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RESEARCH ARTICLE

THE ANTIMICROBIAL ACTIVITY OF DIFFERENT ESSENTIAL OILS ON THE GROWTH OF UROPATHOGENIC *ESCHERICHIA COLI*

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ABSTRACT

In recent years, bacterial resistance to antibiotics has increased significantly. Currently, examples of multiresistant bacteria are known. For this reason, different substances are being investigated for their antimicrobial properties. That is the case of essential oils as products obtained from plants with important antibacterial properties. These substances have been known since antiquity and have been used in industry, in food, among others. This work shows some data related to the antibacterial properties of essential oils.

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INTRODUCTION

The emergence of microbial infections has motivated the search for new drugs to combat pathogenic microorganisms, especially when there are cases of resistance to drugs (such as the bacterial resistance to antibiotics) (Aljeldah, 2022). An alternative has been the use of natural products, through their extracts or essential oils obtained from plants and vegetables (Galgano*et al*., 2022). Since ancient times, different properties of plant extracts have been known. For example, by their anti-inflammatory and antimicrobial properties (Galgano *et al*., 2022; Thitinarongwate *et al.*, 2022). Regarding essential oils, it has been reported that they have antimicrobial properties and their effects have been observed in both Gram-positive and Gram-negative bacteria (Flores-Encarnación *et al*., 2020). The essential oils are substances obtained from plant materials as flowers, leaves, fruits, branches, seeds, bark by different methods. The essential oils are secondary metabolites produced by plants in order to provide a defense function or attraction (Butkiené *et al*., 2015; Burt, 2004; Citarasu, 2010; Cowan, 1999; Flores-Encarnación *et al*., 2016; Solórzano-Santos and Miranda-Novales, 2012). Several authors have reported the mechanisms of action involved in the antibacterial activity of essential oils. One of the reported mechanisms is by altering the permeability of the bacterial cell membrane and causing leaking ions and cytoplasm (bacterial lysis and death) (Flores-Encarnación *et al*., 2020; O'Bryan *et al*., 2015).

For this, in the present work the antimicrobial activity of different essential oils on the growth of uropathogenic *E. coli* was studied.

MATERIAL AND METHODS

Source of material: In this study, the commercial essential oils of mentha, rosemary, eucalyptus and thyme were used. They were obtained from a flavour and fragrance company at Puebla, México.

Biological material: The strain of uropathogenic *E. coli* CFT073 was used. Bacterial strain was stored in cryovials at -40°C until analysis.

Culture: The uropathogenic *E. coli* strain was cultured at 37°C for 18 to 24 h in trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md). For it, sterile Petri dishes (150 mm) were used and tripticasein soy agar plates (containing 20 mL of medium) were prepared. Plates were inoculated by crossstriation with uropathogenic *E. coli*. Each inoculum contained approximately 10^6 CFU mL^{-1}.

Antimicrobial activity: The antimicrobial activity of different essential oils on uropathogenic *E. coli* growth was determined using the technique of disk by diffusion in agar. For this, Petri dishes containing trypticasein soy agar were prepared and seeded as indicated above. Then, sterile filter paper disks (5 mm diameter) were

placed on the surface of tripticasein soy agar plates and different amounts of the essential oil were used: mentha (0.66, 1.32, 2.64, 3.96 and 6.6 mg), rosemary (0.86, 1.72, 3.44, 5.16 and 8.6 mg), eucalyptus (0.84, 1.68, 3.36, 5.04 and 8.4 mg) and thyme (0.75, 1.5, 3, 4.5 and 7.5 mg). The agar plates were incubated at 37°C for 24 h. The diameters of the inhibition halos formed were measured. The analyses were conducted in triplicate. The antimicrobial activity was also determined using the technique of well by diffusion in agar. Briefly, tripticasein soy agar plates containing 20 mL of medium were prepared. Sterile Petri dishes (150 mm) were used. Plates were inoculated by cross-striation with uropathogenic *E. coli*. Each inoculum contained approximately 10^6 CFU mL^{-1}. Subsequently, 6 wells were made on the tripticasein soy agar plate with the aid of the mouthpiece of a sterile glass Pasteur pipette. Then, different concentrations of the essential oil were used: mentha (13.2, 26.4 and 39.6 mg) and eucalyptus (8.4, 16.8 and 42 mg). The agar plates were allowed to stand for about 20 min at room temperature. Then, the plates were incubated at 37°C for 24 h. The analyses were conducted in triplicate.

