

## Research Article

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# Chemical composition and *in vitro* activity of *Origanum vulgare* L., *Satureja hortensis* L., *Thymus serpyllum* L. and *Thymus vulgaris* L. essential oils towards oral isolates of *Candida albicans* and *Candida glabrata*

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**Abstract:** The purpose of this research was to investigate the chemical composition of essential oils (EOs) from: *Origanum vulgare* L., *Satureja hortensis* L., *Thymus serpyllum* L. and *Thymus vulgaris* L. (Lamiaceae) cultivated in Poland, and to study their antifungal activity towards clinical isolates of oral *Candida* spp. The hydrodistilled essential oils were analyzed using the GC-MS method. The antifungal activity was evaluated *in vitro* against oral isolates and reference strains of *Candida albicans* and *C. glabrata*, using the broth microdilution method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) guidelines, allowing for estimation of minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC). GC-MS analysis revealed that carvacrol was the main EO compound in oregano and summer savory, while thymol and linalool were the major ingredients of thyme and wild thyme oils, respectively. The EOs possessed fungicidal activity against *C. albicans* and *C. glabrata*, including oral isolates, with MIC = 125 – 2000 mg/L, MFC = 250 – 4000 mg/L and MFC/MIC = 1 – 4, depending on the yeast and plant species. The most active

was thyme oil – with MIC = 125 – 500 mg/L, MFC = 250 – 500 mg/L and MFC/MIC = 1 – 2.

**Keywords:** *Candida albicans*, *Candida glabrata*, Lamiaceae, essential oil, GC-MS, antifungal activity, oral cavity.

## 1 Introduction

The yeasts from genus *Candida*, covering about 280 species, are a part of the microbiota colonizing mucocutaneous areas, with a reported prevalence of 15-75%, mainly within the oral cavity, upper airways, gastrointestinal tract and vagina of healthy individuals. Simultaneously, *Candida* spp., especially *Candida albicans* and *Candida glabrata* (the last listed under the collective non-*albicans* *Candida* species listing [NAC]) are the most important cause of opportunistic infections worldwide, affecting predominantly immunocompromised or hospitalized patients, as well as the elderly population [1-3]. *Candida* spp. is associated with candidiases, including different diseases that range from superficial infections (e.g. oropharyngeal candidiases), to life threatening systemic disorders. Several factors may contribute to the pathogenic potential of *C. albicans*, e.g. growth in two forms, unicellular and filamentous, to adherence to the mucosal tissues and the production of extracellular enzymes [2]. *C. albicans* is the most common species isolated from the oral cavity (in 40% of the entire population) [1]. In turn, the incidence of *C. glabrata* is difficult to determine since this species is rarely isolated alone and is often co-isolated with *C. albicans*. It currently ranks second or third as the causative agent of *Candida* infections [4].

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During the last decades, a constantly increasing number of fungal diseases caused by resistant strains of different *Candida* species [2] have established themselves within the human population. Insufficient effectiveness of some azole preparations and higher toxicity of polyene antibiotics has stimulated the search for new natural antifungal compounds. One of the most promising natural alternatives to traditional antifungal preparations are the essential oils (EOs) drawn from medicinal plants. Many of the natural products containing EOs and their constituents have antimicrobial properties, while several studies have shown high sensitivity of pathogenic fungi, including yeasts, to EOs [2,5-8]. According to Waller et al. [9-11], 55 botanical species belonging to 27 genus exhibit antifungal effects, especially the aromatic plants, notably *Origanum vulgare* L., *Satureja hortensis* L., *Thymus serpyllum* L. and *Thymus vulgaris* L. from the Lamiaceae family. These plants are a rich source of EOs [6,12,13], the major components of which may belong to several classes of compounds, mainly terpenes, such as thymol and carvacrol [12,14]. Other isolated chemical molecules from Lamiaceae, among others, estragole, 1,8-cineole, terpineol-4-ol,  $\gamma$ -terpinene were also described as promising for use as antifungals in mycoses [9]. However, it is necessary to identify the most effective compounds in order to extract or synthesize in pure form for future use in the pharmaceutical industry. It is also imperative that essential oils should be further examined, so that safe conclusions can be drawn about their potential use the antifungal agents [8].

The purpose of the work was to analyze the chemical composition of EOs from: *Origanum vulgare* L., *Satureja hortensis* L., *Thymus serpyllum* L. and *Thymus vulgaris* L. cultivated in Poland, and to assess their antifungal activity against oral isolates of *Candida* spp.

