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Original Research

Anti-*Candida* and Antibiofilm Activity of Selected *Lamiaceae* Essential Oils

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Abstract

Background: Candidiasis is a common oral and vaginal infection. Some papers have presented that the essential oils of *Lamiaceae* plants can have antifungal activity. This study aimed to investigate the activity of 7 essential oils of the *Lamiaceae* family with known phytochemical compositions against *Candida* fungi. **Methods**: Forty-four strains belonging to six species were tested: *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*. During this investigation, the following methods were used: determination of the minimal inhibitory concentrations (MICs), biofilm inhibition studies, and *in silico* toxicity tests. **Results**: Essential oils of lemon balm (*Melissa officinalis*) and oregano (*Origanum vulgare*) showed the best anti-*Candida* activity, with MIC values below 3.125 mg/mL. Lavender (*Lavandula stoechas*), mint (*Mentha* × *piperita*), rosemary (*Rosmarinus officinalis*), and thyme (*Thymus vulgaris*) essential oils were also very active (0.39 to 6.25 or 12.5 mg/mL). Sage (*Salvia officinalis*) essential oil presented the lowest activity, with MIC values ranging from 3.125 to 100 mg/mL. In an antibiofilm study using MIC values, oregano and thyme essential oils showed the greatest effect, followed by lavender, mint, and rosemary oils. The weakest antibiofilm activity was observed with the lemon balm and sage oils. *In silico* toxicity research suggests that most of main compounds of *Lamiaceae* essential oils probably do not exhibit carcinogenicity, mutagenicity, or cytotoxicity. **Conclusions**: The obtained results showed that *Lamiaceae* essential oils have anti-*Candida* and antibiofilm activity. Further research is required to confirm the safety and efficacy of essential oils in the topical treatment of candidiasis.

Keywords: essential oils; antifungal; antibiofilm; *Lamiaceae*; phytocompounds; drug sensitivity; *in silico*; toxicity

1. Introduction

Fungal infections are the leading cause of changes in oral and vaginal mucous membranes. Among others, the incidence of fungal infections is related to one's status as a carrier of the genus *Candida*, which affects as much as 30% of the population. Oral candidiasis occurs in 4% of people, and its frequency increases in patients with diabetes mellitus, immunodeficiency, or following antibiotic use [1,2]. The most frequently detected yeast-like fungus in the oral cavity is *Candida albicans.* Among non-albicans *Candida* species, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* predominate [2–4]. In the case of mucosal fungal infections, the most frequently identified yeast-like species depend on the study area. In Europe, the most common isolates were *C. albicans* (80–87%), *C. glabrata* (4.2–5.8%), *S. cerevisiae* (5.5%), *C. krusei* (1.6–3.2%) and *C. tropicalis* (2.1–2.2%). Other identified species from patient samples include *C. dubliniensis*, *C. famata*, *C. guilliermondii*, *C. kefyr*, and *C. parapsilosis* [5,6]. However, in Asia the incidence of *C. albicans* infections was lower than in Europe (51–75%). Other *Candida* infections were caused by *C. parapsilosis* (0.7–26%), *C. glabrata* (2.0–23%), *C. tropicalis* (1.8–6.1%), *C. krusei* (2.8–3.9%), *C. krusei* (2.8%) and *C. africana* (1.6%) [3,4,7].

Antifungal drugs are used to treat candidiasis and other yeast-like infections. However, the search for new medicines is constantly ongoing. Many natural substances, including essential oils, have antifungal properties and can be used to treat fungal diseases. Essential oils are found in many plants, including those belonging to the following families: *Acoraceae, Apiaceae, Asteraceae, Cupressaceae, Geraniaceae, Illiciaceae, Lamiaceae, Lauraceae, Myristicaceae, Myrtaceae, Oleaceae, Pinaceae, Poaceae, Rosaceae, Santalaceae*, and *Zingiberaceae* [8]. Essential oils from the *Lamiaceae* family are particularly important in traditional medicine, pharmacology, as well as the food industry. Species in this family are rich in terpenes possessing

Table 1. Phytochemical composition of essential oils used in the studies according to the data obtained from the manufacturer (Etja, Elbląg, Poland).

