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RESEARCH ARTICLE

THE ESSENTIAL OIL OF THYMUS VULGARIS TRAVELS TO THE CELL NUCLEUS

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ABSTRACT

Essential oils have shown interesting properties, such as antimicrobial activity. Some essential oils possess bactericidal and fungicidal activity. Among them, *T. vulgaris* essential oil is a potent inhibitor of the growth of bacteria such as uropathogenic *E. coli*. Some mechanisms of action of essential oils have been reported. The *T. vulgaris* essential oil appears to act in different ways that operate simultaneously, for example, by affecting respiratory activityand forming pores in the cytoplasmic membrane. This work shows some evidence about of a new mechanism of action of *T. vulgaris* essential oil to explain its potent antimicrobial action in *S. cerevisiae*.

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INTRODUCTION

Antimicrobial resistance has emerged as one of the most pressing global public health threats of the 21st century. Antimicrobial resistance manifests when microorganisms, encompassing bacteria, fungi, parasites, and viruses, undergo evolutionary processes leading to their resistance against antimicrobial medications, such as antibiotics, commonly employed for treating such infections (Ahmed *et al*., 2024; Amin *et al.*, 2023; Ferdinand *et al*., 2024; Hu *et al*., 2019; Lim *et al.,* 2024; Prestinaci *et al.*, 2015;Ruckert *et al*., 2024; Salam *et al.*, 2023). In light of this, alternative strategies are currently being sought to combat infectious diseases caused by bacteria or other infectious agents resistant to antimicrobials. One of those strategies is the use of substances of plant origin, such as essential oils (Pan *et al.*, 2023). Essential oils are complex mixtures of volatile compounds obtained from plants where they play aromatic, communicative and defensive roles. They have been used for centuries in medicine, agriculture and industry due to their broad spectrum of biological activity. They exhibit antimicrobial, antiviral, anti-inflammatory, antioxidant and smooth muscle relaxant properties (Gayán *et al.*, 2020; Żukowska and Durczyńska, 2024). Most parts of the aromatic plants contain essential oils: flowers, leaves, roots, rhizomes, fruits, seeds, including wood and bark. Essential oils are stored in complex secretary structures, including glandular trichomes, resin ducts, and secretory cavities. It is considered that the main function of the essential oils for plant organisms is protection against pests, predators, and pollination (Dhifi *et al*. 2016; Ivanova *et al*., 2025; Sharifi-Rad *et al*., 2017). *Thymus vulgaris* essential oil has demonstrated significant antibacterial and antifungal properties. It completely eliminates the bacteria and fungi against which it has been tested. Its mechanism of action is linked to pore formation (Flores-Encarnación *et al.*, 2026). This work shows some evidence of a new mechanism of action of *T. vulgaris* to explain its potent antimicrobial action: *T. vulgaris*reaches the cell nucleus.

MATERIAL AND METHODS

Source of material: In this study, the commercial essential oil of thyme was used. It was obtained from a flavour and fragrance company at Puebla, Mexico.

Biological material: The *Saccharomyces cerevisiae* strain was used. The strain of *S. cerevisiae* used was the yeast marketed for making bread. Yeast was stored in cryovials at -40°C in yeast peptone dextrose (YPD) broth with 20% glycerol until

analysis. The cells of white onion (*Allium cepa*) were used. *A. cepa* was obtained fresh from a commercial store at Puebla, Mexico.

Culture: *S. cerevisiae* strain were cultivated on yeast peptone dextrose broth containing amoxicillin (16µg/mL) and gentamicin (40 µg/mL) and the following components of medium (g/L): 10 yeast extract, 20 peptone and 20 dextrose pH 6.5. The stationary cultures were grown at 30°C for 24 hours in glass tubes containing 5 mL of yeast peptone dextrose broth and were used as precultures. The yeast peptone dextrose agar plates containing 20 mL of medium were prepared. Sterile Petri dishes (100 mm) were used. Plates were inoculated by crossstriation with a stationary 24-hour preculture of *S. cerevisiae* in yeast peptone dextrose broth (Ab_{560nm}=5).

Antifungal activity of *T. vulgaris* essential oil: The antifungal activity of *T. vulgaris* essential oil was determined using the technique of diffusion in agar using paper discs. For it, yeast peptone dextrose agar plates were prepared. Sterile Petri dishes (100 mm) with 3 compartments were used (containing 7 mL of medium per compartment). Plates were inoculated by crossstriation with a stationary 24-hour preculture of *S. cerevisiae* in yeast peptone dextrose broth (Ab_{560nm}=5). Then, sterile filter paper disks (5 mm diameter) were placed on the surface of yeast peptone dextrose agar plates. Different amounts of essential oil were used: 0.65, 1.3 and 2.6mg. The agar plates were incubated at 30°C for 24 h. The diameters of the inhibition zones formed were measured. The analyses were conducted in triplicate.