## 2 Materials and Methods

### 2.1 Raw materials and essential oils

This study used the following commercial raw materials (herbs): oregano (*O. vulgare* L.), summer savory (*S. hortensis* L.), thyme (*T. vulgaris* L.) (mfg. Dary Natury, Poland) and wild thyme (*T. serpyllum* L.) (mfg. Flos, Poland) which were all purchased from a local herbal store (Lublin, Poland). All raw materials were before their expiration date. The EOs were obtained by way of the hydrodistillation method, using the Deryng-type apparatus, according to previously described procedure

[15]. The EOs were dried over anhydrous  $\text{Na}_2\text{SO}_4$ , stored in a dark glass bottle and kept at 4°C until analysis.

### 2.2 GC/MS analysis

Gas chromatography – mass spectrometry (GC-MS) analysis was performed utilizing a Shimadzu GC-2010 Plus, coupled to a Shimadzu QP2010 Ultra mass spectrometer, according to the method described by Skalicka-Wozniak et al. [16]. The separation of compounds was obtained by employing a fused-silica capillary column ZB-5 MS (30 m, 0.25 mm i.d.) with a film thickness of 0.25  $\mu\text{m}$  (Phenomenex) via temperature gradient (50°C for 3 min., then a constant increase up to 250°C during 25 min, after which 250°C was held for 2 min). The temperatures of 250°C, 250°C, and 220°C were kept for the injector, interface and ion source, respectively. Helium was the carrier gas, the flow rate being 1 mL/min, while the split ratio was 1:20. Ions were produced by way of the electron impact method at 70 eV. The scan mode was in the m/z range 40–500, while the scan rate of 0.20 s per scan was applied for data acquisition. Finally, a homologous series of *n*-alkanes ( $\text{C}_8$ – $\text{C}_{24}$ ) were used to determine the retention indices under the same operating conditions. The identification was performed using MS data from the NIST Database [17].

### 2.3 Tested microorganisms

The study used the reference strains – *Candida albicans* ATCC 10231, *C. albicans* ATCC 2091 and *Candida glabrata* ATCC 90030, as well as 30 clinical strains of *C. albicans* (15 isolates) and *C. glabrata* (15 isolates) obtained from the oral mucosa of hospitalized patients, including cancer patients, patients with chronic hepatitis C or diabetes mellitus and elderly people. The isolates were identified by standard diagnostic methods. All the used microbial cultures were stored at -70°C, and then subcultured on the Sabouraud agar at 30°C for 24-48 h.

### 2.4 Estimation of minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

The antifungal activities of EOs were screened *in vitro* by applying the broth microdilution method according to the recommendation of European Committee on Antimicrobial Susceptibility Testing (EUCAST) [18] and Clinical and

Laboratory Standards Institute (CLSI) guidelines [19]. Samples containing these EOs were dissolved in dimethyl sulfoxide (DMSO). The stock solution of EOs was 50 000 mg/L. Subsequently, the MIC (Minimal Inhibitory Concentration) of the compounds was examined by applying the microdilution broth method, using their two-fold dilutions (from 8 000 to 62.5 mg/L) in RPMI 1640 broth with MOPS. In this method, 96-well microtiter plates were used; then, yeast suspensions of 0.5 McFarland standard in sterile saline were prepared in each isolate using a densitometer. The yeast suspension was added (1  $\mu$ l) to each well. The last wells containing only RPMI with the MOPS medium without EOs served as the positive growth control. In turn, the wells with medium and EOs but without yeast inoculum served as the negative control. The microtiter plates were incubated at 35°C for 24 h. The optical density of yeast growth in each well was estimated using the ELx800 microtiter plate reader (Biotek) connected with the KC Juniors for Windows computer program. The MIC was read as the lowest concentration of EOs which prevented growth of a given yeast isolate.

In turn, after MIC readings, MFC (Minimal Fungicidal Concentration) was determined. The inocula from each well which showed a completed growth inhibition and from the last positive well (growth similar to the growth control well), and from the growth control for each isolate were subcultured on Sabouraud agar plates, and incubated as before. The MFC showed the lowest EO concentration that showed either no growth or fewer than three colonies to obtain approximately 99 to 99.5% killing activity. The studies were carried out in accordance with previously described procedures [20]. Nystatin and chlorhexidine (Sigma-Aldrich) were used as reference antifungal compounds (positive controls). The MFC/MIC ratios were calculated to determine the fungicidal (MFC/MIC  $\leq$  4) or fungistatic (MFC/MIC  $>$  4) effect of the tested EOs [20]. Moreover, the values of MIC<sub>50</sub> and MIC<sub>90</sub> or MFC<sub>50</sub> and MFC<sub>90</sub> – minimal inhibitory concentrations or minimal fungicidal concentrations of EOs, which inhibit or kill 50% or 90% of the tested isolates, were also established.