No.	Plant	Composition
1	*Lavandula stoechas* L.	35% linalool, 35% linalyl acetate, 4% caryophyllene, 3% ocimenes, 2% 1,8-cineole, 1.5% α-terpineol, 1% d-limonene, 0.5% camphor
2	*Melissa officinalis* L.	15–30% citral, 3–6% geraniol, 3–5% citronellol, 3–5% citronellal, 1–3% isopulegol, $\leq$1% linalool, $\leq$1% limonene
3	*Mentha* × *piperita* L.	40% menthols, 20% menthones, 5% l-menthyl acetate, 5% 1,8-cineole, 3% d-limonene, 2% α-pinene, 2% caryophyllene, 2% menthofuran, 1% pulegones, 0.4% piperitones
4	*Origanum vulgare* L.	$\geq$50% carvacrol, 1–10% α-terpinene, 1–10% linalool, 1–10% myrcene, 1–10% p-cymene, 1–10% thymol, 0.1–1% α-pinene, 0.1–1% β-pinene, 0.1–1% limonene
5	*Rosmarinus officinalis* L.	45% 1,8-cineole (eucalyptol), 15% α-pinene, 12% camphor, 4% camphene, 3% borneol, 3% caryophyllene, 2% α-terpineol, 1% myrcene, 1% p-cymene, 0.7% linalool
6	*Salvia officinalis* L.	33% thujones, 20% camphor, 10% 1,8-cineole, 8% α-pinene, 6% camphene, 4% caryophyllene, 2% borneol, 2% d-limonene, 1.4% terpinene-4-ol, 1% myrcene
7	*Thymus vulgaris* L.	30–40% p-cymene, $<$30% thymol, 2-4% α-pinene, 0.5–1.5% limonene, $<$1% β-pinene

antifungal properties (e.g., camphor, carvacrol, 1,8-cineole, citral, p-cymene, geraniol, linalool, menthols, pinenes, terpinenes, thujones, and thymol) [9].

Today we know that essential oils have been used for millennia. Archaeological evidence suggests their use as early as the Neolithic Age (before 4000 B.C.). Information about them is also contained in ancient accounts from Mesopotamia and Egypt [10]. Essential oils are used in aromatherapy, bathing, massages, wound healing, as well as in the treatment of headache, muscular pain, respiratory problems, skin changes, and joint inflammation [10,11]. Essential oils are likewise used in toothpastes and mouth rinses to protect against dental caries, periodontal diseases, and candidiasis [12–14]. Furthermore, essential oils are used in the treatment of vaginitis [15,16]. They have a broad range of therapeutically beneficial effects, including antioxidant and anti-inflammatory properties, as well as antibacterial, antifungal, and antibiofilm activity [17–19].

This study aimed to investigate the antifungal and antibiofilm activity of seven essential oils of the *Lamiaceae* family. In total, 44 *Candida* strains, mainly clinical isolates belonging to six species, were tested *in vitro*. Additionally, *in silico* toxicity prediction was performed for the main essential oils used in the investigation.

2. Materials and Methods

2.1 Essential Oils

Essential oils were purchased in Etja (Elbląg, Poland). All oils were stored at 4 °C, with an expiration date of 2023 or 2024. This study made use of seven essential oils from the *Lamiaceae* family, namely from *Lavandula stoechas* L., *Melissa officinalis* L., *Mentha* × *piperita* L., *Origanum vulgare* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L. Table 1 shows the phytochemical composition of the oils according to the data obtained from the manufacturer.

