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Chemical composition and antifungal effect of the essential oil of *Helichrysum italicum* (Roth) G. Don against clinical isolates of *Candida* spp.

Original Article

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Abstract:

This study investigated the chemical composition and antifungal properties of the essential oil derived from *Helichrysum italicum* (Roth) G. Don, a plant belonging to Asteraceae family. A total of 52 compounds were identified using the GC/MS analysis with the main components being neryl acetate (13.4%) and γ-curcumene (10.3%). We examined the antifungal activity of *H. italicum* oil against human *Candida* isolates and its interaction with the conventional antifungal agent, nystatin. Additionally, the effect of oil on biofilm production by *Candida* isolates was investigated. The results revealed a notably strong anti-*Candida* effect, with MIC values ranging from 0.312 to 5.00 mg/mL. The oil exhibited a synergistic effect with the tested antifungal drug and notable antibiofilm activity. These findings support further research into plant secondary metabolites as potential new antifungal agents.

Key words:
candidosis, immortelle essential oil, antifungal activity

Apstrakt:

Hemijski sastav i antifungalni efekat etarskog ulja *Helichrysum italicum* (Roth) G. Don na kliničke izolate *Candida* spp.

U studiji je predstavljen hemijski sastav i antifungalna svojstva etarskog ulja smilja (*Helichrysum italicum* (Roth) G. Don) iz porodice Asteraceae. Ukupno je identifikovano 52 jedinjenja koristeći GC/MS tehnike a među najzastupljenijim komponentama bili su neryl acetat (13.4%) i γ-kurkumen (10.3%). Ispitana je antifungalna aktivnost ulja *H. italicum* na humane izolate kandida, kao i njegova interakcija sa konvencionalnim antifungalnim agensom, nistatinom. Pored toga, ispitan je uticaj etarskog ulja na proces formiranja biofilma kod vrsta *C. albicans* i *C. krusei*. Rezultati su pokazali značajan anti-*Candida* efekat sa MIK vrednostima u rasponu od 0,312 do 5,00 mg/mL. Ulje smilja pokazalo je sinergistički efekat u kombinaciji sa testiranim antifungalnim agensom, kao i statistički značajnu aktivnost u smanjenju biofilm formi. Dobijeni podaci mogu doprineti daljim istraživanjima o biljnim sekundarnim metabolitima kao potencijalno novim antifungalnim agensima.

Ključne reči:
kandidoze, etarsko ulje smilja, antifungalna aktivnost

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Introduction

The genus *Helichrysum* Mill. comprises over a thousand taxa, known for their prolific production of various secondary metabolites, including flavonoids, pyrones, triterpenoids, and sesquiterpenes. These compounds are likely responsible for the remarkable efficacy of the essential oils derived from plants within this genus. The name *Helichrysum* is derived from the Greek words „*helios*" (sun) and „*chrysos*" (gold), reflecting the bright yellow flowers characteristic for these plants (Sala et al., 2003; Rosa et al., 2007).

The most extensively studied species within this genus is *Helichrysum italicum* (Roth) G.Don, a Mediterranean aromatic shrub known for its distinctive yellow flowers. It belongs to the order *Asterales* and the family Asteraceae. Immortelle, a common name for *H. italicum*, is frequently found along the eastern coastline and the islands of the Adriatic Sea. In traditional medicine, it is commonly used as a diuretic, choleretic, and expectorant (Chinou et al., 1996). Ethnopharmacological studies across various regions of Europe have shown that

H. italicum is traditionally used to treat numerous ailments, including those requiring antimicrobial, antioxidant, antiviral, and antiproliferative effects (Sala et al., 2003; Rosa et al., 2007; Antunes Viegas et al., 2014; Ninčević et al., 2019; Mollova et al., 2020; Aćimović et al., 2021; Genčić et al., 2021; Šovljanski et al., 2024).

