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RESEARCH ARTICLE

THE EFFECT OF ESSENTIAL OILS ON THE RESPIRATORY ACTIVITY OF UROPATHOGENIC *Escherichia coli*

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ABSTRACT

As is known, antibiotic resistance is a serious public health problem in the world. More and more types of bacteria are appearing that are more resistant to antibiotics. For it, different substances are being investigated that could be used as antibiotics in the future. Such is the case of essential oils, which have shown important antimicrobial properties. This work presents the action of different essential oils on the growth of uropathogenic *E. coli* and the impact on the respiratory activity.

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INTRODUCTION

The antimicrobial resistance is a critical issue in health care in terms of mortality, quality of services, and financial damage. Numerous bacteria are acquiring resistance to a wide variety of drugs, and these germs cause infections that are not treatable with the currently available antimicrobials (Aljeldah, 2022; D'Costa *et al*., 2011; Irfan *et al*., 2022). Because bacteria quickly develop resistance to antibiotics, the antimicrobial properties of numerous substances of plant origin are being studied. Use of plants as traditional medicine in the treatment of various diseases dates from long before the discovery of the existence of microorganisms (Ali *et al*., 2015; Flores-Encarnación *et al*., 2016; Patra and Baek, 2016). One of the advantages of products obtained from plants as an alternative therapeutic is that it has a wide range of antimicrobial activity because it contains a lot of active ingredients that make it toxic to microorganisms. In that context, antibacterial action of essential oils has been reported. Essential oils are aromatic volatile liquids extracted from plants (flowers, leaves, seeds, peels, branches, bark, wood, roots, underground stems, gums or oily resin) and they contain a collection of secondary metabolites such as phenols, alcohols, esters, aldehydes, oxides, etc.

Currently, essential oils are used in a variety of products for insecticidal, pharmaceutical, cosmetic, virus killing, fungicidal, anti-parasitic (Camele *et al*., 2021; Diao *et al*., 2013; Donato *et al*., 2020; Lianga *et al*., 2023; Soliman *et al*., 2022). Currently, it is known about the bactericidal effect of essential oils. However, few studies have been carried out on its effectiveness. For this, in the present work the effect of different essential oils on growth and the respiratory activity of uropathogenic *E. coli* was studied.

MATERIALS 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 (100 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 48 h. The bacterial growth inhibition halos were observed. The analyses were conducted in triplicate.

Respiratory activity: The respiratory activity was measured polarographically with a Clark oxygen electrode according to the methodology established by Flores-Encarnación *et al*., (2020). For it, complete cells of uropathogenic *E. coli* were used. The complete cells of uropathogenic *E. coli* were obtained from a culture in an Erlenmeyer flask containing 50 mL of trypticase soy broth, incubated at 37 ºC with shaking at 200 r.p.m. for 24 hours. The complete cells of uropathogenic *E. coli* were washed twice with 20 mM phosphate buffer (pH 7.0). The reaction mixture (final volumen= 5 mL) contained: 20 mM phosphate buffer (pH 7.0) and 16.6 mM glucose. The reactions were initiated adding the cell suspension at optical density (OD_{560nm})= 4.27. The oxygen consumption kinetics were recorded for 25 min. The temperature was kept constant at 37°C. In all tests, the respiratory activities of uropathogenic *E. coli* was reported as consumed nmol O_2 min^{-1}. The analyses were conducted in triplicate.

Effect of essential oils on respiratory activity: The effect of different essential oils (mentha, rosemary, eucalyptus and thyme) on respiratory activity of uropathogenic *E. coli* was determinated. For that, washed cells of uropathogenic *E. coli* (cell suspension at OD_{560nm}= 4.27) were centrifuged at 6,500 r.p.m. for 3 min, resuspended in 400 uL of 20 mM phosphate buffer (pH 7.0) and then incubated separately with mentha (1.32 mg), rosemary (1.72 mg), eucalyptus (1.68 mg) and thyme (1.5 mg) eseential oils for 20 min at 37 ºC (facilitating contact between complete cells and essential oils). At the end of incubation, the reaction mixture (final volumen= 5 mL) contained: 20 mM phosphate buffer (pH 7.0) and 16.6 mM glucose. The reactions were initiated adding the uropathogenic *E. coli* cells treated with essential oils. The oxygen consumption kinetics were recorded for 25 min. The temperature was kept constant at 37°C. In all tests, the respiratory activities of uropathogenic *E. coli* was reported as consumed nmol O_2 min^{-1}. The analyses were conducted in triplicate.

