

Antifungal Activity and Inhibition Mechanisms of Various Plant Derived Natural Compounds against Yeast Cells

Bengu ERGUDEN

Gebze Technical University

Hatice Busra KONUK

Gebze Technical University

Abstract: Microorganisms develop resistance due to excessive and improper usage of synthetic chemicals as antimicrobials which have high toxic effects in food industry. Therefore, biological preservatives having antifungal properties began to take place of toxic chemicals. Essential oils (EOs) derived from plants which prevents the deterioration of biofilm formed by yeast and prolongs shelf life as protective agents have recently become important in food industry. However, there is a significant restriction on the usage of essential oils due to variations in their content depending on the extraction methods and storage time. Thus, direct usage of active substances involved in the plant extracts may be preferred for industrial production. On the other hand, knowledge about antifungal mechanisms of substance is an important factor to determine the areas where the components can be used effectively. Cell membrane has a vital role because of providing cellular integrity and homeostasis and carrying out molecular transport. Antifungal agents have activity at very low concentrations via disrupting cell membrane integrity. They can be used for preservation of foods, as well as for additives which are not toxic, and decreasing contamination and biofilm formation via coatings in food industry. Since, EOs can be used as food preservatives and pharmaceutical agents, it is very important to understand their mode of action and their main target sites in the cell. Thus this research not only opens new perspectives to understand antifungal activity mechanisms of EOs, but also help widen their use.

Keywords: *Saccharomyces cerevisiae*, Essential oils, Antifungal activity, Thyme, Carvacrol

Introduction

Essential oils (EOs) are complex mixtures of volatile compounds produced by plants and fruits. They are known to have antioxidant (Yang et al., 2010), antibacterial (Reichling et al., 2009), antifungal (Hammer et al., 2004), antiplasmodial (Schelz et al., 2006), antiviral, antiparasitic and insecticidal properties (Unal et al., 2009). It is clear from different studies on EOs which are defined 'generally regarded as safe' (GRAS) by the FDA that they have potential uses in medicine and applications in the cosmetic, pharmaceutical and food industries and also in cleaning products (Souza et al., 2007; Van Vuuren et al., 2009; Lima de Sousa et al., 2013; Bialon et al., 2014; Rajkowska et al., 2014; Boire et al., 2016).

There are different antimicrobial mechanisms of EOs. Cell wall, cell membrane, intracellular proteins, enzymes and nucleic acids are significant target sites for EO contents (Helander et al., 1998; Burt et al., 2004; Morten et al., 2012). Cell membrane is the first line of defense against environmental stresses. It was suggested that the lipophilic nature of EOs allows them to easily pass through cell membranes to change biological responses of cells (Wang et al., 2015). Especially phenolic compounds and terpenes may accumulate in the cell membrane and result in instant loss of membrane integrity, making it highly permeable to ions that might be responsible for the establishment of antimicrobial activity. It has also been shown that essential oil affects the membrane composition of *Yarrowia lipolytica* yeast and some bacteria (Di Pasqua et al., 2006; Papanikolaou et al., 2008). In other cases, changes in membrane fluidity and integrity of yeast cells were observed upon exposure to various

stress conditions, by regulating the biosynthesis of fatty acids and sterols (Ding et al., 2009; Ta et al., 2010; Dupont et al., 2011; Turk et al., 2011).

Although antifungal activity of EOs was examined before, their effects on cytoplasmic membrane of *S. cerevisiae* have not been extensively studied (Tao et al., 2014a, 2014b). Due to our continuing interest on the mode of action of various chemicals on yeast membranes (Sezen, 2015), we set out to unearth the possible membrane dependent action of essential oils on yeast cells. In this study, we first examined the antifungal effects of *lemon peel*, *orange peel*, *tea tree*, *turpentine*, *rosemary*, *peppermint*, *thyme*, *oregano* and *clove oil* against *S. cerevisiae* by applying the measurement of Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC) and inhibition zone techniques. Then we evaluated the membrane damage by measuring the extracellular pH of glucose-induced cells and conductivity of yeast cells after exposure to different concentrations of above EOs.