Ethical approval: The Ethics Committee of the Medical University of Lublin approved the study protocol (No. KE-0254/75/2011).

### 3 Results and Discussion

Today there are several classes of antifungal drugs available for the treatment of fungal infections in humans. In oral candidiases, various antimycotics have been used as antiseptic agents, with fluconazole, nystatin

suspension and chlorhexidine commonly applied topically as mouthwash [21]. However, several studies have been performed to search for natural compounds with antifungal activity, especially among aromatic plants due to their high content of biologically active compounds, mainly EOs [5,6,14].

It is well known that the composition, quality and content of EOs are subject to a great variation and are influenced by diverse factors such as the geographical and climatic conditions, as well as the conditions used for culture, drying and storage, the harvesting season, or differences in oil extraction techniques. Consequently, these variations can have a repercussion on the biological activity of EOs [7,10,11].

#### 3.1 Chemical composition of essential oils

Using the GC/MS method, 21 to 34 chemical components of the examined oils, were identified. Wild thyme oil (*T. serpyllum* L.) showed the highest number of these ingredients – 34 components. In turn, 23 and 25 components were found in summer savory (*S. hortensis* L.) and thyme oils (*T. vulgaris* L.), respectively. The least number of components (21), were identified in oregano oil (*O. vulgare* L.). The content of individual volatile components of the examined essential oils was presented as their percentage share among the detected compounds. Significant amounts of carvacrol (50.7%),  $\gamma$ -terpinene (24.3%) and p-cymene (12.5%) were found in the savory oil components. Similarly, a high carvacrol content (57.3%) was detected in oregano EO. Additionally, among the identified components of this oil, 1,8-cineole (12.9%) and  $\alpha$ -pinene (7.9%) were in significant amounts. In turn, oil of wild thyme included a large percentage of linalool (30.9%) and thymol (25.1%), and slightly less geraniol (10.5%) and p-cymene (5.3%). Thyme EO showed the largest content of thymol (75.2%), and also showed a lower content of carvacrol (7.7%) and p-cymene (6.3%). In these EOs, the remaining components were present in smaller amounts (Table 1).

According to the GC-MS analysis presented in this article, the concentration of phenolic monoterpenes was higher as compared to that of other compounds prevalent in the studied EOs. We found that oregano EO contained the highest amount of carvacrol (57.3%), 1,8-cineole and  $\alpha$ -pinene (20.9%). Previous reports showed that in Polish oregano cultures, the main components of the EOs were carvacrol, thymol and  $\gamma$ -terpinene [22]. The carvacrol content of different chemotypes of oregano EO is variable and it can be up to 95% [8]. Among the compounds

**Table 1:** Percentage composition of volatile components identified from: *Origanum vulgare*, *Satureja hortensis*, *Thymus serpyllum*, and *Thymus vulgaris* L. essential oils (n=3).