RESULTS

As mentioned above, the effect of different essential oils (mentha, rosemary, eucaliptus and thyme) on growth and the respiratory activity 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, all the essential oils tested produced an inhibitory effect on the growth of uropathogenic *E. coli*. In Fig. 1A, 1B and 1C, halos of growth inhibition were observed. The mentha and eucalyptus essential oils produced the largest growth inhibition zones, while the growth inhibition zones with rosemary essential oil were smaller. Thus, the effect of essential oils on the inhibition of growth of uropathogenic *E. coli* was ordered as follows: rosemary < mentha < eucaliptus, according to the diameter of the growth inhibition halos obtained (data not shown).

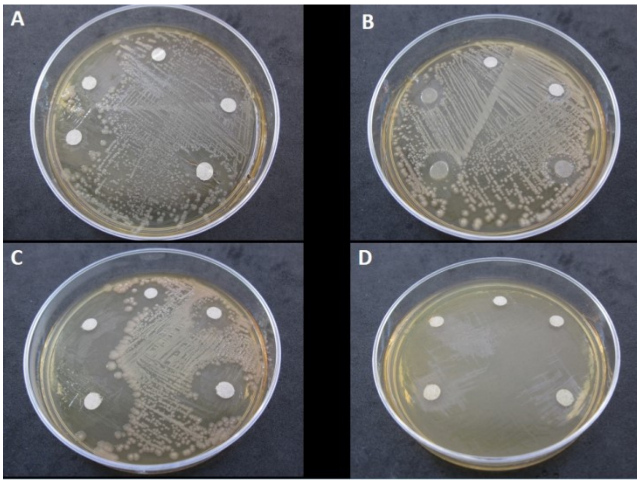

Fig. 1 The effect of different essential oils on growth from uropathogenic *E. coli*. A. Mentha essential oil (0.66 to 6.6 mg). B. Rosemary essential oil (0.86 to 8.6 mg). C. Eucalyptus essential oil (0.84 to 8.4 mg). D. Thyme essential oil (0.75 to 7.5 mg). In all cases, essential oils were placed in increasing amounts in the counterclockwise direction, starting with the top. Agar plates were incubated at 37°C for 48 h

As had already been shown in a previously carried out study, with thyme essential oil total inhibition of the growth of uropathogenic *E. coli* was observed (Fig. 1D). As shown Fig. 1D, 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. Therefore, the highest bactericidal activity was obtained using thyme essential oil, since no growth of *E. coli* was recorded at any of the amounts of essential oil used. From the results shown in Fig. 1, it was concluded that all the essential oils tested showed an inhibitory effect on the growth of uropathogenic *E. coli*, however the effectiveness was different between them, being thyme essential oil the most potent and effective action. As indicated previously, the results were obtained after 48 h of incubation of the culture plates at 37ºC. In previous studies, similar results were obtained by incubating the plates for 24 h at 37ºC. In this case, rosemary essential oil did not show an inhibitory effect on the growth of uropathogenic *E. coli* and it was attributed to problems in the solubility of the essential oil in the agar plates (data not shown), therefore the incubation time was increased to 48 h. Observing that all essential oils caused a negative effect on the growth of uropathogenic *E. coli*, their effect was determined at short times. For it, the respiratory activity of uropathogenic *E. coli* was monitored in the presence of different essential oils, using complete cells and measuring the respiratory activity by a Clark oxygen electrode, as indicated in Materials and Methods. The washed cell pellet of uropathogenic *E. coli* (OD_{560nm}= 4.27) were resuspended in 400 uL of 20 mM phosphate buffer (pH 7.0) and then incubated separately with essential oils for 20 min at 37 ºC. In all tests, the respiratory activities of uropathogenic *E. coli* was reported as consumed nmol O_2 min^{-1}. The oxygen consumption kinetics were recorded for 25 min. The results obtained were shown in Table 1. As shown Table 1, the respiratory activity of uropathogenic *E. coli* was determined in 64 nmol O_2 min^{-1}, using glucose as a substrate (glucose-oxidase activity in whole cells). In general, the respiratory activity of uropathogenic *E. coli* was considerably decreased with all the essential oils tested, especially with thyme essential oil. Exposing uropathogenic *E. coli* cells to rosemary essential oil, respiratory activity decreased by approximately 45%, while eucalyptus and mentha essential oils decreased the respiratory activities by 80 and 84%, respectively. Thyme essential oil produced the highest inhibition of respiratory activity in uropathogenic *E. coli* cells, reducing cellular respiration by 96% at the quantities tested. These results were directly related to what was observed in the agar diffusion tests. However, the respiratory inhibitory action of essential oils could be observed in a shorter time.