2.2 Fungal Strains

During the *in vitro* tests, strains from the Chair and Department of Medical Microbiology collection at the Poznań University of Medical Sciences were used. The tests were performed on *C. albicans* (16 strains), *C. glabrata* (8 strains), *C. krusei* (8 strains), *C. parapsilosis* (4 strains), *C. tropicalis* (4 strains), and *C. guilliermondii* (2 strains). All clinical strains were obtained from patients' mucous membranes and were identified using the Integral System Yeasts Plus (Graso Biotech, Starogard Gdański, Poland) biochemical test. The experiments also included the following reference strains: *C. albicans* ATCC 14053 and *C. glabrata* ATCC 66032. All species were grown at 35 °C for 24 h in Sabouraud dextrose agar (Graso Biotech).

2.3 Antimicrobial Activity (MIC)

The minimal inhibitory concentrations (MICs) of selected essential oils were determined by the micro-dilution method using 96-well plates (NUNC, USA and Nest Scientific Biotechnology, Jiangsu, China). The studies were conducted according to the methodology described in our previous publications [20,21]. Briefly, 90 μL of tryptic soy broth (Graso Biotech) and 10 μL of fungi suspension were placed into each well to a final inoculum concentration of 10^6 CFU/mL. Suspension was performed using McFarland standards and microscopy [22]. Before studies, essential oils were emulsified in tryptic soy broth (1:1) using Omni TH homogenizer (Omni, Kennesaw, GA, USA).

Serial dilutions of each essential oil were performed to obtain concentrations ranging from 200 to 0.1 mg/mL. The plates were incubated at 35 °C for 24 h. The MIC value was the lowest essential oil concentration that inhibited any visible fungal growth. Optical density (OD) was measured at 620 nm using a microtiter plate reader (Eppendorf, Warszawa, Poland). Additionally, 10 μL of a 1% aque-

ous solution of XTT (Sigma Aldrich, Poznań, Poland) was added to each well. Microorganisms convert tetrazolium compounds to a colored water-soluble formazan product [20,21,23,24]. All the experiments were performed in triplicate.

2.4 Biofilm Inhibition

The inhibition of biofilm formation by *C. albicans* (ATCC 14053), the *C. albicans*, *C. glabrata* and *C. krusei* clinical strains was evaluated by means of a crystal violet assay. The influence of essential oils at the concentrations of MIC was determined. The negative control was a sterile culture medium. At first, suspension of each yeast was performed at a concentration of 10^6 CFU/mL [25]. The biofilm was formed in 96-well plates with tryptic soy broth, after the addition of the appropriate essential oil to its previously determined MIC and 10 μL of fungal suspension. The total volume in wells was 100 μL. Next, plates were incubated for 48 hours at 37 °C. After incubation, the wells were washed with 200 μL of PBS three times, and plates were dried by inverting them on absorbent paper for 15 min. Each well was fixed with 200 μL of methanol for 15 min and dried after removing it. Afterward, the wells were stained with 200 μL of 1% crystal violet solution for 20 minutes. Wells were washed thrice with PBS, dried, and 200 μL of 96% ethanol was added to dissolve the crystal violet [26]. To quantify the biofilm, the optical density (OD) was measured at 620 nm, using an Elisa Reader 250 (bioMerieux, Marcy-l'Étoile, France). The percentage of biofilm biomass growth was determined using the following formula:

$$\% \text{ Biofilm growth} = 100 \times (\text{Sample}_{OD620} - \text{Control}_{OD620}) / (\text{Control}_{OD620})$$

2.5 In Silico Toxicity Prediction

The toxicity of the main compounds found in the studied *Lamiaceae* essential oils presented in Table 1 was determined using *in silico* methods. Specifically, the ProTox-II (https://tox-new.charite.de/protox_II) [27] and pkCSM (http://biosig.unimelb.edu.au/pkcsm/) [28] software were used. Studies were performing according to software manuals.

2.6 Statistics

The mean, SD and median of MIC values of essential oils against Candida strains were calculated. The Kruskal–Wallis and post-hoc tests were applied to determine the statistical significance of differences in the MICs of fungi. The results were considered significant at the level of $p < 0.05$. Data were tested using InStat3 software (GraphPad Software, Boston, MA, USA).

