

Modeling the antifungal effect of Menthol and Menthone from *Mentha piperita* essential oil

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Abstract:

Mentha piperita essential oil (MPEO) has shown significant antifungal activity against strains and clinical isolates of *Candida albicans*. The aim of this study is to show the contribution and the role of the majority terpenoids of MPEO in its anti-Candida activity by RSM and QSAR models. The RSM model designed on 30 observations and minimum inhibitory concentrations (MIC) between 0.225 and 0.63 mg/mL has revealed that the different percentages of α -Terpineol, Pulegone, Menthone and Menthol explain 62% of the anti-Candida activity observed *in vitro*. The minimum predicted value of MIC by this model is 0.221 ± 0.098 mg/mL and the corresponding values of the factors are: 1.31% for α -Terpineol, 10.54% for Pulegone, 13.91% for Menthone and 29.57% for Menthol. The QSAR model ($R^2=0.83$, $R^2_{adj}=0.78$) showed that the distribution of atomic Sanderson electronegativity influences the antifungal activity of MPEO oxygenated monoterpenes. Menthol and Menthone were shown to be very active alone and in combination against *C. albicans*, followed by α -Terpineol and Carvone. RSM and QSAR models have a very important utility in predicting the quantitative and qualitative doses of antifungal terpenoids, and also to describe this activity associated with electronic and topological properties of the chemical components of oils.

Keywords: *Mentha piperita*, *Candida albicans*, Menthol, Menthone, RSM, QSAR.

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1 Introduction

Oral Candidiasis is a common fungal infection affecting individuals of all ages. The broad term of Candidiasis refers to cutaneous, mucosal and deep-seated organ infections, caused by yeasts of the genus *Candida*. Candidaemia is an emerging bloodstream invasive infection, widely recognized as a major cause of morbidity and mortality in hospital settings. These infections can be caused by five main pathogens: *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei*. The emergence of antifungal resistance problem led to the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Candida* spp. strains, correlated with extensive use of azoles and echinocandins in hospitals (Akpan & Morgan, 2002; Pappas et al., 2018).

For the purpose of researching new substances capable of controlling and treating *Candida* spp. yeast infections, essential oils (EOs) were considered a promising source of active natural products and having, in most cases, low toxicity on the host. These terpene compounds have several pharmacological properties and by their lipophilic character, they can interact with membranes and lipid structures of yeasts. They can also disrupt the activity of enzymes involved in ergosterol biosynthesis, or disrupt membrane permeability and fluidity by creating

complexes with ergosterol membrane (Ferreira et al., 2021; Samber et al., 2015).

Anti-Candida activity of EOs has been demonstrated by several studies. EOs containing phenols have been shown to be active against fungal growth in solid, liquid and vapor phases. The activity of EOs is also effective against the formation of biofilms and spoilage yeasts. Ketones and monoterpene alcohols are recognized by their fungicidal effect against *C. albicans* which results from the formation of a charge transfer complex with an electron donor from fungal cells. In addition, the phenolic compounds of EOs have been considered to be potent antifungal molecules and it turns out that this activity increases in the presence of alkyl groups on benzene ring (Almeida et al., 2019; Palmeira-de-Oliveira et al., 2009).

The genus *Mentha* L. (Lamiaceae) contains about 42 species recognized by their cultivation worldwide from tropical to temperate climates, and also by their medicinal, aromatic and economic values (Tyagi & Malik, 2011). Over the past decades, several studies have investigated the biological activities of different *Mentha* spp. extracts, such as antioxidant, antiallergic, antiviral, antimicrobial, anti-inflammatory and antihypertensive activities. The antimicrobial effect of *Mentha* EOs varies between moderate to significant depending on the resistance

or sensitivity of the strains and isolates and more importantly depending on the elemental composition of the EO itself (Mahendran & Rahman, 2020).

