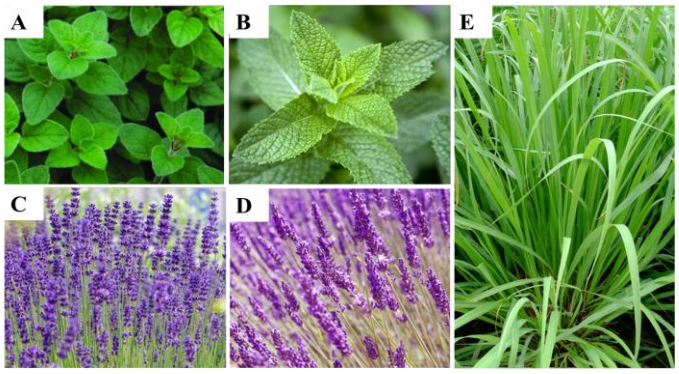

FIG 1. The plants from which the essential oils were investigated: Oregano (A), Peppermint (B), Lavender (C), Lavandin (D), Lemongrass (E)

Effect of lavender essential oil. *Lavandula angustifolia* essential oil is rich in linalool, linalyl acetate, 1,8-cineole, α-ocimene, and camphor, conferring documented antibacterial, antifungal, carminative, sedative, and anti-inflammatory activities

(Cavanagh & Wilkinson, 2002). In our assays (Figure 2), lavender oil exhibited potent antimicrobial effects at the lowest tested volume (1 µL; 1:100 dilution) against *C. albicans* and *E. coli*, completely inhibiting growth. Against *S. aureus*, significant inhibition emerged at 5 µL (1:20 dilution), whereas *E. cloacae* showed moderate susceptibility, with partial growth reduction at 10 µL (1:10 dilution). These results confirm the broad spectrum activity of lavender oil and underscore its efficacy even at high dilution.

Effect of lavandin essential oil. Lavandin, a sterile hybrid of *Lavandula angustifolia* and *Lavandula latifolia*, differs from true lavender by a more complex chemical profile that includes higher levels of camphor and 1,8-cineole in addition to linalool and linalyl acetate (Lesage et al. 2015). These compositional differences enhance both its aroma and antimicrobial potency. In our assays (Fig 3), lavandin oil demonstrated exceptionally strong activity against *Candida albicans* and *Escherichia coli* even at the lowest tested volume (1 µL; 1:100 dilution), fully inhibiting growth. For *Staphylococcus aureus* and *Enterobacter cloacae*, complete inhibition was observed at 5 µL (1:20 dilution). These results confirm lavandin's superior antimicrobial efficacy at low concentrations compared to lavender oil.

Effect of lemongrass essential oil. Lemongrass (*Cymbopogon citratus*) essential oil is enriched in bioactive constituents, primarily citral, isoneral, isogeranial, geraniol, geranyl acetate, citronellal, citronellol, germacrene-D, and elemol, that underpin its documented antifungal, antibacterial, antiviral, anticancer, anti-inflammatory, antioxidant, repellent, insecticidal, and anthelmintic properties (Mukarram et al. 2021). In this study (Fig 4), lemongrass oil exhibited potent antimicrobial activity against all four test strains (*S. aureus*, *E. coli*, *E. cloacae*, and *C. albicans*) at the lowest volume tested (1 µL; 1:100 dilution), completely inhibiting growth. Particularly, application volumes exceeding 15 µL induced caramelization within the wells for all strains, which interfered with optical density measurements; data from these higher concentrations were therefore excluded from graphical analyses.

Effect of peppermint essential oil. Peppermint (*Mentha piperita*) essential oil, rich in menthol, menthone, neomenthol, and isomenthone, is widely used as an adjuvant therapeutic due to its anti-inflammatory, antibacterial, antiviral, immunomodulatory, antitumor, neuroprotective, antioxidant, and antifatigue properties, as well as its protective effects on the gastrointestinal tract, liver, kidneys, skin, respiratory system, brain, and nervous system (Zhao et al. 2022). In Figure 5, peppermint oil demonstrated effective antimicrobial activity against *Candida albicans* and *Escherichia coli* at 1 µL (1:100 dilution), completely inhibiting growth. For *Staphylococcus aureus* and

Enterobacter cloacae, similarly strong inhibition was achieved at 5 µL (1:20 dilution), indicating a slightly higher concentration requirement for these strains.