3. Results

3.1 Antimicrobial Activity (MIC)

Essential oils inhibited the growth of the tested strains of *Candida* at concentrations of 0.1–100 mg/mL (Fig. 1). The essential oils obtained from lemon balm and oregano showed the best anti-*Candida* activity. The MIC value for both oils was below 3.125 mg/mL. Lavender, mint, rosemary, and thyme essential oils were also very active in the range of 0.39 to 6.25 or 12.5 mg/mL. The essential oil obtained from sage exhibited statistically the weakest activity with MIC values ranging from 3.125 to 100 mg/mL for single *C. krusei* and *C. parapsilosis* strains. These results suggest that strains of *C. krusei* are the least sensitive to *Lamiaceae* essential oils. The activity of each essential oil against various *Candida* strains is outlined in Table 2 and **Supplementary Tables 1–7**. Statistical analysis of the mean MICs values (mg/mL) for selected *Lamiaceae* essential oils obtained for all *Candida* strains is presented in Table 3.

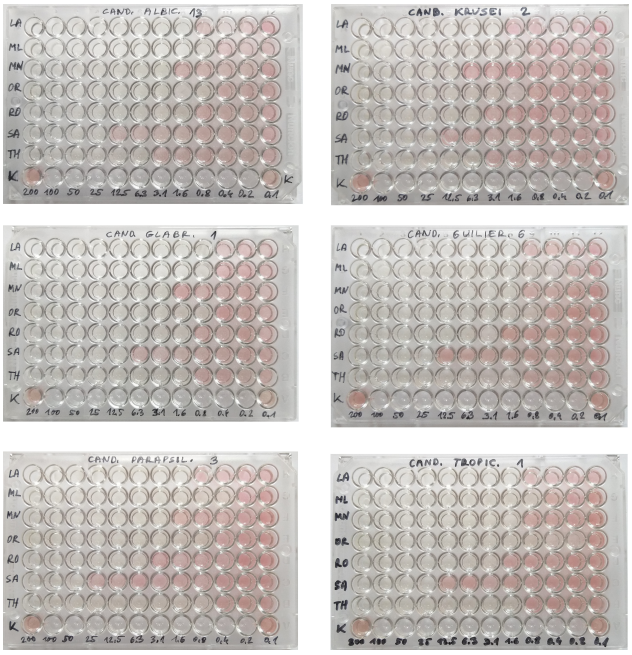

Fig. 1. Representative MIC determination setup for 6 tested *Candida* species on 96-well plates. Pink color indicates fungal growth.

3.2 Biofilm Inhibition

The antibiofilm study using *Lamiaceae* essential oils yielded various results. At the MIC values, oregano and thyme essential oils showed the strongest effect, inhibiting biofilm growth by about 90%. Lavender, mint, and rosemary oils at the MIC concentration inhibited biofilm growth by about 75–85%. The weakest antibiofilm activity was observed with lemon balm and sage oils, which destroy only

Table 2. The activity (MIC — minimal inhibitory concentration) of selected *Lamiaceae* essential oil against *Candida* strains.