No.	Compound	RI*	Relative percentage (%)			
			OV	SH	TS	TV
1	$\alpha$ -Thujene	928	0.2	1.2	0.2	0.1
2	$\alpha$ -Pinene	935	7.9	0.9	0.2	0.2
3	Camphene	953	–	0.1	0.1	0.1
4	$\beta$ -Pinene	980	0.2	0.2	–	–
5	1-Octen-3-ol	983	–	0.2	0.4	–
6	$\beta$ -Myrcene	990	0.3	2.0	0.2	0.1
7	$\alpha$ -Phellandrene	1009	–	0.3	–	–
8	$\alpha$ -Terpinene	1019	0.4	3.6	0.2	0.1
9	p-Cymene	1028	1.4	12.5	5.3	6.3
10	Limonene	1032	1.2	0.4	0.2	–
11	$\beta$ -Phellandrene	1034	–	0.3	–	–
12	1,8 - Cineole	1059	12.9	–	0.5	0.4
13	$\gamma$ -Terpinene	1061	1.6	24.3	0.6	0.3
14	cis-Sabinene hydrate	1075	–	0.2	0.4	–
15	Terpinolene	1089	–	0.1	–	–
16	Linalool	1103	4.0	–	30.9	1.1
17	Camphor	1153	–	–	–	0.4
18	p-Mentha-3-one	1161	–	–	0.3	0.4
19	Borneol	1180	–	–	0.8	1.6
20	Menthol	1184	–	–	0.2	0.8
21	Terpinen-4-ol	1187	–	0.4	0.5	0.8
22	p-Cymene-8-ol	1194	–	0.1	–	–
23	$\alpha$ -Terpineol	1202	2.9	0.2	3.4	0.3
24	Nerol	1229	–	–	0.4	–
25	Thymol methyl ether	1233	–	–	1.4	0.5
26	Carvacrol methyl ether	1242	–	–	1.7	0.3
27	Geraniol	1254	–	–	10.5	–
28	Geranial	1271	–	–	0.3	–
29	Bornyl acetate	1288	–	–	–	0.2
30	Isothymol	1292	–	–	–	0.7
31	Anethole	1301	–	–	0.2	–
32	Thymol	1301	4.7	0.5	25.1	75.2
33	Carvacrol	1312	57.3	50.7	2.2	7.7
34	Myrtenyl acetate	1327	0.2	–	–	–
35	$\alpha$ -Terpinyl acetate	1351	1.4	–	3.8	–
36	Carvacryl acetate	1368	0.2	–	–	–
37	Geranyl acetate	1378	0.3	–	3.2	–
38	$\beta$ -Bourbonene	1392	–	–	0.2	–
39	(E)- $\beta$ -Caryophyllene	1430	0.8	0.6	0.8	0.4
40	$\gamma$ -Muurolene	1483	–	–	0.2	–
41	Isolatedene	1492	–	–	1.3	–
42	$\beta$ -Humulene	1500	0.2	–	–	–
43	$\alpha$ -Acorenol	1506	–	–	0.2	–
44	$\beta$ -Bisabolene	1513	1.3	0.7	4.1	–
45	$\delta$ -Cadinene	1526	–	–	0.3	–
46	Spathulenol	1591	–	0.1	–	0.1
47	Caryophyllene oxide	1596	0.8	0.1	–	1.4
48	$\tau$ -Cadinol	1655	–	–	–	0.5

RI\* - Retention Index; OV – *Origanum vulgare* L.; SH – *Satureja hortensis* L.; TS – *Thymus serpyllum* L.; TV – *Thymus vulgaris* L.

identified in summer savory EO, carvacrol (50.7%) and also  $\gamma$ -terpinene and p-cymene (36.7%) were largely prevalent. This chemotype of the EO cultivated in Poland is very similar to the chemotype of the oil obtained from wild savory that is cultivated in Iran [23]. EOs from savory oil contain variable amounts of the main components to which thymol, carvacrol, as well as  $\gamma$ -terpinene and p-cymene belong [24]. As this paper suggests, wild thyme oil contains a large percentage of linalool (30.9%) and thymol (25.1%), plus geraniol and p-cymene (15.8%). Interestingly, this chemotype is a less frequent chemotype [25,26]. In the study of Wesołowska et al. [26], EOs isolated from wild thyme were composed mainly from carvacrol,  $\gamma$ -terpinene, p-cymene and  $\beta$ -caryophyllene. In other studies, a high content of thymol [27],  $\gamma$ -terpinene [28] or geraniol [25] was found. In contrast, the thyme EO showed a large content of thymol (75.2%), carvacrol (7.7%) and their precursor, p-cymene (6.3%). The composition of this EO is typical for the thymol chemotype. According to other authors [29,30], several chemotypes of thyme, based on EOs compositions, have been established, while the thymol chemotype is very common among essential Polish oil crops [31].

### 3.2 Antifungal assay

The obtained results were summarized in Tables 2, 3 and 4. The listed estimations of  $MIC_{50}$ ,  $MIC_{90}$ ,  $MFC_{50}$  and  $MFC_{90}$  allowed the sensitivity to EOs of the population of *Candida* spp to be assessed. Our results showed that the reference strains of *C. albicans* and *C. glabrata*, as well as the oral cavity isolates, were inhibited and killed by all tested EOs. MIC ranged from 125 to 2000 mg/L and MFC ranged from 125 to 4000 mg/L, depending on the yeast and plant species. Moreover, the MFC/MIC ratio was 1 to 4, indicating that the studied EOs held a positive fungicidal effect. The effect of the selected EOs on the studied oral isolates was similar. The values of  $MIC_{50}$ ,  $MIC_{90}$ ,  $MFC_{50}$  and  $MFC_{90}$  were in the range of 250 – 500 mg/L for oregano, savory and thyme EOs, while higher values were obtained for wild thyme EO.