Bactericidal or bacteriostatic activity: The bactericidal or bacteriostatic activity of essential oils was determined by scraping with a sterile bacteriological loop in the areas of the growth inhibition halos shown produced by the essential oils. For this, dishes containing trypticasein soy agar were used and inoculated with the scraping obtained from the growth inhibition zones (from the halos obtained with the essential oils of mentha, eucalyptus and thyme). Then, the agar plates were incubated at 37°C for 24 h. The absence of growth denoted an evident bactericidal activity, while the bacterial growth on the agar plate after incubation denoted a bacteriostatic activity. The analyses were conducted in triplicate.

Antimicrobial synergistic effect: The antimicrobial synergistic effect was determined using the technique of well by diffusion in agar. Briefly, tripticasein soy agar plates containing 20 mL of medium were prepared as described above. Plates were inoculated by cross-striation with uropathogenic *E. coli*. Each inoculum contained approximately 10^6 CFU mL^{-1}. Subsequently, 6 wells were made on the tripticasein soy agar plate with the aid of the mouthpiece of a sterile glass Pasteur pipette. Then, 3 mixtures containing essential oil of mentha and eucalyptus were prepared. The mixes contained (mentha/eucaliptus): 13.2 mg/8.4 mg, 26.4 mg/16.8 mg, 39.6 mg/42 mg). The agar plates were allowed to stand for about 20 min at room temperature. Then, the plates were incubated at 37°C for 24 h. As a reference, the eucalyptus essential oil was used and different amounts (8.4, 16.8 and 42 mg) were placed in 3 wells of the trypticasein soy agar plate. The analyses were conducted in triplicate.

RESULTS

The antibacterial activity of commercial different essential oils (mentha, rosemary, eucaliptus and thyme) on the growth of uropathogenic *E. coli* was determinated. So, tripticasein soy agar plates were inoculated with uropathogenic *E. coli* and different concentrations of the essential oil were added as indicated in the Materials and Methods. The results were shown in Fig. 1. As shown, most of the essential oils tested produced an inhibitory effect on the growth of uropathogenic *E. coli*. The Fig. 1A shows a lower inhibitory effect of mentha essential oil on the growth of uropathogenic *E. coli.* The growth inhibition halos showed a diameter around 12 to 18 mm, when 2.64, 3.96 and 6.6 mg of the essential oil were tested. Fig. 1C shows the inhibitory effect produced by eucaliptus essential oil on the growth of uropathogenic *E. coli*. In this image, a greater inhibitory effect on the growth of the bacteria was registered than that observed with the essential oil of mentha. The growth inhibition halos showed a diameter around 18 to 36 mm, when 0.84 to 8.4 mg, of the essential oil were used. Fig. 1D shows the inhibitory effect produced by thyme essential oil. Fig. 1D showed the strongest inhibitory effect on the growth of uropathogenic *E. coli*. In this image, the trypticasein soy agar surface lacked bacterial growth and the surface of the agar acquired a bright appearance. No bacterial growth was recorded in any of the amounts (0.75 to 7.5 mg) of thyme essential oil tested. Fig. 1B shows the results obtained when rosemary essential oil was used. As seen in the image, this essential oil did not inhibit the growth of uropathogenic *E. coli*. By the results obtained, the bactericidal or bacteriostatic effect of essential oils was determined according to the methodology described in Materials and Methods. The results were shown in Fig. 2A. As seen in the image, the growth of uropathogenic *E. coli* after the agar plates were incubated at 37°C for 24 h was not observed, indicating that the effect of essential oils was a bactericidal effect. Secondly, the antimicrobial activity was also determined using the well technique by diffusion in agar, with the objective of knowing the effect of the essential oils of eucalyptus and mint on the growth of uropathogenic *E. coli* in larger amounts. Briefly, tripticasein soy agar plates containing 20 mL of medium were prepared. Plates were inoculated by cross-striation using 10^6 CFU mL^{-1} of uropathogenic *E. coli*. Subsequently, 6 wells were made on the tripticasein soy agar plate with the aid of the mouthpiece of a sterile glass Pasteur pipette. Then, different concentrations of the essential oil were used: eucalyptus (8.4, 16.8 and 42 mg) and mentha (13.2, 26.4 and 39.6 mg). The plates were incubated at 37°C for 24 h. Results are shown in Fig. 2B. As seen on the left side of Fig. 2B, the growth of uropathogenic *E. coli* was inhibited when the eucalyptus essential oil was used. However, the inhibition halos obtained showed a smaller diameter than the halos observed when using the same essential oil and the disk diffusion technique (Fig. 1C).