The genus *Candida* comprises over 200 species, though only a few are pathogenic to humans, all are opportunistic pathogens. Typically, *Candida* species are part of the normal microbiota of the human body, classifying them as commensal microorganisms. In healthy individuals, they are considered a harmless component of the microbiome and do not cause infection. However, *Candida albicans* is the most prevalent cause of human fungal infections and is a leading cause of nosocomial infections, particularly in immunocompromised patients (Mayer et al., 2013; Sawant & Khan, 2017; Dadar et al., 2018; Talapko et al., 2021). In recent years, research on natural substances as potential antifungal agents has intensified due to the rising resistance of *Candida* isolates to antifungal drugs and the occurrence of recurrent infections. Given the prolonged antifungal therapy often required for individuals with confirmed candidosis, alternative treatments could significantly improve treatment efficacy (Cid-Chevecich et al., 2022; Blanc et al., 2023; Karpiński et al., 2023).

Our study aimed to investigate the chemical composition and inhibitory effect of immortelle essential oil against clinical human *Candida* isolates, as well as a reference strain of *C. albicans* ATCC 24433. The study also examined the potential synergistic interaction between this essential oil and the commercial antifungal agent nystatin. Additionally, it evaluated the oil's effectiveness in reducing biofilm production when treated with varying concentrations of the tested substances.

Materials and Methods

Microorganisms

Clinical strains of *Candida* were isolated from various sites of patients with confirmed candidosis. The isolates were identified as *C. krusei* and *C. albicans* using Chromogenic Candida Agar (HiCrome™ Candida Differential Agar; HiMedia) based on their growth characteristics, as well as the observation of their morphological and biochemical properties. The isolates were cultured on Sabouraud dextrose agar (SDA) (HiMedia, Mumbai, India) in a thermostat before being used for the experiment. Following the acquisition of overnight cultures, yeast suspensions were prepared, and their density was adjusted to 10^7 CFU/mL by using a densitometer (DEN-1, BioSan). These cultures were then used to conduct experiments outlined in our research.

Essential oils

The essential oil (EO) used in the study was commercially available and purchased from a local store in Niš, Serbia. This oil was obtained through the distillation of species *H. italicum* (Siempre Viva Oils, Niš District, Serbia). The oil was dissolved in dimethyl sulfoxide (DMSO) solvent for use in the experiments.

GC and GC/MS analyses

GC/MS analyses (performed in triplicate) were conducted using a Hewlett-Packard 6890N gas chromatograph (Agilent Technologies, Santa Clara, California, USA) equipped with a DB-5MS fused silica capillary column ((5%-phenyl)-methylpolysiloxane, 30 m × 0.25 mm, film thickness 0.25 µm) and coupled to a 5975B mass selective detector. The injector and interface temperatures were set at 250 °C and 300 °C, respectively. The oven temperature was programmed to increase from 70 °C to 290 °C at a rate of 5 °C/min, followed by an isothermal period of 10 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Samples (1.0 µL of essential oil solutions, prepared by dissolving 10.0 mg of essential oil in 1.0 mL of diethyl ether) were injected in pulsed split mode (with an initial flow rate of 1.5 mL/min for 0.5 min, then reduced to 1.0 mL/min for the remainder of the analysis; split ratio 40:1). The MS conditions included an ionization voltage of 70 eV, an acquisition mass range of m/z 35-650, and a scan time of 0.32 s. The percentage composition of the essential oils was calculated based on the GC peak areas without any corrections. Constituents were identified by comparing their linear retention indices (relative to C8-C33 n-alkanes on the DB-5MS column) with literature values (Adams, 2007) and mass spectra with those of authentic standards, as well as databases such as Wiley 6, NIST11, and MassFinder 2.3, and a custom MS library containing spectra of known oils and pure substances. Wherever possible, identification was further confirmed through co-injection with authentic samples.