Among the tested EOs, thyme oil, was the most active, with MIC ranging from 125 – 500 mg/L and 125 – 250 mg/L against oral isolates of *C. albicans* and *C. glabrata*, respectively. In turn, the value of MFC was in the range of 250 – 500 mg/L and MFC/MIC in the range of 1 – 2 towards isolates of both *Candida* species. The values of  $MIC_{50}$ ,  $MIC_{90}$ ,  $MFC_{50}$  and  $MFC_{90}$  were in the range of 250 – 500 mg/L.

We found that all tested EOs showed activity against yeasts belonging to *C. albicans* and *C. glabrata* reference strains and those isolated from the oral cavity of different groups of patients. These EOs were able to inhibit the growth of pathogenic yeasts and also killed them at the same or slightly higher concentrations (MFC/MIC in the range of 1 – 4), hence, indicating their fungicidal activity. The following order of EOs antifungal activity against oral isolates of *C. albicans* and *C. glabrata* was found as follows: thyme EO (MIC = 125 – 500 mg/L)  $\geq$  oregano EO (MIC = 250 – 500 mg/L) = summer savory EO (MIC = 250 – 500 mg/L)  $\geq$  wild thyme EO (MIC = 500 – 1000 mg/L).

The thyme essential oil had the highest effect towards oral isolates of *Candida* spp. among the tested oils. Numerous investigations have reported it to be a highly antimicrobial compound [5,7,30,32,33]. Furthermore, Cosentino et al. [30] showed similar activity of thyme EO (MIC = MFC = 225 – 450 mg/L) as reported above. In turn, the study of Al-Shahrani et al. [32] exhibited a much lower antifungal activity (MIC = 500 – 10 000 mg/L and MFC = 2 500 – 10 000 mg/L). Perhaps, lower MIC and MFC values were associated with a lower content of active ingredients in the EO. However, this cannot be confirmed because the authors did not analyze the chemical composition. A particularly high activity of this oil with MIC = 16.3 mg/L against *C. albicans* was demonstrated by Fani et al. [33]. In contrast, other results [7] indicated that thyme EO was not able to inhibit the growth of the *C. glabrata* isolates at the tested concentrations (MIC > 3200 mg/L). However, Giordani et al. [5] assessed the potential effect of various chemotypes of thyme EOs against *C. albicans* and showed that the thymol chemotype was the most active, with a MIC 80% of 14.7 mg/L, wherein the efficacy was mainly due to the high level of thymol (63.2%). This aligns with our data.

The antifungal effect of the remaining EOs showed a lower activity against the oral isolates of *C. albicans* and *C. glabrata* compared to that of the thyme oil. According to other data, the values of MIC of oregano EO against strains of *Candida* spp. differentiated, ranging from 62.5 to 1600 mg/L [7,34,35-39]. Our data are consistent with the earlier studies by other authors [34,39] who showed that MIC of *O. vulgare* EO ranged from 62.5 to 500 mg/L against clinical *C. albicans* strains. Çoşkun et al. [35] found that the oil was also very effective against different strains of *Candida* spp. and its values of MIC were relatively low, ranging between 62.5 – 125 mg/L. Moreover, Delić et al. [37] compared the activity of oregano oil against *C. albicans* indicating MIC at 110 mg/L and MFC at 230 mg/L. Vahedi G. et al. [36] revealed that the MIC of oil for *C. glabrata* isolates obtained from healthy individuals and patients

**Table 2:** The activity of the tested essential oils against reference strains of *Candida albicans* and *C. glabrata*.

EOs	<i>C. albicans</i> ATCC 2091			<i>C. albicans</i> ATCC 10231			<i>C. glabrata</i> ATCC 90030		
	MIC	MFC	MFC/MIC	MIC	MFC	MFC/MIC	MIC	MFC	MFC/MIC
OV	250	500	2	250	500	2	250	500	2
SH	250	250	1	500	500	1	500	500	1
TS	500	1000	2	500	1000	2	1000	1000	1
TV	250	500	2	125	250	2	250	250	1
Positive controls									
CHX	3.91	3.91	1	3.91	7.81	2	3.91	3.91	1
NY	0.24	0.24	1	0.48	0.48	1	0.24	0.48	2

The values of MIC and MFC were expressed in mg/L; NY: nystatin; CHX: chlorhexidine; OV: *Origanum vulgare* L.; SH: *Satureja hortensis* L.; TS: *Thymus serpyllum* L.; TV: *Thymus vulgaris* L.