Methylene blue staining: Methylene blue staining was performed as follows: 1 mL of an active culture of *S. cerevisiae* (18-24 hours of culture, Ab_{560nm}=5) was centrifuged at 3,000 r.p.m. for 10 min at 4 °C. The supernatant was removed and 200 µL of phosphate-buffered saline solution pH 7.0 were added (cell suspension). Methylene blue staining was made by mixing 10 µL of cell suspension and 10 µL of 0.1% methylene bluedye. This mixture was incubated for 10 minutes at room temperature. Then, 10 µL of mix was observed at 40X power. All determinations were made in triplicate.

Effect of *T. vulgaris* essential oil on *S. cerevisiae* cells stained with methylene blue: The effect of *T. vulgaris* essential oil on *S. cerevisiae* cells stained with methylene bluedye was determined as follows. The cell suspension was prepared and mixed with the methylene blue dye as described before. Before this, 1.3 mg of *T. vulgaris* essential oil was added to cell suspension (10 µL) and mixture was incubated at room temperature for 5 min. Finally, 10 µL of the mix were applied on amicroscope slide observing at 40X power. All determinations were made in triplicate.

Trypan blue stainingon *A. cepa* cells: The *A. cepa* cells were obtained from a white onion. For this, the surface of a fresh white onion was cleaned with 70% alcohol. Then, the outermost layer of the onion was mechanically removed and an incision (3–4 mm deep) was madein the remaining body of the onion, delimiting a 1 cm^2 area. Using sterile forceps, a thin layer containing *A. cepa* cells was removed and placed on a microscope slide. Immediately, 10 µL of 0.1% trypan blue dye were added to *A. cepa* cells and observing at 35X power. Dead cells were observed in a deep blue color. All determinations were made in triplicate.

Effect of *T. vulgaris* essential oil on *A. cepa* cells stained with trypan blue dye: The *A. cepa* cells were obtained as previously indicated. Then, 10 µL of phosphate-buffered saline solution pH 7.0 were added to the *A. cepa* cells. Subsequently, 2.6 mg of *T. vulgaris* essential oil mixed with 10 µL of 0.1% trypan blue were added; a coverslip was placed, and the preparation was incubated for 1 minute at room temperature. The *A. cepa* cells were observed at 35X. All determinations were made in triplicate.

RESULTS

In this study, *S. cerevisiae* was grown on yeast peptone dextrose agar plates as described in Materials and Methods and the antifungal activity of *T. vulgaris* essential oil was determined using the technique of diffusion in agar. So, sterile filter paper disks were placed on the surface of yeast peptone dextrose agar plates and different amounts of essential oil were used: 0.65, 1.3 and 2.6 mg. The agar plates were incubated at 30°C for 24 h. The results obtained are shown in Fig. 1A. As can be seen, the growth of *S. cerevisiae* was inhibited in all cases at the tested quantities of the essential oil. In this study, yeast peptone dextrose agar plates were used with 3 compartments in order to observe the effect of *T. vulgaris* independently. The growth of *S. cerevisiae* was inhibited even at the lowest concentrations of the essential oil. Subsequently, *S. cerevisiae* cells were stained with methylene blue. The preparation was incubated for 10 minutes at room temperature as indicated in Materials and Methods. The results are shown in Fig. 1B. In this case, *S. cerevisiae* cells did not exhibit a blue color indicating viable cells; the absence of blue coloration is due to the fact that methylene blue is reduced within the cytoplasm of these cells, resulting in a colorless state. When *S. cerevisiae* cells were treated with *T. vulgaris* essential oil, the cytoplasm stained an intense blue, indicating that the *T. vulgaris* essential oil damaged the *S. cerevisiae* cytoplasmic membrane, thereby facilitating the dye's penetration into the cytoplasm.