Microdilution method

The microdilution method was used to examine the potential activity of the tested essential oil against *Candida* isolates (Stojanović-Radić et al., 2020; Cid-Chevecich et al., 2022). The method involves preparing serial dilutions of the substances used in the experiment to determine the minimum concentrations required to inhibit the growth of *Candida* cells. The inhibitory activity test was conducted in Sabouraud dextrose broth (SDB) (HiMedia, Mumbai, India),

which was used to inoculate the microtiter plates. The initial concentration of the tested essential oil was 10 mg/mL, with the antifungal agent nystatin serving as a positive control. Prepared cell suspensions were applied to microtiter plates, where a series of dilutions of the immortelle oil had already been transferred. The plates were then incubated at 35 °C for 48 h, after which each substance's MIC (Minimum Inhibitory Concentration) was determined. The experiment was conducted in triplicate.

Checkerboard assay

The checkerboard method involves using various dilutions of two substances, applied in a cross-pattern on microtiter plates to create different combinations of agents at different doses (Silva et al., 2011; Stringaro et al., 2014; De Castro et al., 2015). This method is used to examine the type of interaction between potential antifungal agents, determining whether their effects are synergistic, additive, or antagonistic. In our research, the immortelle oil was tested in combination with a commercial antimycotic drug (nystatin). The prepared plates were incubated at 35 °C for 48 h, after which FIC (Fractional Inhibitory Concentration) values were recorded. The data were then used to calculate the FIC index, which determined the outcome of the experiment. The FICI was interpreted as a synergistic effect when it is ≤0.5, as additive or indifferent when it is >0.5 and ≤2, and as antagonistic when its value is >2.

Anti-biofilm assay

The ability to produce biofilms and the efficacy of essential oil in reducing biofilm formation were assessed using the crystal violet (CV) method (Stepanović et al., 2007; Stojanović-Radić et al., 2016, 2020). This method was carried out in 96-well microtiter plates, with yeast suspensions adjusted to a final concentration of 5×10^5 CFU/mL. The test was conducted in a sterile RPMI-1640 medium (Sigma-Aldrich) containing 0.8% glucose, while a sample of immortelle essential oil was dissolved in DMSO solvent for the experiment. Two concentrations of the test agent, MIC and half-MIC, were used, with nystatin as the positive control.

The prepared plates were incubated at an optimal temperature of 35 °C for 48 h. After the incubation period, the contents of the plates were aspirated, and the wells were washed twice with sterile phosphate-buffered saline (PBS, pH 7.4). The plates were then dried, and a 0.8% crystal violet solution was added for 20 min. After staining, the plates were washed, and 250 µL of 96% ethanol (v/v) was added to each well for decolorization, which lasted 45 min. The resulting contents were then transferred to new sterile plates for absorbance reading. The absorbance was measured at 595 nm using an ELISA reader (Multiscan™ FC Microplate Reader, Thermo Scientific).

Statistical analysis

The data from our study were statistically analyzed using Prism software (GraphPad Software, version 5.01). The statistical analysis employed was ANOVA, followed by a Tukey post hoc test. All experiments were conducted in triplicate, with a 95% confidence level. Statistical significance was defined as $p<0.05$.

Results and discussion

Chemical composition

A total of 52 compounds were identified using the GC/MS analysis (**Tab. 1**), with the most abundant components being neryl acetate (13.4%), *γ*-curcumene (10.3%), *β*-selinene (9.3%) and *α*-pinene (8.3%). All other isolated components were present in significantly lower concentrations in the composition of the commercial immortelle essential oil sample.