Table 1. Respiratory activities of uropathogenic *E. coli* in the presence of different essential oils

Essential oils	Respiratory activity* (oxygen consumed, nmol O_2 min^{-1})	Inhibition of respiration (%)
None	64	0
Rosemary	35	45.32
Eucalyptus	13	79.69
Mentha	10	84.37
Thyme	4	96.00

*Glucose-oxidase activity in complete cells.

Thus, the inhibitory effect of essential oils on the respiratory activity of uropathogenic *E. coli* was ordered as follows: thyme essential oil > mentha essential oil > eucaliptus essential oil > rosemary essential oil, according to glucosa-oxidase activity which was measured.

DISCUSSION

Essential oils are composed particularly of liophilic and highly volatile secondary plant metabolites, principally mono- and sesquiterpenes but other compounds such as allyl and iso allyl phenols may also presents (Mustafa *et al*., 2020). The essential oils have demonstrated antioxidant, insecticidal, antibacterial, antifungal and antiviral properties (Cowan, 1999; Kim and Park, 2013; Flores-Encarnación *et al*., 2016; Kordali *et al*., 2005; Wojnicz *et al*., 2012). It has been shown that *Lippia berlandieri* (oregano), *Thymus vulgaris* (thyme) and *Cinnamomum verum* (cinnamon) have antioxidant properties related with phenolic compounds such as carvacrol and thymol and these can be used under certain conditions as fungicides and bactericides (Abdalá and Roozen, 2011; Burt, 2004; Cowan, 1999; Kordali *et al*., 2005). In this study, the effect of different essential oils such as mentha, rosemary, eucaliptus and thyme on growth and the respiratory activity of uropathogenic *E. coli* was determinated. The results indicated that all the essential oils tested produced an inhibitory effect on the growth of uropathogenic *E. coli*. With rosemary, mentha and eucaliptus essential oils, halos of growth inhibition of uropathogenic *E. coli* were observed.

The mentha and eucalyptus essential oils produced the largest growth inhibition zones, while the growth inhibition zones with rosemary essential oil were smaller. The thyme essential oil produced the total inhibition of the growth of uropathogenic *E. coli*. The thyme essential oil is a potent inhibitor of the growth of uropathogenic *E. coli.* It was concluded that all the essential oils tested showed an inhibitory effect on the growth of uropathogenic *E. coli*, however the effectiveness was different between them, being thyme essential oil the most potent and effective action. Prabuseenivasan *et al*., (2006) reported that essential oils showed antibacterial activity. They demostrated a significant inhibitory effect by cinnamon, clove, geranium, lemon, lime, orange and rosemary oils. Cinnamon oil showed the highest activity at low concentrations; aniseed, eucalyptus and camphor oils showed the lowest antibacterial activity against the tested bacteria both Gram-negative and Gram-positive: *Klebsiella pneumoniae*, *Proteus vulgaris*, *Bacillus subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*. It has been reported also the potent inhibitory effect of essential oil of oregano, thyme and basil on the growth of *E. coli* and *P. aeruginosa* (Al-Bayati, 2008; Flores-Encarnación *et al*., 2016; Flores-Encarnación *et al*., 2020; Sateriale *et al*., 2022). In this study, the effect of essential oils was also determined in a short time interval. So, as an indicator the respiratory activity of uropathogenic *E. coli* was monitored in the presence of different essential oils. The results showed that glucose-oxidase activity in whole cells of uropathogenic *E. coli* was considerably decreased at the essential oils tested, especially with thyme essential oil. Thyme essential oil produced the highest inhibition of respiratory activity in uropathogenic *E. coli* cells (it decreased cellular respiration by up to 96%). This result allowed us to understand why thyme essential oil is more efficient in inhibiting the growth of uropathogenic *E. coli*, which was observed in the culture plates. The rosemary essential oil decreased the respiratory activity approximately by 45%; eucaliptus essential oil decreased the respiratory activity approximately by 80% and mentha essential oil decreased the respiratory activities by 84%. From these results, it was proposed that the effectiveness of each essential oil should be determined by the chemical composition of each one, as well as their mechanisms of action in bacterial cells. It has been reported that the essential oil of *T. vulgaris* is a bacterial growth inhibitor; it has potent bactericidal properties including efficacy against antibiotic-resistant strains (Al-Shuneigat *et al*., 2014; Flores-Encarnación *et al.*, 2019). The essential oil of *T. vulgaris* was found to be able to interfere with bacterial colonization and the formation of biofilm on surfaces for uropathogenic *E. coli* (Flores-Encarnación *et al.*, 2018). Camele *et al*., (2021) reported the major components of *Mentha* x *piperita* cv. 'Kristinka' essential oil: menthol (70.08%), menthone (14.49%), limonene (4.32%), menthyl acetate (3.76%) and β-caryophyllene (2.96%). The essential oil of *Thymus vulgaris* (thyme) which contains monoterpenes as thymol (49%), ρ-cimene (18%), carvacrol (6%), γ- terpinene (9%), linalool (3%), car-3-eno (2%), β- mirceno (2%), α- pinene (1%), limonene (1%) and camphane (0.5%) (Ben *et al*., 2019; Sakkas and Papadopoulou, 2007). Pellegrini *et al.* (2018) reported that camphor was the principal compound (22.07%) of rosemary essential oil, followed by α-pinene (16.64%), eucalyptol (15.71%), and borneol (11.99%). Almas *et al*., (2021) reported the major compounds in the leaf essential oil of *Eucalyptus maculata* and were: eucalyptol (54.29%), *p*-cymene (10.10%), *α*-pinene (7.78%), β-myrcene (7.78%), γ-terpinene (1.73%) and citronellal (1.62%).