The diameter of the inhibition halo (using the highest amount of eucalyptus essential oil) was around 23 mm. This led to the proposal that eucalyptus essential oil shows solubility problems on trypticasein soy agar. Therefore, despite using larger amounts of essential oil, it could not diffuse freely through the culture medium. Something similar occurred with the essential oil of mentha, which did not produce any effect on the growth of uropathogenic *E. coli*, as seen on the right side of Fig. 2B. In order to determine if the essential oil of eucalyptus and mentha could show a synergistic effect, both were mixed in different quantities. The antimicrobial synergistic assay was determined using the well technique by diffusion in agar as described in Materials and Methods. Briefly, tripticasein soy agar plates were inoculated with uropathogenic *E. coli* and then 3 mixtures containing essential oil of mentha and eucalyptus were prepared. As shown above, the mixes contained (mentha/eucaliptus): 13.2 mg/8.4 mg, 26.4 mg/16.8 mg, 39.6 mg/42 mg). The plates were incubated at 37°C for 24 h. Results are shown in Fig. 2C. As seen on the right hand side of Fig. 2C, the essential oil of eucalyptus produced the halos of inhibition of the growth of the bacteria as it had already been shown before. On the left side of Fig. 2C, the results obtained by mixing the essential oils of mentha and eucalyptus in different amounts were shown. In this case, it can be seen that the mixture of essential oils produced halos of inhibition of growth of uropathogenic *E. coli*, nevertheless diameter is smaller than that registered using only the essential oil of eucalyptus. This could suggest that mentha essential oil shows low solubility with eucalyptus essential oil and it showed interference to inhibit the growth of uropathogenic *E. coli*.

DISCUSSION

Aromatic plants and their essential oils have been used since ancient times in food, agriculture, medicine, cosmetic applications, as condiments and spices, in therapeutic uses, as antimicrobials (Bakkali *et al.*, 2008; Stojiljkovic *et al*., 2018). Due to antibiotic resistance and failure of chemotherapy by pathogenic microbial agents, search for plant products has increased for their potential antimicrobial activity (Flores-Encarnación *et al*., 2020; Hammer *et al.*, 1999; Inayatullah *et al*., 2017). In the present work, the antibacterial activity of commercial different essential oils on the growth of uropathogenic *E. coli* was studied. As shown in Fig. 1, most of the essential oils tested produced an inhibitory effect on the growth of uropathogenic *E. coli*. However, the inhibitory effect on the growth of uropathogenic *E. coli* was stronger using the eucalyptus and thyme essential oils.