These components (neryl acetate, *γ*-curcumene, *β*-selinene, and *α*-pinene) were also the most abundant in the *H. italicum* essential oils examined by other authors (Staver et al., 2018; Tzanova et al., 2018; Aćimović et al., 2021). In their study, Tzanova et al. (2018) investigated the chemical composition of essential oils from *H. italicum*, which was introduced from Corsica (France) and cultivated in South Bulgaria. Using the GC/MS method, they identified a total of 41 components. The main compounds in the essential oil were *α*- and *γ*-curcumene (12.46–25.76%), neryl acetate (12.02–20.6%), and *α*-pinene (5.59–19.52%). Staver et al. (2018) used fresh aerial parts of *H. italicum* from Central Dalmatia, Croatia, which were hydrodistilled to identify its chemical composition. A total of 38 compounds were identified, with α-pinene (21.6%) and neryl acetate (7.9%) being the most abundant components of the essential oil. In contrast, the results of the study by Ćavar Zeljković et al. (2015) revealed a distinct chemical composition of *H. italicum* essential oil from various locations along the Adriatic coast in Croatia. The oils were extremely rich in sesquiterpene hydrocarbons, with isomers of curcumene being the dominant compounds (Ćavar Zeljković et al., 2015). The chemical composition of *H. italicum* essential oil in the study conducted by Acimović et al. (2021) was analyzed using GC/FID and GC/MS methods. A total of 70 compounds were detected, with 17 unidentified components making up 8.5% of the total composition. The dominant

Table 1. Chemical composition of *H. italicum* essential oil tested for anti-*Candida* activity

RI[a]	RI[b]	Constituent[c]	Content (%)[d]
932	932	*α*-Pinene[e]	8.3
946	945	*α*-Fenchene	0.5
948	946	Camphene[e]	tr
951	953	Thuja-2,4(10)-diene	tr
976	974	*β*-Pinene[e]	0.3
995	1000	Isobutyl 2-methylbutanoate[e]	tr
1022	1020	*p*-Cymene[e]	tr
1026	1024	Limonene[e]	0.7
1028	1026	1.8-Cineole[e]	0.7
1042	1045	Isobutyl angelate[e]	tr
1066	1067	*cis*-Linalool oxide (furanoid)[e]	tr
1083	1084	*trans*-Linalool oxide (furanoid)[e]	0.1
1095	1095	Linalool[e]	1.0
1118	1118	*exo*-Fenchol[e]	tr
1122	1119	*trans*-*p*-Mentha-2.8-dien-1-ol	tr
1125	1122	*α*-Campholenal	0.8
1142	1135	*trans*-Pinocarveol[e]	0.5
1145	1143	Isoamyl angelate[e]	2.5
1164	1160	Pinocarvone[e]	tr
1173	1165	Borneol[e]	tr
1182	1174	Terpinen-4-ol[e]	tr
1197	1186	*α*-Terpineol[e]	tr
1199	1195	Myrtenal[e]	0.6
1211	1204	Verbenone[e]	0.7
1224	1226	*cis*-Carveol	tr
1250	1239	Carvone[e]	tr
1281	1280	(*Z*)-3-Hexenyl angelate[e]	0.4
1288	1291	*trans*-Verbenyl acetate[e]	tr
1350	1343	Limonene glycol	tr
1355	1359	Neryl acetate[e]	13.4
1371	1376	*α*-Ylangene	0.4
1378	1379	Isoitalicene	tr
1409	1405	Italicene	3.5
1413	1411	*α*-*cis*- Bergamotene	1.1
1434	1432	*α*-*trans*- Bergamotene	0.7
1441	1438	4,6,9-Trimethyldec-8-ene-3,5-dione (diketo tautomer)[e]	tr
1444	1452	Neryl propionate	3.4
1468	1464	*α*-Acoradiene	0.4
1472	1469	*β*-Acoradiene	0.4
1475	1471	4,5-di-epi-Aristolochene	tr
1477	1485	Selina-4,11-diene	

1481	1481	γ-Curcumene	10.4
1485	1491	2,4,6,9-Tetramethyldec-8-ene-3,5-dione, diastereomer I (diketo tautomer)	tr
1494	1491	β-Selinene	9.3
1501	1498	α-Selinene	1.5
1517	1513	γ-Cadinene	0.6
1527	1528	*cis*-Calamenene	tr
1555	1547	Italicene epoxide	0.8
1572	1578	3,5,7,10-Tetramethylundec-9-ene-4,6-dione, diastereomer I (diketo tautomer)	1.7
1581	1582	*ar*-Tumerol	0.8
1589	1582	Caryophyllene oxide[e]	6.3
1616	1608	Humulene epoxide II	0.8