**Table 3:** The activity of the tested essential oils against *Candida albicans* isolates from oral cavity.

EOs	Range of MIC*	MIC <sub>50</sub>	MIC <sub>90</sub>	Range of MFC*	MFC <sub>50</sub>	MFC <sub>90</sub>	MFC/MIC
OV	250 – 500	250	500	250 – 500	500	500	1 – 2
SH	250 – 500	250	500	250 – 500	250	500	1 – 2
TS	500 – 1000	500	1000	1000 – 2000	1000	2000	1 – 4
TV	125 – 500	250	500	250 – 500	250	500	1 – 2
Positive controls							
CHX	1.98 – 7.81	3.91	7.81	1.98 – 15.62	7.81	15.62	1 – 8
NY	0.06 – 0.48	0.24	0.48	0.12 – 0.48	0.24	0.48	1 – 2

Explanations as above; \* - results is significant at  $p < 0.05$

**Table 4:** The activity of the tested essential oils against *Candida glabrata* isolates from oral cavity.

EOs	Range of MIC*	MIC <sub>50</sub>	MIC <sub>90</sub>	Range of MFC*	MFC <sub>50</sub>	MFC <sub>90</sub>	MFC/MIC
OV	250 – 500	250	500	500 – 1000	500	500	1 – 2
SH	250 – 500	500	500	500	500	500	1 – 2
TS	500 – 2000	1000	2000	1000 – 4000	2000	4000	1 – 4
TV	125 – 250	250	250	250 – 500	500	500	1 – 2
Positive controls							
CHX	1.98 – 7.81	3.91	7.81	3.91 – 31.25	15.62	15.62	1 – 8
NY	0.06 – 0.48	0.24	0.48	0.12 – 0.98	0.48	0.98	1 – 4

Explanations as above; \* - results is significant at  $p < 0.05$

with oropharyngeal candidiasis were between 150 – 200 mg/L and 150 – 250 mg/L, respectively. In turn, Pozzatti et al. [38] observed values in the range of 400 to 800 mg/L for both parameters MIC and MFC, while Soares et al. [7] indicated that this EO showed varying levels of antifungal activity, with MIC ranging from 400 – 1600 mg/L and an even higher MFC ranging from 400 – 3200 mg/L.

Our data are in agreement with these results. However, the results obtained by Sartoratto et al. [40] and by Ignatova-Ivanova et al. [41] showed a much weaker oregano EO activity at 2000 mg/L or even at 2500 mg/L against both *C. albicans* and *C. glabrata* strains. In contrast to our data, Adiguzel et al. [24] showed that summer savory oil had no activity against *Candida* spp. In turn, Wesołowska et al. [26] reported the significant activity of wild thyme oil, with values of MIC at 45.5 mg/L and MFC at 91 mg/L, against *C. albicans*. This is contrary to our results – which indicated a lower antifungal activity. However, there is little literature available that correlates the activity of these EOs against *Candida* spp. and their chemical composition.

In our study, there was no difference in the MIC ranges of EOs between *C. albicans* and *C. glabrata* isolates, but the MIC values of EOs against these strains from other studies varied extensively. These variations might be explained by the different chemical composition of the EOs and the type of isolates used in these investigations. According to İşcan [42], terpene alcohols, phenols aldehydes and ketones appeared to be the most active components of EOs, while terpene hydrocarbons and esters appeared to be the least active. The antimicrobial activity of EO constituents was ranked as follows: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons [36]. The observed antimicrobial properties of the tested EOs may be related to the high content of the terpene alcohols and their high solubility ratio in both aqueous media and bio membranes due to the alcohol moiety. Some differences were observed regarding the antimicrobial effect of stereoisomers [42].