Method

Inhibition Zone Measurement

The agar diffusion method (Skocibusic et al., 2006) was employed for the determination of antifungal activities of the essential oils. 500 μ L of fresh culture of *S. cerevisiae* was spread on the YPD agar media plates and allowed to dry for 2 hours. Later, 5 mm diameter wells were opened in solid media plates. EOs were diluted in DMSO at different concentrations and 55 μ L of essential oils were filled into the each well. Plates were placed in the incubator at 25 °C for 48 hours. After incubation, the diameters of the inhibition zones were measured in centimeter.

Extracellular pH Measurement

The permeability of *S. cerevisiae* cell membranes is expressed in terms of their electric conductivity and extracellular pH value (Gaskova et al., 2013) and was determined by following method. *S. cerevisiae* strain was cultured overnight at 25°C in 20 mL of YPD broth. After incubation, the yeast cells were centrifuged at 3200 rpm for 5 min and pellet was washed twice with sterilized dH₂O. The pellet was then resuspended in sterilized dH₂O. About 50 mg wet weight of yeast cells were used for each experiment. The essential oils dissolved in DMSO were diluted to the mentioned concentrations. %2 glucose (zero point) and essential oils (18. min) were manually injected to the final concentrations. Extracellular pH was recorded with an HI 98127 water proof pH meter (HANNA, USA).

Results and Discussion

The main purpose of this study was to unearth the dependence of antifungal activity of various essential oils on the integrity of cell membrane. After a thorough search of the literature we uncovered that the information on the antifungal activity against *S. cerevisiae* was limited. More importantly experimental data reported in the literature were based on studies with different experimental conditions preventing a simple comparison of the data with each other. Thus at the beginning of our studies we set out to determine the antifungal activity of *lemon peel*, *orange peel*, *tea tree*, *turpentine*, *rosemary*, *peppermint*, *thyme*, *oregano* and *clove oils* against *S. cerevisiae* via MIC, MFC and inhibition zone measurements.

The inhibition zones of essential oils are presented in Table 1. According to inhibitory zone measurements five oils with the highest efficacy were oregano oil (for 20% dilution, 3.6 ± 0.1), orange peel oil (for 20% dilution 3.3 ± 0.9), thyme oil (for 20% dilution 3.0 ± 0.5), turpentine oil (for 20% dilution 2.5 ± 0.2), and clove oil (for 20% dilution 2.2 ± 0.5). The inhibition zone values generally confirmed the results obtained in the MIC and MFC data. Slight differences between the two data sets may be caused by different rates of diffusion of particular oil components into the agar medium or by evaporation of some of the components during the incubation time (Kunicka-Styczyńska, 2011). Recent studies highlight the role of water-soluble and vaporized components in the assessment of antimicrobial activity of essential oils (Inouye et al., 2006; Fisher et al., 2006).

Table 1. Antifungal activity of essential oils presented as Zones of Inhibition. Essential oils were mixed with DMSO to increase solubility. The values are mean of four replicates \pm standard deviation

#	Essential Oil	%	Zones of Inhibition (cm)
1	lemon peel	100	$1,3 \pm 0,3$
		20	$<0,5$
2	orange peel	20	$3,3 \pm 0,9$
3	tea tree	100	<1
		20	$<0,5$
4	turpentine	20	$2,5 \pm 0,2$
5	rosemary	100	<1
		20	$<0,5$
6	peppermint	100	$1,7 \pm 0,3$
		20	<1
7	thyme	20	$3,0 \pm 0,5$
8	oregano	20	$3,6 \pm 0,1$
9	clove	20	$2,2 \pm 0,5$

Later, extracellular pH and conductivity measurements were performed to determine their effect on membrane integrity and membrane permeability of yeast cells (Gaskova et al., 2013). It is well known that glucose-induced cells show a decrease in extracellular pH values (Souza et al., 2007). In order to reach a maximal pH gradient across the cell membrane, yeast cells were glucose-induced before extracellular pH measurement experiments. Figure 1 shows the changes in extracellular pH of orange peel, turpentine, thyme and oregano oil treated yeast cells for 0-60 min. Upon addition of essential oils, an increase in extracellular pH was observed.