Mentha piperita L. is a hybrid between *Mentha spicata* L. and *Mentha aquatica* L., it is a herbaceous with stems up to 60 to 90 cm long with lance-shaped leaves and purple flowers (Barceloux, 2008). It is a perennial plant indigenous to Europe and distributed by cultivation throughout the world. Since ancient times, *M. piperita* has been used by North African and Egyptian people as a flavoring agent for food and tea. Furthermore, this species has been used in traditional pharmacopoeia in Iran as a carminative, stimulant, tonic, anti-viral and anti-fungal agent (Mahendran & Rahman, 2020) and in Algeria as anthelmintic and for fever, intestinal colic, indigestion (Tahri et al. 2020). Furthermore, *M. piperita* and its EOs have been used as anti-spasmodic, anti-septic and in treatments for colds, nausea and sore throat. Moreover, the leaves of *M. piperita* have been used against coughs, bronchitis and its EO against menstrual cramps, rheumatism and muscle pain relief (Anwar et al. 2019).

In addition to its use in perfumery, cosmetics, spices, aromatherapy and nutrition, studies have shown interesting biological activities of *M. piperita* EO (MPEO) such as antiviral, antioxidant, antifungal, antibacterial, antitumor, and antiallergic (Rosato et al. 2018). Thereby, studies have investigated the inhibitory effect of MPEO against strains and clinical isolates of *C. albicans*. This EO has been shown to be active with significantly effective concentrations (Benzaid et al. 2019). The aim of this study is to show the contribution and the role of the majority terpenoids of MPEO in its anti-*Candida* activity by Response Surface Methodology (RSM) and Quantitative Structure Activity Relationship (QSAR) models.

2 Materials and methods

2.1 Response surface methodology model

This model aims to study of the percentages influence of four components of MPEO on Minimum Inhibitory Concentrations (MIC) of *C. albicans* growth. The percentages and activities of α -Terpineol, Pulegone, Menthone and Menthol representing 35.45 to 70.7% of the total composition of six MEPOs obtained from commercial sources (Işcan et al. 2002; Nikolić et al. 2014; 4). These percentages enabled the establishment of the experimental design with Design Expert 13.0.1.0. Table 1 represents the MIC values at the different experimental settings for this factorial analysis of 2 level design type with 4 factors and 30 runs.

Table 1: Experimental design and the MIC values with different percentages of MPEO chemical constituents.

S t d	R u n	Factors				Respo nse MIC (mg mL ⁻¹)
		A: α - Terpine ol (%)	B:Pule gone (%)	C:Men thone (%)	D:Me nthol (%)	
22	1	0.16	0.47	9.1	34.82	0.225
30	2	0.16	0.47	9.1	34.82	0.225
73	3	0.16	0.47	9.1	34.82	0.225
21	4	0.16	0.47	9.1	34.82	0.225
12	5	0.16	0.47	9.1	34.82	0.225
29	6	0.16	0.47	9.1	34.82	0.225
10	7	2.4	0.7	27.9	27.5	0.625
26	8	1.6	1	21.2	42.3	0.625
27	9	1.7	1.9	27.2	39.9	0.312
13	10	0.7	14.4	18.4	34.6	0.625
13	11	0.4	0.0	21.7	47.5	0.320
22	12	0.4	0.0	21.7	47.5	0.39
15	13	0.4	0.0	21.7	47.5	0.51
33	14	0.4	0.0	21.7	47.5	0.51
11	15	0.4	0.0	21.7	47.5	0.51
28	16	0.4	0.0	21.7	47.5	0.249
20	17	0.4	0.0	21.7	47.5	0.39
44	18	0.4	0.0	21.7	47.5	0.39
88	19	0.4	0.0	21.7	47.5	0.63
19	20	0.4	0.0	21.7	47.5	0.51
66	21	0.4	0.0	21.7	47.5	0.51
23	22	0.4	0.0	21.7	47.5	0.320
16	23	0.4	0.0	21.7	47.5	0.320
17	24	0.4	0.0	21.7	47.5	0.320
14	25	0.4	0.0	21.7	47.5	0.51
55	26	0.4	0.0	21.7	47.5	0.51

6						
2	2	0.4	0.0	21.7	47.5	0.51
4	7					
9	2	0.4	0.0	21.7	47.5	0.63
	8					
1	2	0.4	0.0	21.7	47.5	0.320
8	9					
2	3	0.4	0.0	21.7	47.5	0.51
5	0					

The statistical significance of the model and the interactions between variables were estimated by analysis of variance (ANOVA).