Plant species	Yeast-like fungi					
	MICs (mg/mL) mean ± SD [median]					
	C. albicans	*C. glabrata*	*C. krusei*	*C. parapsilosis*	*C. tropicalis*	*C. guilliermondii*
Lavandula stoechas	1.47 ± 0.9 [1.56]	1.08 ± 0.47 [0.78]	3.12 ± 1.45 [3.125]	1.37 ± 0.39 [1.56]	1.76 ± 0.98 [1.56]	0.78 ± 0.0 [0.78]
Melissa officinalis	0.78 ± 0.9 [0.39]	0.78 ± 0.34 [0.78]	1.41 ± 0.83 [1.56]	0.49 ± 0.2 [0.39]	0.68 ± 0.2 [0.78]	0.39 ± 0.0 [0.39]
Mentha × piperita	2.27 ± 1.37 [1.56]	2.78 ± 0.69 [3.125]	6.64 ± 3.9 [6.25]	2.34 ± 0.9 [2.343]	2.73 ± 0.78 [3.125]	1.56 ± 0.0 [1.56]
Origanum vulgare	0.83 ± 0.71 [0.78]	1.08 ± 0.47 [0.78]	2.05 ± 0.93 [1.56]	0.88 ± 0.5 [0.78]	0.83 ± 0.56 [0.78]	0.59 ± 0.28 [0.59]
Rosmarinus officinalis	1.91 ± 1.38 [1.56]	1.56 ± 0.68 [1.56]	7.81 ± 4.09 [6.25]	3.52 ± 1.97 [3.125]	2.73 ± 0.78 [3.125]	2.34 ± 1.11 [2.34]
Salvia officinalis	16.36 ± 11.02 [12.5]	20.14 ± 13.18 [12.5]	29.69 ± 29.08 [25]	50 ± 35.36 [37.5]	14.06 ± 7.86 [12.5]	18.75 ± 8.84 [18.75]
Thymus vulgaris	4.16 ± 3.63 [3.125]	1.56 ± 0.68 [1.56]	6.45 ± 4.13 [6.25]	0.88 ± 0.49 [0.78]	2.15 ± 1.17 [2.343]	1.56 ± 0.0 [1.56]

Table 3. Statistical analysis (*p* values) of the mean MICs values (mg/mL) for selected *Lamiaceae* essential oils against *Candida* strains.

Plant species (mean of MICs)	*L. stoechas*	*M. officinalis*	*M. × piperita*	*O. vulgare*	*R. officinalis*	*S. officinalis*	*T. vulgaris*
	(1.67)	(0.84)	(3.186)	(1.1)	(3.15)	(22.5)	(3.44)
Lavandula stoechas	-	<0.05	<0.05	ns	ns	<0.001	ns
Melissa officinalis	<0.05	-	<0.001	ns	<0.001	<0.001	<0.001
Mentha × piperita	<0.05	<0.001	-	<0.001	ns	<0.001	ns
Origanum vulgare	ns	ns	<0.001	-	<0.001	<0.001	<0.001
Rosmarinus officinalis	ns	<0.001	ns	<0.001	-	<0.001	ns
Salvia officinalis	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001
Thymus vulgaris	ns	<0.001	ns	<0.001	ns	<0.001	-

about 60–70% of biofilm. Interestingly, lemon balm essential oil exhibited the strongest anti-*Candida* activity in the planktonic form, while it exerted a much weaker inhibitory effect in the antibiofilm study. The antibiofilm activity of the studied *Lamiaceae* essential oils at the MICs are presented in Fig. 2.

3.3 In Silico Toxicity Prediction

In silico toxicity studies revealed that most of the main compounds found in the *Lamiaceae* essential oils used in this investigation did not exhibit carcinogenicity, mutagenicity, or cytotoxicity. Only p-cymene has carcinogenic activity, while menthone exhibited mutagenic activity. 16 out of 21 compounds were found to have the potential to lead to skin sensitization (Table 4, Ref. [27,28]). This means that *Lamiaceae* essential oils can irritate the skin and should only be applied for short periods of time. With the exception of p-cymene, all of the tested compounds have high lethal dose 50 (LD50) values, which means that they are relatively safe for use in the oral cavity, even if ingested.

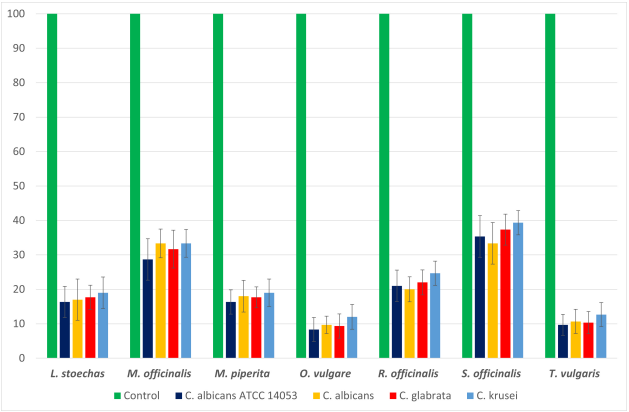

Fig. 2. Antibiofilm activity of *Lamiaceae* essential oils against *Candida albicans* ATCC 14053 and three clinical strains of *C. albicans*, *C. glabrata* and *C. krusei*.