There is evidence about antimicrobial activities attributed to specific compounds related to thyme, carvacrol, α- pinene, linalool, methyl salicylate, eugenol and geraniol (Monzote-Fidalgo *et al*., 2004; Prasanth *et al.*, 2014; Scalas *et al.*, 2018; Wińska *et al*., 2019). On the other hand, some mechanisms from antibacterial activity of essential oils have been reported. One of the reported mechanisms of action is the the ability to alter and to penetrate the lipid membrane of bacteria, making it more permeable and causing leaking ions and cytoplasm (bacterial lysis and death). There are few reports about the bactericidal mechanism of the essential oil of *T. vulgaris*. It is believed that this essential oil alters the permeability of membrane as do other oils (Hussein *et al.*, 2018; O'Bryan *et al.*, 2015). It has been 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 (Lee *et al.*, 2005; Tural and Turhan, 2017). It has been reported that carvacrol is a hydrophobic compound that influences cell membranes by altering the composition of fatty acids, which then affects the membrane fluidity and permeability (Rudramurthy *et al.*, 2016). However, its exact mechanism of action is still unclear. It was reported that carvacrol significantly depleted the internal ATP pool of bacterial cells and induced the leakage and loss of ATP from bacterial cells (Rudramurthy *et al*., 2016; Swamy *et al*., 2016; Ultee *et al.*, 2002). In a previous study, it was reported in uropathogenic *E. coli* a new possible mode of action of essential oil of *T. vulgaris*, which consists of the blockage of the respiratory chain of the bacterium and that could explain the loss of ATP from bacterial cells reported by other authors (Flores-Encarnación *et al*., 2020). In the present study, the inhibitory effect of essential oils on the respiratory activity of uropathogenic *E. coli* was ordered as follows: thyme essential oil > mentha essential oil > eucaliptus essential oil > rosemary essential oil, according to glucosa-oxidase activity registered. It is possible that thyme essential oil has several mechanisms of action that favor its antibacterial action. Therefore, it is necessary to carry out more studies that inform about the mechanisms of action of the compounds of essential oils.

CONCLUSION

In this study, essential oils significantly inhibited the growth of uropathogenic *E. coli*, especially thyme essential oil.

Thyme essential oil completely inhibited the growth of the bacteria at all amounts tested. Similarly, the respiratory activity of uropathogenic *E. coli*, measured as glucose oxidase, decreased considerably when the cells were incubated in the presence of thyme essential oil. The other oils tested also decreased respiratory activity but their contribution was smaller. It is possible that thyme essential oil has several mechanisms of action that favor its antibacterial action.

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REFERENCES

Abdalá A.E. and Roozen J.P. 2011. The effects of stabilized extracts of sage and oregano on the oxidation of salad dressings. Eur. Food Res. Technol. 212:551-560.

Al-Bayati F.A. 2008. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. J. Ethnopharmacol. 116:403-406.

Ali B., Al-Wabel N.A. Shams S., Ahamad A., Khan S.A. and Anwar F. 2015. Essential oils used in aromatherapy: a systemic review. Asian Pac. J. Trop. Biomed. 5:601-611.