The tested EOs in our study, as mentioned earlier, were rich in the following compounds: thymol, carvacrol, p-cymene,  $\alpha$ -pinene, geraniol, linalool,  $\gamma$ -terpinene and 1,8-cineole. The study of İşcan [42] indicated that the activity of thymol (MIC = 250 – 500 mg/L), carvacrol and p-cymene (MIC = 250 – 500 mg/L) or  $\alpha$ -pinene (MIC = 500 – 1000 mg/L) was similar compared to the *C. albicans* and *C. glabrata* strains. Herein, geraniol possessed a particularly high activity, with MIC ranging from 60 to 500 mg/L and 1000 mg/L compared to *C. albicans* and *C. glabrata*, respectively, while linalool showed a slightly weaker effect at MIC = 500 – 1000 mg/L and 2000 mg/L

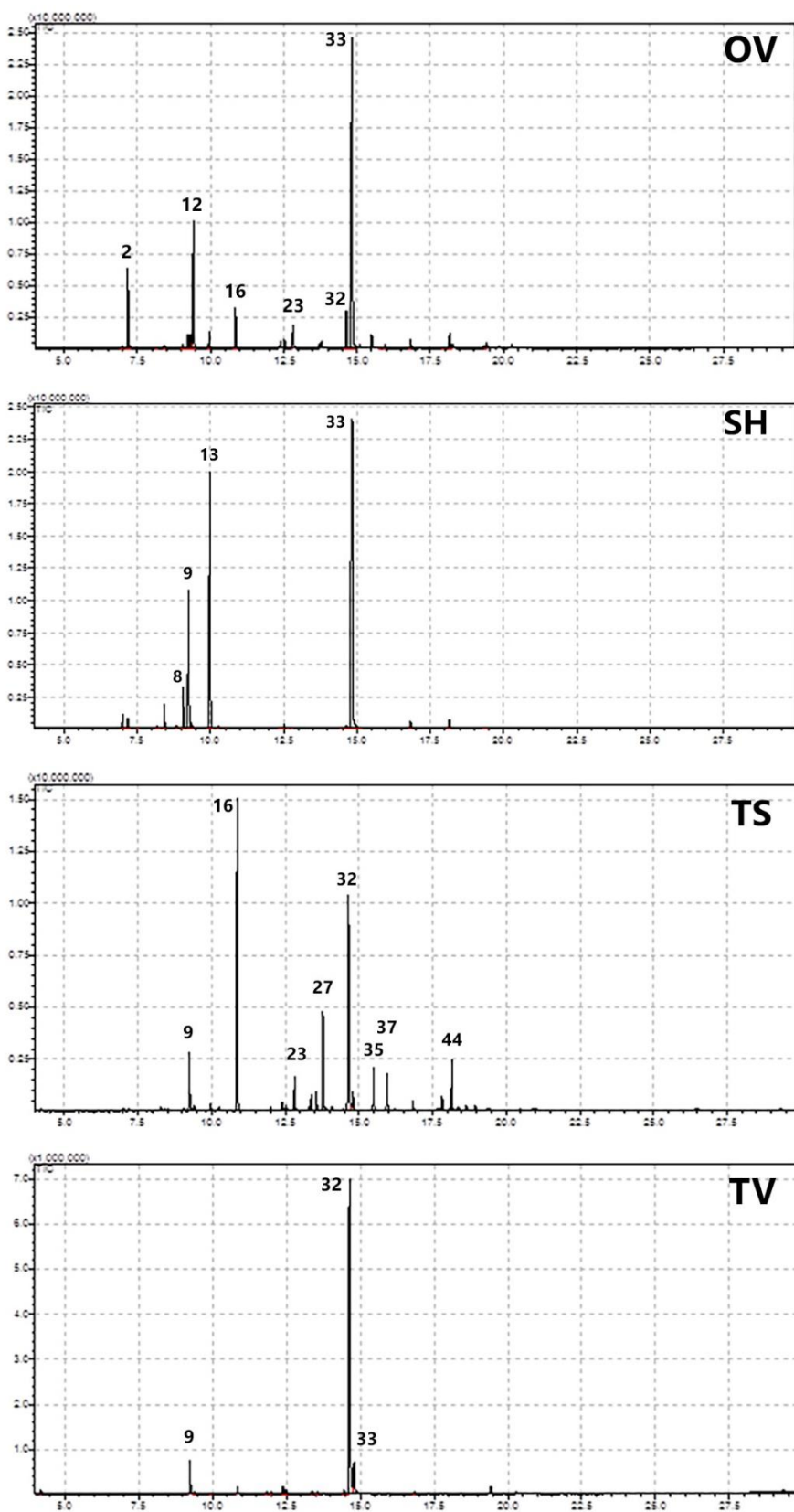
towards *C. albicans* and *C. glabrata*, respectively. In turn, 1,8-cineole and  $\gamma$ -terpinene had the lowest activity, with MIC ranging from 2000 – 4000 mg/L and 4000 mg/L against *C. albicans* at 2000 mg/L or 8000 mg/L relating to *C. glabrata*, respectively.