Effect of oregano essential oil. Oregano (*Origanum vulgare*) essential oil contains the phenolic compounds carvacrol and thymol as its principal bioactive constituents, which confer broad-spectrum antimicrobial, antioxidant, anti-inflammatory, antitumor, antigenotoxic, cardioprotective, antispasmodic, antiurolithic, antinociceptive, antidiabetic, hepatoprotective, antidepressant, antiplatelet aggregation, acaricidal, insecticidal, and larvicidal activities (Karadayi et al. 2020). In this work (Figure 6), oregano oil exhibited strong inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae*, and *Candida albicans* at the lowest tested volume of 1 µL (1:100 dilution). Remarkably, in YPD medium, even 1 µL of oregano oil induced caramelization, precluding optical density measurements at higher concentrations.

Across all five essential oils, lemongrass, lavender, and oregano exhibited the strongest inhibitory effects against *E. coli, E. cloacae, S. aureus,* and *C. albicans* at the lowest tested volume (1 µL; 1:100 dilution). In contrast, under the same conditions, lavender oil displayed the weakest inhibition, closely followed by peppermint oil.

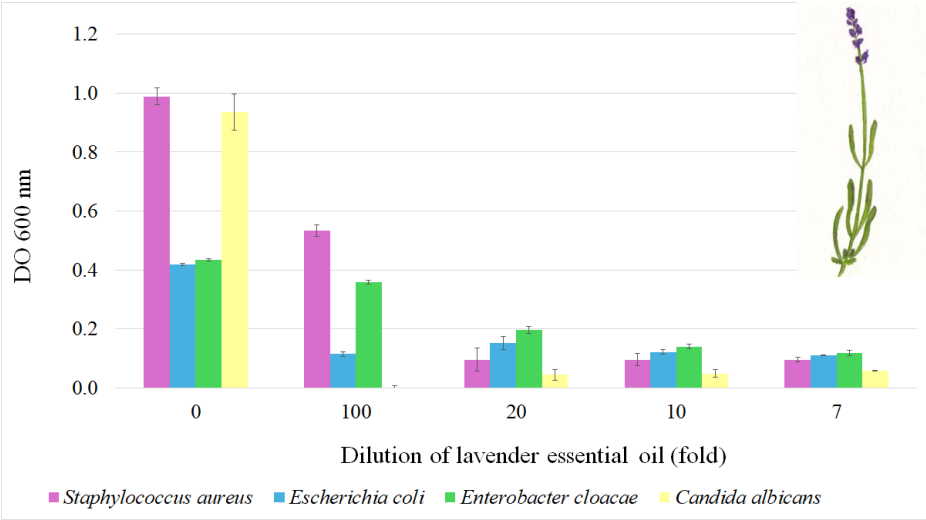

FIG 2. Effect of lavender essential oil on tested microbial strains (*Escherichia coli, Enterobacter cloacae, Staphylococcus aureus*, and *Candida albicans).* The value "0" on the graph represent the negative control, the specific culture medium for each microorganism, without the addition of essential oil.