However, the *S. cerevisiae* cells lost their viability; consequently, the methylene blue dye could not be reduced, causing the cytoplasm to retain its intense blue color (Fig. 1C). In this figure, it was also observed that the *S. cerevisiae* cells exhibited some regions that stained more intensely. These were likely the nuclei of the *S. cerevisiae* cells and vacuoles present in the cytoplasm. Due to the size of *S. cerevisiae* cells, the biological model was changed to use larger cells, such as those of *A. cepa*. The *A. cepa* cells were obtained as described in Materials and Methods. These cells were stained with another dye, 0.1% trypan blue (another vital dye) (Fig. 1D). Then, 10 µL of phosphate-buffered saline solution pH 7.0 were added to the *A. cepa* cells and 2.6 mg of *T. vulgaris* essential oil mixed with 10 µL of 0.1% trypan blue were added. The results are shown in Fig. 1E. As can be seen in Fig. 1D, the *A. cepa* cells appeared larger. They exhibited a different architecture, and the outlines corresponding to the cell wall and cytoplasmic membrane were clearly visible, stained by the trypan blue dye, which was not internalized into the cells. Cells treated with *T. vulgaris* essential oil exhibited blue coloration, indicating that the dye was able to penetrate the cytoplasm de *A. cepa*; most notably, the nuclei were also stained an intense blue (Fig. 1E). Although plant cells have a more complex structure than yeast cells, the essential oil of *T. vulgaris* created pores in the cell

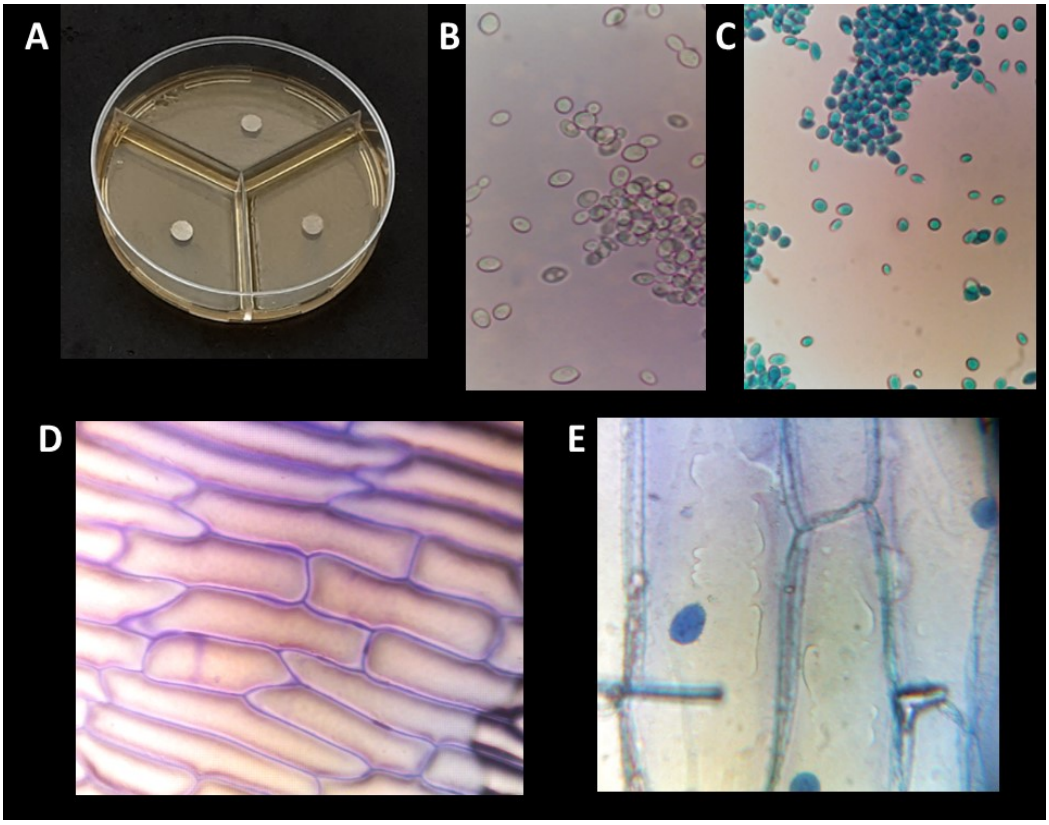

Fig. 1 The effect of *T. vulgaris* essential oil on *S. cerevisiae* and *A. cepa* cells. A. Inhibition of *S. cerevisiae* growth by *T. vulgaris* essential oil. B. *S. cerevisiae* cells stained with 0.1% methylene blue. C. *S. cerevisiae* cells treated with *T. vulgaris* and stained with methylene blue. D. *A. cepa* cells stained with 0.1% tripan blue. E. *A. cepa* cells treated with *T. vulgaris* and stained with tripan blue (migration of essential oil to cell nucleus)

membrane and was able to diffuse through the dense envelope, reaching the nucleus of the plant cells and, presumably, the nucleus of *S. cerevisiae* as well.