4. Discussion

Volatile oils obtained from Lamiaceae plants contain various groups of chemical compounds such as monoterpenes, sesquiterpenes as well as phenolics. Depending on

Table 4. Toxicity of the main *Lamiaceae* essential oil compounds determined using the *in silico* ProTox-II and pkCSM online software tools [27,28].

Compound	Predicted LD50 [mg/kg]	Skin sensitization	Carcinogenicity	Mutagenicity	Cytotoxicity
Camphene	5000	No	No	No	No
Camphor	775	No	No	No	No
Carvacrol	810	Yes	No	No	No
Caryophyllene	5300	Yes	No	No	No
1,8-Cineole (Eucalyptol)	2480	Yes	No	No	No
Citral	500	Yes	No	No	No
Citronellal	2420	Yes	No	No	No
Citronellol	3450	Yes	No	No	No
p-Cymene	3	Yes	Yes	No	No
Geraniol	2100	Yes	No	No	No
Limonene	4400	Yes	No	No	No
Linalool	2200	Yes	No	No	No
Linalyl Acetate	12,000	Yes	No	No	No
Menthol	940	Yes	No	No	No
Menthone	500	Yes	No	Yes	No
L-Menthyl Acetate	3200	Yes	No	No	No
Myrcene	5000	No	No	No	No
α-Pinene	3700	No	No	No	No
α-Terpinene	1680	No	No	No	No
Thujone	500	Yes	No	No	No
Thymol	640	Yes	No	No	No

the major chemical compounds, several chemotypes have been described [29]. It has been well established, that the chemical composition of essential oils is influenced by environmental factors, geographical regions of plant growth, harvesting time, the stage of plant development [30]. Popular extraction methods used to isolate essential oils, include conventional hydrodistillation (HD) [31–33], and vacuum distillation [34]. These methods of distillation are described in the European Pharmacopoeia. Other methods of distillation are likewise used and include enzyme-assisted hydrodistillation [35], and water microwave assisted hydrodistillation (MAHD) techniques [36,37]. However, no significant differences were found in composition of *Lamiaceae* essential oils after the comparison of MAHD and HD [36]. Another innovative method is supercritical fluid extraction (SFE) of volatile oils, which allows for higher essential oil yield and the isolation of more chemical compounds when compared to the more traditional hydrodistillation process [38]. In contrast, Rodriguez-Solana *et al*. [39] found that the highest extraction yields were obtained using the Soxlet and accelerated solvent (ASE) techniques (but not SFE) in case of *Mentha piperita* and *Rosmarinus officinalis*. To identify and quantity of chemical constituents of a given essential oil, the method of choice is gas chromatography with mass spectrometry (GC-MS) [33] and GC with flame ionization detector (GC-FID) [33,39].

Data found in the literature indicate considerable variability regarding the antifungal activity of essential oils. These differences are likely related to the different biochemical composition of essential oils, which depend on the place of harvest, soil and light conditions, and the harvest date [40]. Another critical factor is the method of obtaining essential oils, which can lead to significantly different concentrations of active compounds [41].

In this study, the active concentrations of essential oils ranged between 0.1 to 100 mg/mL. Essential oil from lavender acted against *Candida* strains at concentrations between 0.39–6.25 mg/mL. These values differ markedly from those available in the published literature available to date. In the study by Khoury *et al*. [42], the MIC of *Lavandula stoechas* oil against *C. albicans* was found to be 0.5 mg/mL. This value was similar to that obtained during this investigation. However, Zuzarte *et al*. [43] reported that the MICs of *Lavandula stoechas* oil against *C. albicans*, *C. guillermondii*, *C. krusei*, *C. parapsilosis,* and *C. tropicalis* were very low, ranging between 0.64–2.5 μg/mL.