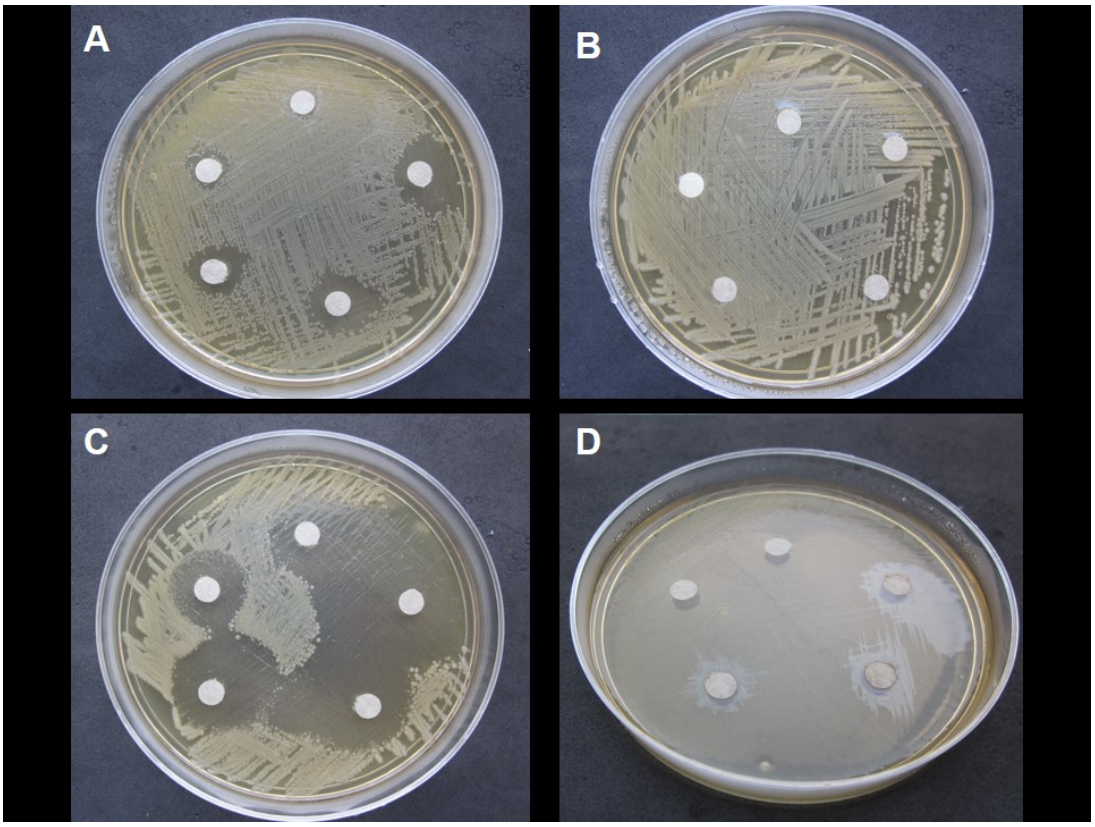

Fig. 1. The inhibition of growth from uropathogenic *E. coli* using commercial different essential oils. A. Antibacterial activity of mentha essential oil (0.66 to 6.6 mg). B. Antibacterial activity of rosemary essential oil (0.86 to 8.6 mg). C. Antibacterial activity of eucalyptus essential oil (0.84 to 8.4 mg). D. Antibacterial activity of thyme essential oil (0.75 to 7.5 mg). In all cases, essential oils were placed in increasing amounts in the counterclockwise direction, starting with the top

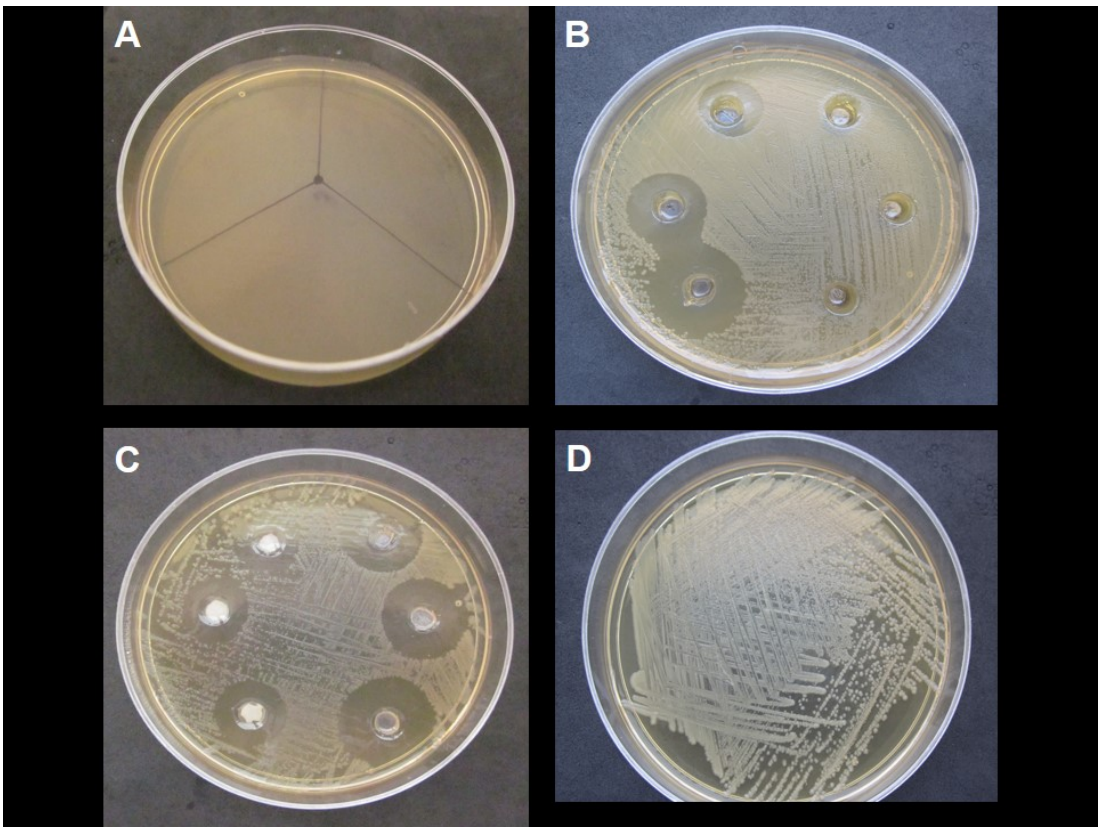

Fig. 2 The antimicrobial activity of essential oils of mentha, eucalyptus and thyme. A. Bactericidal effect of essential oils (each third of the agar plate was inoculated with the scraping obtained from the growth inhibition zones). B. The well technique by diffusion in agar using: the eucalyptus essential oil (8.4 to 42 mg; left, top to bottom) and mentha essential oil (13.2 to 39.6 mg; right, top to bottom). C. Synergistic effect of essential oils: mentha and eucalyptus in different amounts (left, top to bottom) and only eucalyptus (right, top to bottom). D. Control condition