2.2 Quantitative Structure Activity Relationship modeling

Three-dimensional structures (.sdf) of α -Terpineol, Linalool, Menthofuran, Menthone, Menthol, Pulegone and Carvone have been downloaded from PubChem database. MIC values of the selected components, tested alone against the growth of *C. albicans*, were obtained from published studies (Boni et al., 2016; Dias et al., 2018; Fernandes et al., 2021; Samber et al., 2015). 2D descriptors were obtained from Mold2 software. A total of 777 molecular descriptors were calculated, allowing the selection of descriptor that can explain the variation of antifungal activity in the training set (Hong et al. 2008). BuildQSAR 2.1 was used to calculate the multiple linear regression model (de Oliveira and Gaudio 2000).

2.3 Statistical analysis

The statistical significance of the models obtained was assessed by examining the correlation coefficients ($R > 0.6$) between the observed and predicted activities, the standard deviation, F-value and P-value. The predicted residual sums of cross-validation coefficient Q^2 ($Q^2 > 0.5$), squares standard deviation (SPress) and standard deviation error in prediction (SDEP) were used for the predictive ability validation of the obtained models.

3 Results and Discussion

3.1 In vitro inhibitory effect of *M. piperita* EO against *C. albicans*

In vitro studies have shown moderate to significant anti-Candida potency by MPEOs. Compared to a standard antifungal agent (Ketoconazole), the effect of MPEOs from different regions was moderate with a MIC of 0.635 mg/mL (Işcan et al. 2002). However, commercial MPEO exerted significant inhibition against 55 isolates of *C. albicans*, interpreted by MIC values between 0,083 and 0,21 mg/mL (Nikolić et al. 2014). Similarly, the effect of MPEO against three ATCC strains of *C. albicans* was also significant (0.22 mg/mL) and higher than the effect of synthetic compounds (Carvone, Menthone, and Menthol) (Samber et al., 2015).

In addition, *C. albicans* biofilm formation was inhibited by MPEO at MICs between 1 and 10 mg/L, this is not different from the EOs of *Artemisia judaica*, *Lawsonia inermis*, and *Thymus vulgaris* (Karpiński et al. 2021). Taking into consideration the variability of antibiofilm activity between species and within the same species. For MPEO, the study of Benzaid et al (2019) has shown that this oil inhibits the formation of *Candida* biofilm at a concentration of 10 μ L/mL. However, the study of Agarwal et al. (Agarwal et al. 2008) showed low activity with 800 μ L/mL. Moreover, in the study of Tyagi and Malik (2011), the MIC values of MPEO against *C. albicans* using the broth dilution method was 1.13 mg/mL. Furthermore, the inhibition was complete in the presence of a vapor generated by 40 μ L of MPEO. Therefore, the viability of *C. albicans* was reduced to 100% following exposure to this vapor for 8 h.

A significant synergistic effect was noted by the combination of MPEO and Fluconazole against strains of *C. albicans* sensitive and resistant to Fluconazole, with fractional inhibitory concentration index (FICI) between 0.26 and 0.41 (Samber et al., 2015). In another study, the FICI values resulting from the combination of MPEO with Amphotericin B and Miconazole were respectively 0.46 and 0.16-0.46 against three ATCC strains of *C. albicans* (Rosato et al. 2018). This synergistic effect was also observed by the combination of MPEO and Itraconazole with FICI of 0.24 (Tullio et al. 2019) and also by the combination with Diclofenac Sodium Salt with FICI of 0.3 against five strains of *C. albicans* (Rosato et al. 2021).