Based upon the results obtained in this investigation, the essential oils from melissa and oregano have the best antifungal activity (MICs from 0.2 up to 3.125 mg/mL). Other Polish studies have shown the sensitivity of yeast-like fungi to melissa oil to be in the concentration range of 0.25–2.0 mg/mL. Most strains of *C. albicans*, *C. glabrata,* and *C. humicola* were inhibited at concentrations of 0.25–0.5 mg/mL. In contrast, MIC values for *C. kefyr*, *C. krusei*, *C. lusitaniae* and *C. tropicalis* strains were in the range of 0.5–2.0 mg/mL [44]. Similarly, a study from the USA found that melissa oil exhibited a MIC of 0.3 mg/mL against *C. albicans* [45]. In research from Italy, MICs against *C. krusei*, *C. parapsilosis*,

C. valida, *C. lusitaniae*, and *C. norvegensis* were found to be 0.3–1.2 mg/mL [46]. In the case of oregano oil, the literature shows a similar MIC range (0.26–2.5 mg/mL) against *Candida* species [45–48] as that obtained in this study.

The present study found that peppermint oil, rosemary oil, and thyme oil exhibit similar strength against *Candida* strains, with MIC values ranging from 0.39 to 12.5 mg/mL. In other publications, these values were at the level of 0.23–1.1 mg/mL for *Mentha piperita* oil [45,49,50] and 0.22–3.13 mg/mL for *Rosmarinus officinalis* oil [42,45,47,48,51]. In the study by Kędzia and Hołderna-Kędzia, rosemary oil inhibited the growth of yeast-like fungi at a concentration of 7.5–15 mg/mL. Strains of *C. utilis*, *C. guilliermondii,* and *C. kefyr* are most susceptible to rosemary oil, while *C. tropicalis*, *C. krusei,* and *C. lusitaniae* are the most resistant [52]. In the case of thyme, there are large differences in the MIC values. The lowest reported MIC values of thyme oil against *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. guillermondii,* and *C. parapsilosis* were 0.08–0.32 μg/mL [53]. In contrast, another paper reported that the MIC of thyme oil against *C. albicans* was 313 μg/mL [45]. In two articles published in 2021, the MIC values for *Thymus vulgaris* oil against *Candida* species were 0.4–0.6 mg/mL [46,48].

Essential oil from a sage was the only one for which this study revealed a MIC value of up to 100 mg/mL. High active concentrations of *Salvia officinalis* oil against *Candida* were also reported by Mandras *et al*. [46] and Proškovcová *et al*. [48]. The MIC values obtained in those studies were 2.5–10 mg/mL and 3.13–50 mg/mL, respectively. A study by Sookto *et al*. [54] demonstrated a MIC value of 2.78 mg/mL. However, one study from 2013 reported very low MICs in the range of 1.25–5 μg/mL against *C. albicans*, *C. tropicalis*, *C. krusei*, *C. guillermondii,* and *C. parapsilosis* [55].

The presented results of anti-biofilm activity indicate that essential oils from *Origanum vulgare* and *Thymus vulgaris* have the strongest effect, which at a concentration of 1 MIC destroyed about 90% of biofilm. Oils from *Lavandula stoechas*, *Mentha* × *piperita* and *Rosmarinus officinalis* showed a weaker effect, destroying about 75–85% of the biofilm. Essential oils from *Melissa officinalis* and *Salvia officinalis* had the weakest activity, destroying only about 60–70% of the biofilm.