DISCUSSION

Essential oils are very complex mixtures of volatile substances obtained from plant material. The primary function of essential oils is to provide scent and flavour to plants. They also play an important communicative role, attracting pollinators and repelling pests. Sometimes, they also act as signals for other plants of the same species (Gershenzon and Dudareva, 2007; Vigan *et al.*, 2010; Żukowska and Durczyńska, 2024). *T. vulgaris* is rich in essential oils, phenolic compounds, and flavonoids. These components give it powerful antioxidant, antimicrobial, and anti-inflammatory properties. Thymol and carvacrol, phenolic compounds are the main constituents of *T. vulgaris* essential oil, are particularly effective in neutralizing reactive oxygen species and exhibit antimicrobial activity against a broad spectrum of pathogens (El-Sayed and El-Sayed, 2020; Farhadi *et al*., 2024; Gavaric *et al*., 2015; Gómez-Llorente *et al*., 2025; Jilani *et al.*, 2025). Plant essential oils exert antimicrobial activity through multiple concurrent mechanisms, including membrane disruption, biofilm inhibition, quorum sensing interference, and suppression of antimicrobial resistance determinants such as efflux pumps and β-lactamases. This multi-target activity distinguishes essential oils from conventional singletarget antibiotics and may explain the limited development of bacterial resistance observed in vitro (Iskandar *et al*., 2025; Kim and Rhee, 2016; Tariq *et al*., 2019).

There is little information regarding the antimicrobial mechanism of action of *T. vulgaris* essential oil. In this study, it was shown that *T. vulgaris* essential oil is a potent inhibitor of *S. cerevisiae* growth. There is evidence about antimicrobial activities attributed to specific compounds related to monoterpenes such as thyme, carvacrol, α- pinene, linalool, methyl salicylate, eugenol and geraniol, compounds present in *T. vulgaris* essential oil (Monzote -Fidalgo *et al*., 2004; Prasanth *et al*., 2014; Scalas *et al*., 2018; Wińska *et al*., 2019). In previous studies, we found that *T. vulgaris* essential oil reduced respiratory activity in *S. cerevisiae* cells and also formed pores in the yeast plasma membrane (Flores-Encarnación and Hernández-Hernández, 2024; Flores-Encarnación *et al.*, 2026). In the present study, *T. vulgaris* essential oil damaged the *S. cerevisiae* cytoplasmic membrane, thereby facilitating the penetration of methylene blue dye into the cytoplasm. The *S. cerevisiae* cells lost their viability, consequently the methylene blue dye could not be reduced causing the cytoplasm to retain its intense blue color. Living cells possess active metabolic pathways that can reduce methylene blue dye to its colorless form. In contrast, dead cells lack this enzymatic activity and remain stained blue (Kwolek-Mirek and Zadrag-Tecza, 2014). In this study, it was also also observed that *S. cerevisiae* cells exhibited some regions that stained more intensely (nuclei and vacuoles of *S. cerevisiae*). Thus, *T. vulgaris* essential oil permeated other structures in *S. cerevisiae*. To better visualize this effect, *A. cepa* cells were used; these exhibited an ordered symmetry and were larger in size. In *A. cepa* cells, it was observed that the addition of *T. vulgaris* essential oil altered cell membrane permeability, facilitating the entry of the trypan blue (vital dye).

In addition to the cytoplasm, the nuclei of the *A. cepa* cells were also stained, indicating that the essential oil of *T. vulgaris* permeated the nuclear membrane in these plant cells. This finding provided a better understanding of the potent antimicrobial effect observed when using *T. vulgaris* against bacteria and fungi: this essential oil can simultaneously affect various key sites and structures within microbial cells (bacteria and yeasts). The entry of *T. vulgaris* essential oil into the cell nucleus could be highly significant, as *T. vulgaris* might alter the function of the genetic material- thereby inhibiting cell replication- and consequently facilitate the death of *S. cerevisiae*. In bacteria have been reported that antibacterial properties of the essential oils might be related to their hydrophobicity, which causes a rise in cell permeability and the subsequent leakage of cell components. The disturbance of cell walls and membranes, modification of ATP generation and protein synthesis, pH disturbance, intracytoplasmic alterations (Cox *et al.*, 2000; Taheri *et al.*, 2023).

CONCLUSION

The antimicrobial properties of certain plant-derived substances have attracted the attention of various researchers. In particular, essential oils- such as the one extracted from *T. vulgaris*- have proven to be potent inhibitors of bacterial and fungal growth. As demonstrated in this study, *T. vulgaris* acts on *S. cerevisiae* through various mechanisms, making it highly effective as an antifungal agent. Further research is needed to understand the effects of this essential oil on the nucleus of *S. cerevisiae* or other yeast-like fungi.

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