In addition to reducing the growth of *C. albicans* and inhibiting biofilm formation, MPEO downregulated the transition of this yeast from the blastopore form to hyphal phenotype. A large reduction in hyphae density was observed after direct contact of 10 μ L/mL of the oil with *C. albicans* culture. Treatment with MPEO and its vapor distorts the external morphology of *C. albicans*, reduces the expression of different Secreted Aspartyl Proteinases (SAP 1, 2, 3, 9 and 10) at 5 μ L/mL and completely inhibits Hyphal Wall Protein 1 gene expression (Benzaid et al. 2019). On the other hand, the study of Samber et al. suggests that MPEO reduces ergosterol levels and inhibits PM-ATPase leading to intracellular acidification which will end in cell death.

3.2 Prediction MIC of *M. piperita* EOs on *C. albicans*

The RSM model was designed on 30 observations with responses ranging from 0.225 to 0.63 mg/mL. Statistical significance, ANOVA results and fit statistics were summarized in Table 2. The high F-value and the very low p-value indicate that the model obtained is significant at a confidence level of

95%. The model's coefficient of determination ($R^2 = 0.62$) explains 62% of the antifungal activity of MPEO. While the small difference between R^2 and adjusted R^2 indicates a strong correlation between observed and anticipated data. The adequate precision value is a ratio between predicted values at the design points to the average prediction error. A ratio greater than 4 indicates adequate discrimination of the model and an appropriate signal-to-noise ratio (Elyemni et al. 2020).

Table 2: Results of ANOVA and fit statistics for RSM model.

Source of variance	Sum of Squares	Degrees of freedom	Mean Square	F-value	p-value	Significance
Model	0.3757	5	0.0751	7.7849	0.0000	Significant
A- α -Terpineol	0.0299	1	0.0299	3.0950	0.03	
B-Pulegone	0.0828	1	0.0828	8.5739	0.007	
C-Menthone	0.0375	1	0.0375	3.8880	0.06	
D-Menthol	0.0772	1	0.0772	8.0013	0.009	
CD	0.0829	1	0.0829	8.5880	0.007	
Pure Error	0.2316	24	0.0097			
Cor Total	0.6073	29				
R^2	0.619					
Adjusted R^2	0.539					
C.V. %	23.755					
Adequate precision	9.104					

MTSAa3, Moran topological structure autocorrelation length-3 weighted by atomic Sanderson electronegativities; s, standard deviation; F, Fisher value; p, probability value; Q2, cross-validated correlation coefficient; SPRESS, standard deviation of cross-validation; SDEP, standard deviation error in prediction.

The predicted minimum value of MIC is 0.221 ± 0.098 mg/mL and the corresponding values of the

factors are: 1.31% for α -Terpineol, 10.54% for Pulegone, 13.91% for Menthone and 29.57% for Menthol. This combination is closer to the composition of MPEO obtained from Erdoğan, Perfume Industry, imported from India (Işcan et al. 2002). The final equations in terms of coded factors and actual factors are:

$$\text{MIC} = 0.8084 + 0.2493 \cdot A + 0.2331 \cdot B - 0.4072 \cdot C + 0.8842 \cdot D - 1.07 \cdot CD$$

$$\text{MIC} = -6.62798 + 0.222556 \cdot \alpha\text{-Terpineol} + 0.032373 \cdot \text{Pulegone} + 0.258239 \cdot \text{Menthone} + 0.195352 \cdot \text{Menthole} - 0.007665 \cdot \text{Menthone} \cdot \text{Menthol}$$

The estimated coefficients make it possible to deduce the effects of the factors on the value of MIC. The coded equation shows the positive impact of variables A, B and C and the inverse effect of variables C and CD on the final response. Figure 1 shows contour plot and 3D response surface obtained from RSM model. This figure represents the influence of the percentages of Menthone and Menthol on the inhibitory activity against *C. albicans*. The MIC increases with an increase in the percentage of Menthol and with a moderate percentage of Menthone. On the other hand, this response decreases with a low to moderate percentage of Menthone and a moderate percentage of Menthol. Considering that Pulegone, Menthole and the Menthone/Menthol combination have significant effects ($p < 0.05$) in the RSM model.