Taking into account the chemical composition of the studied EOs, it is clear that there is a relationship between the high activity of the thyme EO and the presence of phenolic components such as thymol, carvacrol and their precursor p-cymene [24,36]. Carvacrol was also one of the most important ingredients of oregano and summer savory EOs. In addition, linalool and thymol were found in wild thyme. The antifungal activity of these EOs could be explained by the higher percentage of these compounds. Furthermore, EOs oils are complex mixtures of numerous molecules, and it is to be determined whether their antimicrobial effect is the result of a synergism of all molecules or whether it reflects only those of the main molecules present at the highest levels based on gas chromatographic analysis. Therefore, it is possible that the activity of the main components is also modulated by other minor molecules [41].

The antifungal activity of these EOs may be altered by synergistic and antagonistic effects between some components because some authors reported the synergistic activity of carvacrol and thymol [36,41]. Moreover, p-cymene increases the antimicrobial activity of thymol or carvacrol [36].

The mode of action of these phenolic components, is mostly related to the reaction with the microbial cell membrane. They initiate modifying effects on the outer and inner membrane, interact with or unfold the outer membrane proteins, cause changes in cell membrane fluidity, increase the membrane permeability and leakage of necessary ions, and change the pH hemostasis. The mechanisms of their antifungal action are related to the disruption of the fungal cell wall integrity and weakening of the synthesis procedure of ergosterol [36].

Because the antimicrobial activity of EO seems to depend on the presence of certain components, it is important to note that their chemical composition can vary within the same species of plant due to, among other causes, the presence of different chemotypes, harvest times, and extraction methods [7]. In addition, differences in the activity of EOs may exist between reference *Candida* spp. strains and isolates from various clinical specimens from healthy individuals or hospitalized patients. Additional research is, therefore, needed to address the antimicrobial activity of the EO majority fractions, the synergism between different EOs and antifungal agents, and to test the susceptibility of other opportunistic fungal species [7].



**Figure 1:** Total ion chromatograms (TIC's) of tested essential oils. OV: *Origanum vulgare*, SH: *Satureja hortensis*, TS: *Thymus serpyllum*, TV: *Thymus vulgaris*. (Number of compounds see Tab. 1).



Also, most of the oral *Candida* spp. isolates are rarely isolated on their own, and often exist as a community of microbes. They can, however, occur alone, eventually, in immunocompromised or hospitalized patients, and patients undergoing chronic antibiotic therapy. Therefore, the additional advantage of EOs is their general antibacterial activity. According to some authors [26,33,42], they show a very wide spectrum of activity against other human pathogens such as Gram-positive and Gram-negative bacteria but are relatively more active against Gram-positive than Gram-negative bacteria. The data of Fani et al. [33] revealed the strong inhibitory activity of these EOs on some oral bacteria, including *Streptococcus pyogenes*, *Streptococcus mutans*, and also the periodontopathic anaerobic Gram negative rods *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* that are the most prevalent etiological agents of periodontal diseases. However, generally, these components demonstrated better inhibitory effects on *Candida* strains than the bacteria [42].

Our results are of clinical importance since *C. albicans* is responsible for the great majority of the infections in patients with recurrent oral candidiasis. In addition, *C. albicans* is considered the most pathogenic species of the genus *Candida* [43] and *C. glabrata* is resistant to a majority of azole drugs.

Despite the fact that *in vitro* studies cannot be directly extrapolated to *in vivo* effects, the results suggest that the use of these EOs should be further explored. It is difficult to find any research on the activity of these EOs on human subjects; however, some studies were performed on rats [44]. In an immunosuppressed rat of oral candidiasis, carvacrol or eugenol treatment significantly reduced the number of colony counts sampled from the oral cavity of the treated rats, compared to the untreated control rats. Further studies are needed to evaluate the *in vivo* potential of EOs both in human and animal subjects.

## 4 Conclusions

The obtained results indicated that the studied EOs, due to the high content of phenolic monoterpenes compounds responsible for their antifungal activity, may be regarded as useful natural components within formulations applied for the prevention and treatment of oral candidiasis. Moreover, these EOs can substitute for many common synthetic antifungal preparations.

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