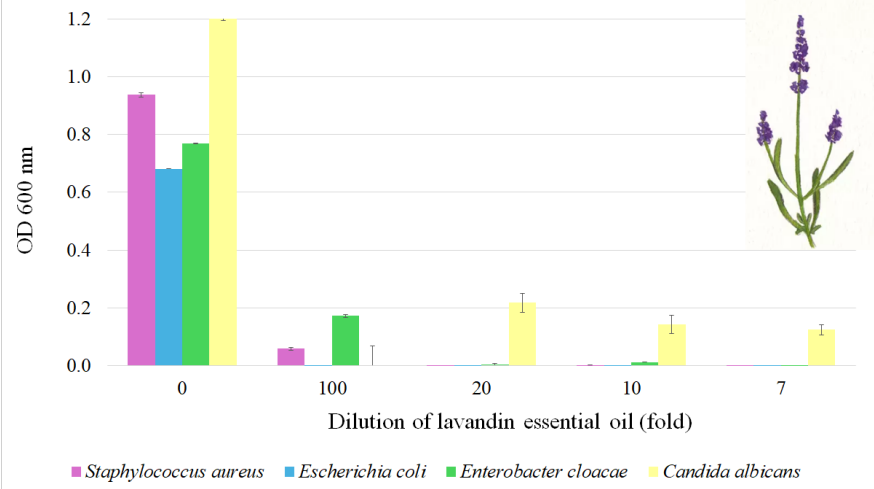

FIG 3. Effect of lavandin essential oil on tested microbial strains (*Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, and *Candida albicans).* The value "0" on the graph represent the negative control, the specific culture medium for each microorganism, without the addition of essential oil.

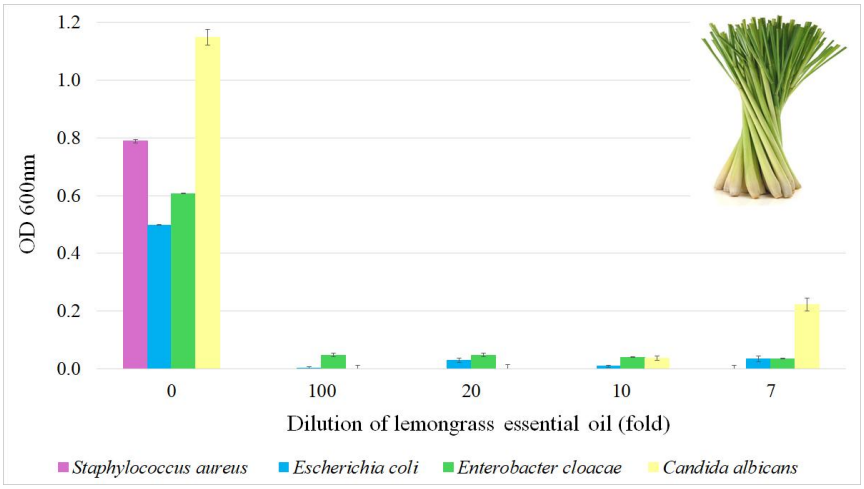

FIG 4. Effect of lemongrass essential oil on tested microbial strains (*Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, and *Candida albicans).* The value "0" on the graph represent the negative control, the specific culture medium for each microorganism, without the addition of essential oil.

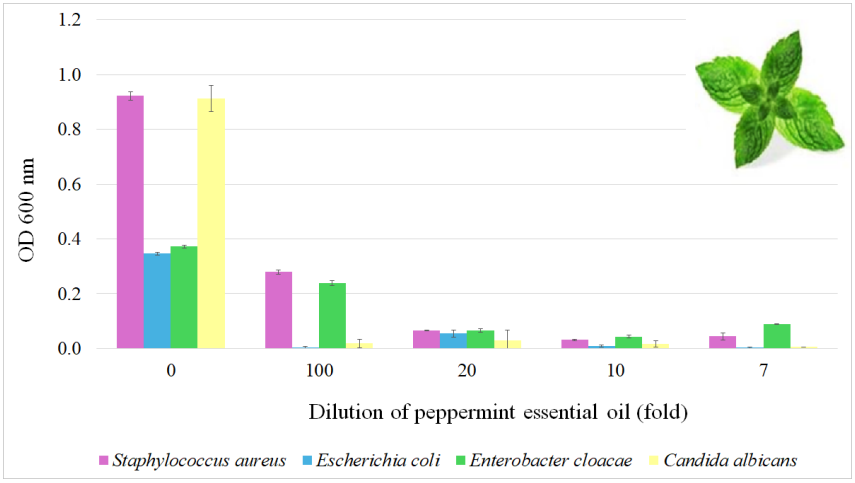

FIG 5. Effect of peppermint essential oil on tested microbial strains (*Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, and *Candida albicans*). The value "0" on the graph represent the negative control, the specific culture medium for each microorganism, without the addition of essential oil.