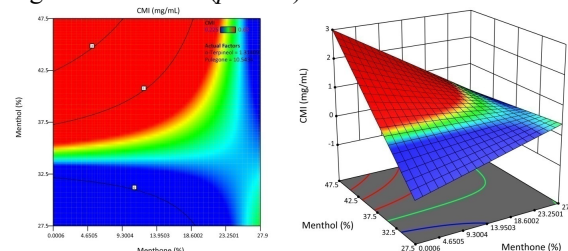


Figure 1: Contour plot and 3D response surface for the effect of Menthone and Menthol on MIC.

In the experiments investigated in this study, the four oxygenated monoterpenes represent 35.45 to 70.7% of the total composition of the MPEOs analyzed. Menthone and Menthol were known to be the major components of MPEO with percentages in the oils investigated in this study going to 69.2%. The effect of α -Terpineol alone against the growth of six strains of *C. albicans* was significant with MIC values between 0.078 and 0.31 mg/mL (Fernandes et al. 2021). On the other hand, the inhibitory power of Menthone and Pulegone on the fungal growth of ten clinical isolates of *C. albicans* was shown to be weak with MICs of 4 to 8 mg/mL and 1 to 2 mg/mL for these two compounds respectively (Boni et al. 2016). However, Menthol exerted a significant effect against 20 isolates of *C. albicans*, an effect revealed

by MIC values ranging from 0.012 to 0.1 mg/mL. This effect was shown to be greater when combining Menthol with standard antifungals like Fluconazole (0.003-1 mg/mL), Amphotericin B (0.003-0.05 mg/mL), Itraconazole (0.001-0.025 mg/mL) and Voriconazole (0.003-0.075 mg/mL) (Norouzi et al. 2021).

Bioactivity is interdependent on bioaccessibility, which is defined as the quantity or fraction released from the food matrix in the digestive tract that is available for absorption. These two parts make the term bioavailability which is the measure of the rate and fraction of the initial dose of a drug that successfully reaches the site of action or body fluids of the target drug to produce the desired biological effect (Masyita et al. 2022; Thakur et al. 2020). Terpenoids with lipophilic properties require the incorporation of a lipid phase either during digestion or during food processing, since this lipophilic behavior influences their solubility in the aqueous phase of the gut lumen (Masyita et al. 2022). The administration of terpenoids can be done intravenously, cutaneously, orally and by inhalation. In these last two routes, the upper gastrointestinal tract had no significant role in the absorption. However, via the inhalation route, terpenoids can be absorbed through the lungs so that they are available systemically (Kohlert et al. 2000; Masyita et al. 2022).

Metabolism and biotransformation of terpenoids occurs in the liver by phase I and phase II reactions and in most cases in a single phase. Elimination of terpenoids therefore occurs in the form of feces, expired air, and urine. After oral administration of Menthol or MPEO, 35-50% of the original Menthol content was excreted renally as menthol glucuronide (Kohlert et al. 2000). The elimination of menthol glucuronide, after administration of MPEO to ileostomized patients, was less than after administration to healthy subjects, indicating that the absorption of menthol mainly took place in the small intestine (Somerville et al. 1984). Other compounds like Menthone and Menthyl acetate can be metabolized into Menthol and also excreted as menthol glucuronide. It has been noted that terpenoids have a short elimination half-life, making it impossible for their metabolites to accumulate (Masyita et al. 2022). On the other hand, terpenoids exhibit excellent permeation-enhancing effects that facilitate transdermal drug delivery. As a result of their lipophilic character, Menthone and Menthol enhance the penetration of ligustrazine hydrochloride, an anti-platelet aggregation, by promoting skin permeation through mechanisms including disruption and extraction of the hydrogen bond connection of lipids (Wang et al. 2017).