In the article by Proškovcová *et al*. [56], *C. albicans* biofilm reduction by *Origanum vulgare*, *Rosmarinus officinalis* and *Thymus vulgaris* essential oils was 63.8–69.2%, which means that it was lower than that obtained in our study. It is interesting, that biofilm reduction by *Salvia officinalis* essential oil was the highest (70.6%) [56], but in our results was least, notwithstanding percentage was similar. Unfortunately, the results of biofilm formation inhibition may differ significantly, as exemplified by 2 publications. In paper by Benzaid *et al*. [57] the MIC concentration of *Mentha x piperita* essential oil causing *C. albicans* biofilm inhibition was 10 μg/mL, and Agarval *et al*. [58] demonstrated this effect at a MIC of 800 μg/mL. These differences may be related among others to with different composition of essential oils or other research methodology [9]. It is surprising, however, that also in the case of individual chemical compounds these differences can be very large. A study of thymol on biofilm formation by Jafri and Ahmad [59] indicated that it was active at a concentration of 3.12 μg/mL. However, in the studies of Braga *et al*. [60] the required concentration was as high as 125 μg/mL.

A significant advantage of this investigation is the inclusion of a large number of both essential oils and *Candida* strains. Very often, only single reference strains are tested by other authors [42,45,50,51,54,55]. Additionally, many publications do not include clinical strains [42,45,47,49–51,54,55]. Unfortunately, as can be seen in the tables presented above there are often notable differences in the sensitivity of fungi from the same species to the same essential oil. An excellent example of this variability is the activity of *Thymus vulgaris* essential oil against *C. albicans*, for which the MIC values range from 0.39 up to 12.5 mg/mL. Such differences cannot be readily appreciated when testing only one strain per species.

The use of several species allows for the demonstration of interspecies differences in the sensitivity to plant compounds within a given genus. One such notable example from this study is the low sensitivity of *C. krusei* to essential oils compared to other species of the *Candida* genus. Such differences cannot be elucidated by studies in which only one species of a given genus was tested [42,45,47,48,50,51,54]. Furthermore, in the case of natural substances, phytochemical analysis is important, allowing for the comparison of substances originating, for example, from different regions of the world or isolated using different methods. Unfortunately, in some publications, phytochemical data are not included or data from the general literature is provided, this does not accurately reflect the actual composition of the substances used in the studies [44,52,54].

The large number of clinical *Candida* isolates used in this investigation lends to its broad applicability. Most of the available literature focuses on single reference strains with clinical isolates being excluded entirely [42,45,47,49–51,54,55]. To the best of our knowledge, this is the first study to investigate the *in silico* toxicity prediction of the main compounds of *Lamiaceae* essential oils. The present investigation also demonstrates via *in silico* methods that the majority of the compounds in the *Lamiaceae* essential oils used in this study do not exhibit toxicity. Unfortunately, there are few publications assessing the toxicity of the essential oils or their constituent compounds used in the present study. In the available studies on mice and rats, essential oils and extracts from *Lamiaceae* are non-toxic or slightly toxic [61–63]. These studies lend support to the *in silico* findings reported here.

5. Conclusions

Essential oils from lemon balm and oregano exerted the most potent anti-*Candida* activity, with MIC values below 3.125 mg/mL. Lavender, mint, rosemary, and thyme essential oils also inhibit growth in the range of 0.39 to 6.25 or 12.5 mg/mL. The weakest activity was observed with sage essential oil, for which the MIC values ranged from 3.125 to 100 mg/mL for single *C. krusei* and *C. parapsilosis* strains. All tested essential oils can also inhibit *Candida albicans* biofilm formation. The majority of main compounds found in the *Lamiaceae* essential oils used in this study did not exhibit toxicity *in silico,* but probably can sensitize the skin.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

Conceptualization—TMK and MO; methodology—TMK; investigation—TMK, MO and ASM; writing - original draft preparation—TMK, ASM and HW; writing - review and editing—MO and HW; supervision—TMK and ASM; funding acquisition—TMK and MO. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

Given their role as Guest Editors, Tomasz M. Karpiński and Marcin Ożarowski had no involvement in the peer-review of this article and have no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Graham Pawelec. The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2802028.

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