Aljeldah M.M. 2022. Antimicrobial resistance and its spread is a global threat. Antibiotics. 11:1082-1096.

Almas I., Innocent E., Machumi F., Kisinza W. 2021. Chemical composition of essential oils from *Eucalyptus globulus* and *Eucalyptus maculata* grown in Tanzania. Scientific African. 12:e00758.

Al-Shuneigat J., Al-Sarayreh S., Al-Saraireh Y., Al-Qudah M., Al-Tarawneh I. 2014. Effects of wild *Thymus vulgaris* essential oil on clinical isolates biofilm-forming bacteria. IOSR J. Dental Med. Sci. 13: 62-66.

Ben G., Herrera R., Lengliz O., Abderrabba M. and Labidi J. 2019. Effect of the chemical composition of free-terpene hydrocarbons essential oils on antifungal activity. Mol. 24:1-11.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. Inter. J. Food Microbiol. 94:223-253.

Camele I., Grulova D., Elshafie H.S. 2021. Chemical composition and antimicrobial properties of *Mentha x piperita* cv. 'Kristinka' essential oil. Plants. 10:1567.

Cowan M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12:564-565.

D'Costa V.M., King C.E., Kalan L., Morar M., Sung W.W.L., Schwarz C., Froese D., Zazula G., Calmels F., Debruyne R., Golding G.B., Poinar H.N. and Whigth G.D. 2011. Antibiotic resistance is ancient. Nature. 477:457-461.

Diao W.R., Hu Q.P., Feng S.S., Li W.Q. and Xu J.G 2013. Chemical composition and antibacterial activity of the essential oil from green huajiao *Zanthoxylum schinifolium* against selected foodborne pathogens. J. Agric. Food Chem. 3:6044-6049.

Donato R., Sacco C., Pini G., Bilia A.R. 2020. Antifungal activity of different essential oils against *Malassezia* pathogenic species. J. Ethnopharmacol. 249:112376.

Flores-Encarnación M., Acosta-Báez, L.A., Aguilar-Gutiérrez G.R., Cabrera-Maldonado C., Gallardo-Soto H.M. and Sánchez-Herrera A.J. 2019. The effect of essential oil of *Thymus vulgaris* on the growth of bacterial environmental and clinical isolates. Inter. J. Res. Stud. Biosci. 7:1-8.

Flores-Encarnación M., Espino-Benítez A.S., Aguilar-Gutiérrez G.R., Martínez-Flores L.D., Xicohtencatl-Cortes J., Carreño-López R. and Cabrera-Maldonado C. 2020. The effect of *Thymus vulgaris* on the respiratory activity of uropathogenic *Escherichia coli*. Internat. J. Res. Studies Biosci. 8:1-6.

Flores-Encarnación M., Nava-Nolazco R.M., Aguilar-Gutiérrez G.R., Carreño-López R. and García-García S. C. 2018. The effect of *Thymus vulgaris* on growth and biofilm formation of uropathogenic *Escherichia coli*. Afr. J. Microbiol. Res. 12:237-242.

Flores-Encarnación M., Nava-Nolazco R.M., Carreño-López R., Aguilar-Gutiérrez G.R., García-García S.C. and Cabrera-Maldonado C. 2016. The antibacterial effect of plant-based essential oils. Internat. J. Res. Studies Biosci. 4:1-6.

Hussein H.J., Hadi M.Y. and Hameed I.H. 2018. Cytotoxic activity of *Thymus vulgaris*: antibacterial and antifungal activity. Inter. J. Pharm. Quality Assurance. 9:166-169.

Irfan M., Almotiri A. and AlZeyadi Z.A. 2022. Antimicrobial resistance and its drivers- a review. Antibiotics. 11:1362-1380.

Kim H.S. and Park H.D. 2013. Ginger extract inhibits biofilm formation by *Pseudomonas aeruginosa* PA14. PLoS One. 8:9: e76106.

Kordali S., Kotan R., Mavi A., Cakir A., Ala A. and Yildirim A. 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. J. Agric. Food Chem. 53:9452- 9458.

Lee S.J., Umano K., Shibamoto T. and Lee K.G. 2005. Identification of volatile components in basil *Ocimum basilicum* L. and thyme leaves *Thymus vulgaris* L. and their antioxidant properties. Food Chem. 91:131-137.

Lianga J., Zhang Y., Chi P., Liua H., Jinga Z., Caoa H., Dua Y., Zhaoa Y., Qinb X., Zhang W. and Kong D. 2023. Essential oils: Chemical constituents, potential neuropharmacological effects and aromatherapy- A review. Pharmacological Res.- Modern Chinese Med. 6:100210.