3.3 QSAR of oxygenated monoterpenes and MIC

The values of MIC, 2D descriptor and QSAR model fitting parameters for oxygenated monoterpenes are shown in Table 3. Representation of regression model of the antifungal activities of MPEO oxygenated monoterpenes is shown in Figure 2. QSAR equation obtained from MIC's multiple linear regression model ($R^2=0.83$, $R^2_{adj}=0.78$, $P<0.05$) is as follows:

$$\text{MIC} = + 4.8155 (\pm 3.0572) \text{MTSAA3} - 1.1038 (\pm 2.0899)$$

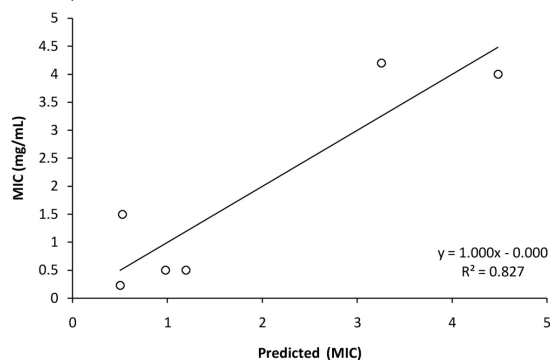


Figure 2: Correlation plot of predicted vs. observed antifungal activities of oxygenated monoterpenes.

The QSAR model showed the positive contribution of Moran topological structure autocorrelation length-3 weighted by atomic Sanderson electronegativities, which is an index of spatial autocorrelation, defined over the Sanderson electronegativities of atoms. This descriptor is given by:

$$I(3) = \frac{\frac{1}{\Delta} \sum_{i=1}^A \sum_{j=1}^A \delta_{ij} (\chi_i - \bar{\chi}) \cdot (\chi_j - \bar{\chi})}{\frac{1}{A} \sum_{i=1}^A (\chi_i - \bar{\chi})}$$

Where χ_i is the Sanderson electronegativity of every atom in the molecule, $\bar{\chi}$ is the average electronegativity over the molecule, A is the number of atoms in the molecule, 3 is the topological distance used in this case, δ_{ij} is a Kronecker delta ($\delta_{ij}=1$ if $d_{ij}=3$, 0 otherwise), and Δ is the sum of the obtained Kronecker deltas (Benfenati 2011). The structural variables introduced by Moran were defined to quantify the contribution of an atomic property considered to the property analyzed, which corresponds to two-dimensional autocorrelations between pairs of atoms in the molecule (Duchowicz et al. 2007). MTSAA3 assumes that the distribution of atomic Sanderson electronegativity influences the antifungal activity of MPEO oxygenated monoterpenes.

Menthone is cyclohexanone substituted by a methyl and an isopropyl group at positions 5 and 2 respectively. Menthol is a non-planar cyclohexane ring which has methyl, hydroxyl and isopropyl

substitutes. Pulegone is cyclohexan-1-one substituted by a methyl group at position 5 and a propan-2-ylidene group at position 2. Carvone is cyclohex-2-enone having methyl and isopropenyl substituents at positions 2 and 5, respectively. α -Terpineol is cyclohexane with a 4-methylcyclohex-3-en-1-yl group at position 2. In both conformations of cyclohexane; the chair and the boat, chair type is the most thermodynamically stable. It has been suggested that substituents in the equatorial positions are important in antifungal activity (Dambolena et al. 2010). Menthofuran is 4,5,6,7-tetrahydro-1-benzofuran substituted by methyl groups at positions 3 and 6. It can be oxidized by CYP enzymes to create a reactive and hepatotoxic form resulting in liver damage (Tisserand and Young, 2013). Linalool is octa-1,6-diene substituted by methyl groups at positions 3 and 7 and a hydroxy group at position 3. (-)-Linalool was much more common than the (+)-Linalool in the essential oils. However, both enantiomers demonstrated approximately 50% growth inhibition of *Botrytis cinerea* at 0.3 μ M in 48 hrs (Ozek et al. 2010).

Menthol with its 3 asymmetric carbon atoms occurs as four optical isomer pairs: namely, (+)- and (-)-isomenthol, (+)- and (-)-menthol, (+)- and (-)-neomenthol, (+)- and (-)-neoisomenthol. While its biosynthesis goes through eight stages from geranyl diphosphate to (+)-Pulegone, (-)-Menthone then (-)-Menthol. The latter form is commonly used for its cooling properties greater than the other isomers. However, like all terpenes alcohols, Menthol does not absorb light well in the 290-320 nm range and upon oxidation it will transform into Menthone (Kamatou et al. 2013). The antifungal activity of five stereoisomers of Menthol has shown that (-)-Menthol and (+)-Menthol are more active against the growth, sporulation and biosynthesis of Fumonisin B1 from *Fusarium verticillioides* (Dambolena et al. 2010).