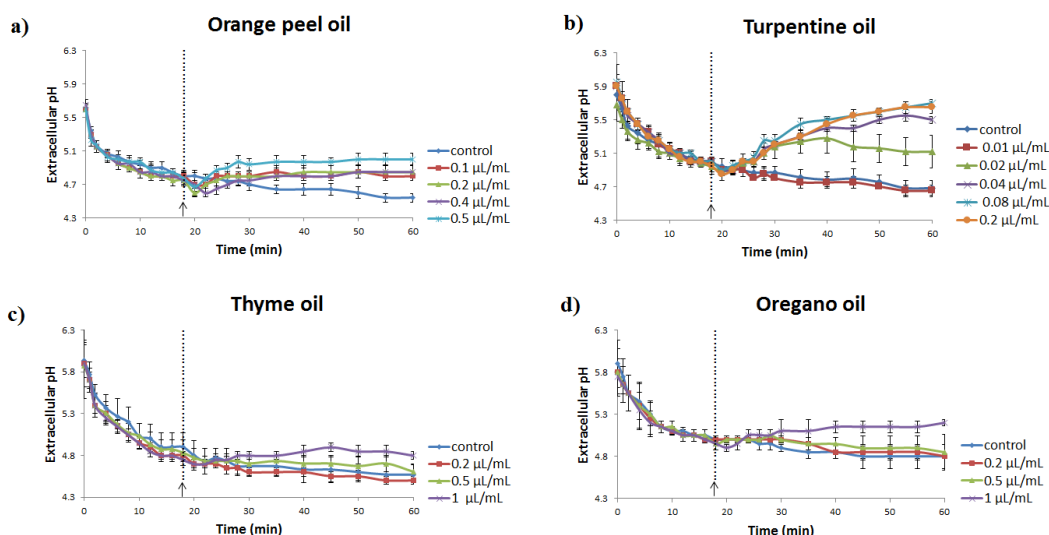


Figure 1. Effects of essential oils on the extracellular pH of *S. cerevisiae*. Concentration dependent effects of essential oils on yeast cells in glucose-induced medium are shown. The arrows indicate the time of addition of a) orange peel oil: 0.1; 0.2; 0.4; 0.5 $\mu\text{L/mL}$, b) turpentine oil: 0.01; 0.02; 0.04; 0.08; 0.2 $\mu\text{L/mL}$, c) thyme oil: 0.2; 0.5; 1 $\mu\text{L/mL}$, d) oregano oil: 0.2; 0.5; 1 $\mu\text{L/mL}$. The data represent the average of at least two independent experiments.

Especially extracellular pH of yeast cells treated with various concentrations of turpentine oil dramatically increased in a concentration dependent-manner. Higher concentrations than 0.2 $\mu\text{L/mL}$ could not be tested due to solubility problems. Addition of orange peel, thyme and oregano oils also caused an increase in the extracellular pH of yeast cells, possibly due to the neutralization of the glucose-induced pH gradient upon impairment of the cell membrane.

The interest in EOs has significantly grown in recent years and there has been an increase in the number of scientific publications of essential oils. Our results demonstrate that essential oils extracted from different plants show wide spectrum of antifungal activity against *S. cerevisiae* and that the cell membrane is the main target for the antifungal agents in the content of EOs, while disruption of yeast cell membrane integrity is the basic mode of action of these agents. These results will augment our knowledge about the mechanism of action of EOs against *S. cerevisiae* cells and help us widen their usage in food, cosmetic and pharmaceutical industries.

Acknowledgements

*This work was supported by GTÜ-BAP, project number: 2018-A108-70.