Monzote-Fidalgo L., Sariego-Ramos I., Montalvo-Álvarez A.M., Garrido-Lorente N., Scull-Lizama R. and Abreu-Payrol J. 2004. Propiedades antiprotozoarias de aceites esenciales extraídos de plantas cubanas. Rev. Cub. de Med. Tropical. 56:230-233.

Mustafa A., Alvi A.I., Siddiqui Z.A. and Meena R.P. 2020. Brief literature on essential oils- a systematic review. World Journal of Pharmaceutical Research. 9:1282-1294.

O'Bryan C.A., Pendleton S.J., Crandall P.G. and Ricke S.C. 2015. Potential of plant essential oils and their components in animal agriculture- in vitro studies on antibacterial mode of action. Frontiers Vet. Sci. 2:1-8.

Patra J.K. and Baek K.H. 2016. Antibacterial activity and action mechanism of the essential oil from *Enteromorpha linza* L. against foodborne pathogenic bacteria. Molecules. 21:1-11.

Pellegrini M., Ricci A., Serio, A., Chaves-López C., Mazzarrino G., D'Amato S., Lo Sterzo C. and Paparella A. 2018. Characterization of essential oils obtained from *Abruzzo autochthonous* plants: antioxidant and antimicrobial activities assessment for food application. Foods. 7:19.

Prabuseenivasan S., Jayakumar M. and Ignacimuthu S. 2006. In vitro antibacterial activity of some plant essential oils. BMC Compl. Altern. Med. 6:39.

Prasanth R.V., Ravi V.K., Varsha P.V. and Satyam S. 2014. Review on *Thymus vulgaris* traditional uses and pharmacological properties. Medicinal Aromat. Plants. 3:1-3.

Rudramurthy G.R., Swamy M.K., Sinniah U.R. and Ghasemzadeh A. 2016. Nanoparticles: alternatives against drug-resistant pathogenic microbes. Molecules. 21:836-866.

Sakkas H. and Papadopoulou C. 2007. Antimicrobial activity of basil, oregano, and thyme essential oils. J. Microbiol. Biotechnol. 27:429-438.

Sateriale D., Forgione G., De Cristofaro G.A., Facchiano S., Boscaino F., Pagliuca C., Colicchio R., Salvatore P., Paolucci M. and Pagliarulo C. 2022. Towards green strategies of food security: antibacterial synergy of essential oils from *Thymus vulgaris* and *Syzygium aromaticum* to inhibit *Escherichia coli* and *Staphylococcus aureus* pathogenic food isolates. Microorganisms. 10:2446-2463.

Scalas D., Mandras N., Roana J., Tardugno R., Cuffini A.M., Ghisetti V., Benvenuti S. and Tullio V. 2018. Use of *Pinus sylvestris* L. *Pinaceae*, *Origanum vulgare* L. *Lamiaceae*, and *Thymus vulgaris* L. *Lamiaceae* essential oils and their main components to enhance itraconazole activity against azole susceptible/not- susceptible

Cryptococcus neoformans strains. BMC Complement. Alt. Med. 18:1-13.

Soliman S.A., Hafez E.E., Al-Kolaibe A. 2022. Biochemical characterization, antifungal activity, and relative gene expression of two mentha essential oils controlling *Fusarium oxysporum*, the causal agent of *Lycopersicon esculentum* root rot, Plants. 11:189.

Swamy M.K., Akhtar M.S. and Sinniah U.R. 2016. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evidence-Based Compl. Altern. Med. 2016:3012462.

Tural S. and Turhan S. 2017. Antimicrobial and antioxidant properties of thyme *Thymus vulgaris* L., rosemary *Rosmarinus officinalis* L. and laurel *Lauris nobilis* L. essential oils and their mixtures. GIDA The J. Food. 42:588-596.

Ultee A., Bennik M.H.J. and Moezelaar R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68:1561-1568.

Wińska K., Mączka W., Łyczko J., Grabarczyk M., Czubaszek A. and Szumny A. 2019. Essential oils as antimicrobial agents-myth or real alternative?. Mol. 24:1-21.

Wojnicz D., Kucharska A.Z., Sokól-Letowska A., Kicia M. and Tichaczek-Goska D. 2012. Medicinal plants extracts affect virulence factors expression and biofilm formation by the uropathogenic *Escherichia coli*. Urol Res. 40:683-697.

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