The compounds α -Terpineol, Linalool, Pulegone and carvone tend to have a higher water solubility and H-bonding capacity. On the other hand, the discriminating variables of the anti-Candida activity between (-)-Menthone and (+)/(-)-Menthols are: H-bond acceptor capacity, H-bonding capacity, Polarity and Hydrophilic area (%). As a result, Griffin et al. (Griffin et al. 1999) reported that the MIC value of Linalool was lower (1300 ppm) compared to Linalool oxide and Linalyl acetate (5500 and 19300 ppm, respectively). It has been suggested that the addition of an acetate moiety to the molecule decrease the antimicrobial activity. However, (-)-Menthol and (+)-Menthol, having the same MIC value (500 ppm), were more active than (-)-Menthone (4000 ppm). Moreover, α -Terpineol activity was shown to be important against *C.*

albicans with MIC of 930 ppm. The anti-Candida activity of Pulegone is greater than that of Carvone. But despite being a major compound in EOs of several mint species, it has been reported that Pulegone cause gastritis, induce abortion, and result in hepatic failure, central nervous system, renal and pulmonary toxicity (van Zyl et al. 2006; Zárybnický et al. 2018).

(+)-Pulegone is of central importance in the biosynthesis of MPEO monoterpenes. It is the precursor of (-)-Menthol, but it can be reduced to (-)-Menthone by pulegone reductase or oxidized to Menthofuran by menthofuran synthase. However, it has been suggested that Menthofuran down-regulate pulegone reductase activity leading to an increase in the concentration of Pulegone in the oil (Mahmoud and Croteau 2003). The ratio of Menthone/Pulegone, Menthol/Pulegone and (Menthone+Menthol)/Pulegone was increased by the application of bioregulators to *M. piperita* plants at a concentration of 40 ppm gibberellic acid + 40 ppm indole butyric acid + 5%v/v methanol (Pourhadi et al. 2018). On the other hand, high contents of MPEOs, and percentage of Menthol and Menthone/Menthol were obtained by spraying Chitosan (5 g/L) and inoculating arbuscular mycorrhizal fungi (Goudarzian et al. 2020).

The physicochemical properties of the major compounds of MPEO have helped explain its significant activity against *C. albicans*. The synergistic effect between these components and especially between Menthone and Menthol played an interesting role in this activity. The resulting QSAR model indicates its predictive power for the activity of oxygenated monoterpenes of MPEO based on topological and electronic properties. The RSM and QSAR models elucidate the important anti-Candida capacity of the oxygenated components of the essential oil of this easy-to-grow plant, found worldwide and has been used in traditional medicine since ancient times.

4 Conclusions

The RSM model showed that the percentages of α -Terpineol, Pulegone, Menthone and Menthol representing 35.45 to 70.7% of the total composition of MPEOs analyzed explains 62% of the anti-Candida activity of MPEO. The minimum predicted MIC value is 0.221 ± 0.098 mg/mL and the corresponding percentages of the other factors are: 1.31% for α -Terpineol, 10.54% for Pulegone, 13.91% for Menthone and 29.57% for Menthol. The QSAR model ($R^2 = 0.83$, $R^2_{adj} = 0.78$) predicted the activity of seven components of MPEO in relation to their molecular properties. Menthol alone or in combination with Menthone was found to be the most active, followed by α -Terpineol and Carvone. It

is important to note that antifungal activity varies between susceptible and resistant strains and isolates. Therefore, the results of this study can be used in the preparation of the optimal combinations of these oxygenated monoterpenes to improve their antifungal power against the different strains of *C. albicans*.

Declarations

Disclosure of potential conflicts of interest

The authors declare no conflict of interest.

Research involving Human Participants and/or Animals

This article does not contain any studies with human participants or animals performed by any of the authors.

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