References

- Bialon M, Krzysko-Lupicka T, Koszałkowska M, Wieczorek PP (2014) The influence of chemical composition of commercial lemon essential oils on the growth of candida strains. *Mycopathologia*;177:29–39.
- Boire N, Zhang S, Khuvis J, Lee R, Rivers J, Crandall P, Kevin Keel M, Parrish N. (2016) Potent inhibition of *Pseudogymnoascus destructans*, the causative agent of White-Nose Syndrome in bats, by cold-pressed, terpeneless, valencia orange oil. *PLoS One*;11:1-10.
- Burt S. (2004) Essential oils: Their antibacterial properties and potential applications in food, A review. *Int J Food Microbiol*;94:223-53.
- Di Pasqua R, Hoskins N, Betts G, Mauriello, G. (2006) Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. *J Agric Food Chem*;54:2745–9.
- Fisher K, Phillip CA. (2006) The effect of lemon, orange and bergamot essential
- Gaskova D, Plasek J, Zahumensky J, Benesova I, Buriankova L, Sigler K. (2013) Alcohols are inhibitors of *Saccharomyces cerevisiae* multidrug-resistance pumps Pdr5p and Snq2p. *FEMS Yeast Res*;13:782–95.
- Hammer KA, Carson CF, Riley TV. (2004) Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans*, *Candida glabrata* and *Saccharomyces cerevisiae*. *J Antimicrob Chemother*;53:1081–5.
- Helander IM, Akakomi H, Latva-Kala K, Mattila-Sandholm T, Pol I, Eddy J. (1998) Characterization of the action of selected essential oil components on gram-negative bacteria. *J Agric Food Chem*;46:3590–5.
- Kunicka-Styczyńska A. (2011) Activity of essential oils against food-spoiling yeast. A review. *Flavour Fragr J*;26:326–8.
- Lima de Sousa L, Albuquerque de Andrade SC, Aguiar Athayde AJA, Vasconcelos de Oliveira CE, Veríssimo de Sales C, Madruga MS, Leite de Souza E. (2013) Efficacy of *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils in combination to control postharvest pathogenic *Aspergilli* and autochthonous mycoflora in *Vitis labrusca* L. (table grapes). *Int J Food Microbiol*;165:312–8.
- Martos AI, Romero A, Gonzalez MT, Gonzalez A, Serrano C, Castro C. (2010) Evaluation of the Etest method for susceptibility testing of *Aspergillus spp.* and *Fusarium spp.* to three echinocandins. *Med Mycol*;48:858–61.
- Morten H, Mygind TL, Rikke M. (2012) Essential oils in food preservation: mode of action, synergies and interactions with food matrix components. *Front Microbiol*;3:1-24.
- Papanikolaou S, Gortzi O, Margeli E, Chinou I, Galiotou-Panayotou M, Lalas S. (2008) Effect of Citrus essential oil addition upon growth and cellular lipids of *Yarrowia lipolytica* yeast. *Eur J Lipid Sci Technol*;110:997–1006.
- Rajkowska K, Kunicka-Styczyńska A, Maroszyńska M, Dabrowska M. (2014) The effect of thyme and tea tree oils on morphology and metabolism of *Candida albicans*. *Acta Biochim Pol*;61:305-10.
- Reichling J, Schnitzler P, Suschke U, Saller R. (2009) Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties – an overview. *Forsch Komplementmed*;16:79–90.
- Schelz Z, Molnar J, Hohmann J. (2006) Antimicrobial and antiplasmid activities of essential oils. *Fitoterapia*;77:279–85.
- Sezen B. (2015) Reduction of *S. cerevisiae* Pom34 protein level by SESA network is related to membrane lipid composition. *FEMS Yeast Res*;15:fov089.
- Souza EL, Stamford TLM, Lima EO, Trajano VN. (2007) Effectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts. *Food Control*;8:409-13.
- Tao N, Liu Y, Zhang M. (2009) Chemical composition and antimicrobial activities of essential oil from the peel of bingtang sweet orange (*Citrus sinensis* Osbeck). *Int J Food Sci Technol*;44:1281–5.

- Unal MU, Ucan F, Şener A, Dincer S. (2009) Research on antifungal and inhibitory effects of DL-limonene on some yeasts. *Turk J Agric For*;36:576-82.
- Van Vuuren SF, Suliman S, Viljoen AM. (2009) The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. *Lett Appl Microbiol*;48:440–6.
- Wang Y, Zeng X, Zhou Z, Xing K, Tessema A, Zeng H, Tian J. (2015) Inhibitory effect of nerol against *Aspergillus niger* on grapes through a membrane lesion mechanism. *Food Control*, 55, 54-61.
- Yang SA, Jeon SK, Lee EJ, Shim CH, Lee IS. (2010) Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. *Nat Prod Res*, 24, 140–51.

Author Information

Bengu Erguden

Gebze Technical University
Department of Bioengineering
41400 Çayırova, Gebze, Kocaeli, TURKEY
Contact E-mail: b.sezen@gtu.edu.tr

Hatice Busra Konuk

Gebze Technical University
Department of Bioengineering
41400 Çayırova, Gebze, Kocaeli, TURKEY
Contact E-mail: b.sezen@gtu.edu.tr