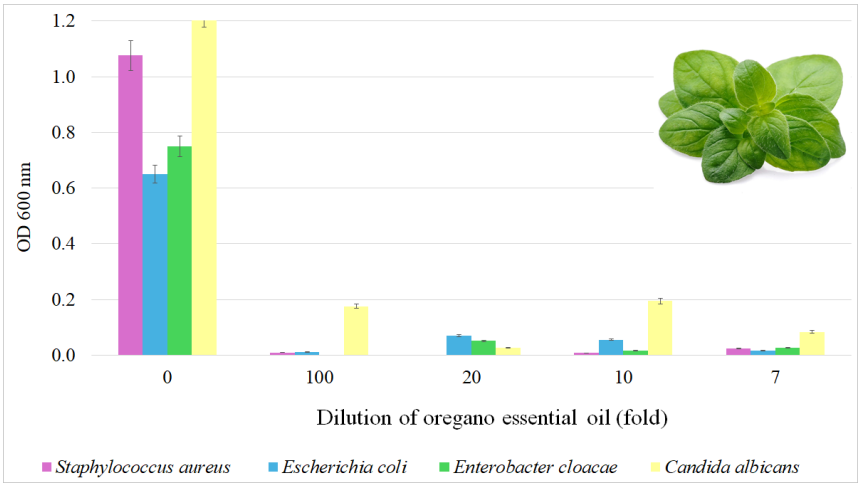

FIG 6. Effect of oregano essential oil on tested microbial strains (*Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, and *Candida albicans*). The value "0" on the graph represent the negative control, the specific culture medium for each microorganism, without the addition of essential oil.

Microbial viability assay. To corroborate the spectrophotometric MIC data, viability of each microbial strain was assessed following exposure to the minimal inhibitory concentration (1 µL; 1:100 dilution) of each essential oil. Aliquots were taken corresponding to the lowest oil volume, and plated onto solid LB or YPD media. Plates were incubated at 30°C for 48 h, after which colony formation was recorded. Figure 7 illustrates the viability results. This assay confirmed that lemongrass, lavender, and oregano oils effectively prevented colony development, whereas residual growth was noted following treatment with peppermint and lavandin oils at the same concentration.

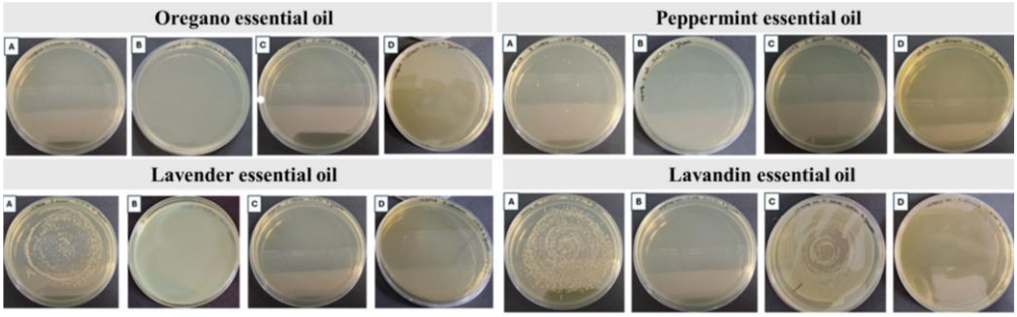

FIG 7. Viability test of different microorganisms (A- *Staphylococcus aureus*, B- *Escherichia coli*, C- *Enterobacter cloacae*, D- *Candida albicans*) after 48 h of incubation on oregano, peppermint, lavender and lavandin essential oils, 100 fold diluted.

CONCLUSIONS

The present study has demonstrated that lemongrass and oregano essential oils exhibit strong bactericidal activity against all tested strains (*Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, and *Candida albicans*) at a 1:100 dilutions. Lavender and peppermint oils were bactericidal against *E. coli*, *E. cloacae*, and *C. albicans*, but only bacteriostatic against *S. aureus*. Lavandin oil showed bactericidal effects on *E. coli* and *C. albicans*, while it was bacteriostatic against *S. aureus* and *E. cloacae*. These results highlight the high potential of specific essential oils as antimicrobial agents, with efficacy that varies according to the microbial target.

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