As shown in Fig. 1D, the thyme essential oil showed the strongest inhibitory effect on the growth of uropathogenic *E. coli*, looking everywhere a trypticasein soy agar surface lacked bacterial growth. The surface of the agar acquired a bright appearance. This is in agreement with other authors, who have reported that the essential oils derived from plants have antimicrobial activity against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus* (Burt, 2004). Specifically, the antimicrobial activity of *T. vulgaris* is has been reported against Gram negative bacteria such as *Salmonella enteritidis*, *S. choleraesuis*, *S. typhimurium*, *Proteus mirabilis*, *P. vulgaris*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *E. coli*, and Gram positive bacteria such as *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis* (Kon and Rai, 2012; Lević *et al*., 2011; Mohsenipour and Hassanshahian, 2015; Rattanachaikunsopon and Phumkhachorn, 2010; Soković *et al.* 2010; Tohidpour *et al.* 2010). It has been also reported that quantitatively thymol and carvacrol are the major components of essential oil of *T. vulgaris* and that they are largely responsible of antimicrobial properties (Flores-Encarnación *et al*., 2020; Lee *et al.*, 2005; Tural and Turhan, 2017). In this study, the essential oil of mentha showed less antibacterial activity. Among the properties of the genus *Mentha* the following have been reported: antiviral, antibacterial, antifungal, high antioxidant, cytotoxic, contraceptive, antiinflammatory and antiallergic (Chávez-González *et al.* 2016; Giménez-Santamarina *et al.*, 2022; Sharma *et al*. 2014). As shown in Fig. 2A of this study, the observed effect of essential oils was a bactericidal effect because the growth of uropathogenic *E. coli* was not observed after the agar plates were incubated at 37°C for 24 h. It has been reported that the essential oil of *T. vulgaris* contains several chemical components. Thymol, carvacrol and eugenol are the most active constituents with a wide antimicrobial spectrum. Both constituents destabilizes the bacterial cytoplasmic membrane (Bassolé *et al*. 2010; Al-Shuneigat *et al*., 2014; Flores-Encarnación *et al*., 2017; Soković *et al*., 2010; Ultee *et al.*, 2002). Several mechanisms have been proposed to explain their mechanism of action. It has been reported that the hydrophobicity of the essential oils produce changes in bacterial membrane structure and wall structures. Alteration of the cell permeability, disturbance to respiration, modification of bacterial quorum sensing, potassium leakage from cells, effects on membrane potential (proton translocation), changes in pH gradient and ATP production of bacterial cell bacterial lipid membrane, lead to the lysis and death of bacteria (Flores-Encarnación *et al*., 2018; O'Bryan *et al.*, 2015). Another example is the hydroxyl group of eugenol which react with proteins and inhibit action of enzymes; hydrophobic thymol and carvacrol may damage the outer membrane of Gram-negative and bacterial cell wall releasing lipopolysaccharides (Gómez-Estaca *et al*. 2010; Kon and Rai, 2012). Finally, in this study was observed that the antimicrobial activity determined using the well technique in agar was lower than that observed using the disk diffusion test (Fig. 2B and Fig. 2C). In this case, the eucalyptus essential oil produced inhibition halos smaller than the halos observed when using the same essential oil and the disk diffusion technique. While mentha essential oil did not produce any effect on the growth of uropathogenic *E. coli* using the well technique in agar. This led to the proposal that eucalyptus essential oil shows solubility problems on trypticasein soy agar (especially the essential oil of mentha). It has been reported that *T. vulgaris* extract strongly inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but it inhibited poorly the growth of *Bacillus cereus* and *E. coli*. *T. vulgaris* extracts are more efficient in broth media than in solid media; they have low diffusion in solid media compared with broth media. Therefore, greater concentration is required in solid media compared with broth media (Flores-Encarnación *et al*., 2017; Mohsenipour and Hassanshahian, 2015).

CONCLUSION

Essential oils show different antimicrobial properties. Its effect is bactericidal, which means that bacteria are incapable of generating resistance to essential oils, unlike antibiotics where resistant strains are known. Some essential oils show greater bactericidal power, such as thyme essential oil on the growth of uropathogenic *E. coli.*

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