[a]Retention indices determined experimentally on a DB-5MS column relative to a series of C7-C40 n-alkanes. [b]Literature values of retention indices taken from Adams (Adams, 2007) or NIST (NIST Mass Spectrometry Data Center, William E. Wallace, n.d.) collection, if not stated otherwise. [c]Compound identified based on mass spectra and retention indices matching with literature data, if not stated otherwise. [d]Values are means of three individualanalyses. [e]Compound identity confirmed by a co-injection experiment with a standard. [f]tr, trace amounts (<0.05%)

compounds included γ-curcumene (13.6%), β-selinene (12.2%), and α-pinene (11.8%), followed by β-caryophyllene (6.7%) and ar-curcumene (5.0%). It is important to emphasize that the efficacy of immortelle essential oil is highly dependent on the proportions of its chemical components, which can vary based on the chemotype (Aćimović, 2023).

Anticandidal activity

The results of our investigation into the antifungal potential of the tested essential oil are presented in **Tab. 2**. The table shows the minimum concentrations of the natural agent required to inhibit the growth of clinical *Candida* isolates and the reference strain *C. albicans* ATCC 24433. The data obtained through the microdilution method demonstrated a strong antifungal potential of the tested essential oil, with MIC values ranging from 0.312 to 5 mg/mL. The *C. krusei* species were particularly sensitive to the oil, with cell growth inhibition occurring at 0.312 mg/mL in isolates C_3 and C_{10}. Candidosis poses a significant challenge due to increasing resistance to conventional drugs, primarily as a result of the prolonged therapy required for such infections.

Table 2. Antifungal activity of *H. italicum* essential oil against clinical isolates of *Candida* spp. and reference strain *C. albicans* ATCC 24433

	Tested yeasts	*H. italicum* oil	Nystatin
		MIC (mg/mL)	MIC (mg/mL)
C_1	*Candida albicans*	5.00	0.009
C_2	*Candida albicans*	1.25	0.004
C_3	*Candida krusei*	0.31	0.009
C_4	*Candida albicans*	2.50	0.004
C_5	*Candida albicans*	1.25	0.004
C_6	*Candida albicans*	0.62	0.004
C_7	*Candida krusei*	0.62	0.004
C_8	*Candida albicans*	2.50	0.009
C_9	*Candida albicans*	1.25	0.004
C_{10}	*Candida krusei*	0.31	0.002
C_{11}	*Candida albicans*	0.62	0.004

MIC – Minimum inhibitory concentration.

Comparing our obtained results with those previously reported in the literature, the essential oil of *H. italicum* demonstrates a remarkable inhibitory effect against *Candida* strains (Mastelic et al., 2005; Djihane et al., 2017; Staver et al., 2018; Bacic et al., 2021; Zheljazkov et al., 2022). The study by Mastelic et al. (2005) investigated the chemical composition and antimicrobial potential of *H. italicum* essential oil from Croatia. The experiment tested selected strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *C. albicans*. The findings revealed that the essential oil significantly inhibited the growth of the yeast *C. albicans* and the Gram-positive bacteria *S. aureus*. Research by Djihane et al. (2017) found that *C. albicans* ATCC 10231 exhibited the highest sensitivity (6.32 µg/mL) to the action of immortelle essential oil. In their study, the aerial parts of *H. italicum* were collected in June 2013 during the flowering period

from Bejaia, northern Algeria. The essential oil from this collection was characterized by a high content of oxygen-containing sesquiterpenes (76.7%) and monoterpenes (61.42%). Major constituents included *α*-cedrene (13.61%), *α*-curcumene (11.41%), ceranyl acetate (10.05%), limonene (6.07%), nerol (5.04%), neryl acetate (4.91%), and *α*-pinene (3.78%). Staver et al. (2018) described the effects of *H. italicum* essential oil, sourced from Central Dalmatia (Croatia), against various microorganisms. Notably, at a concentration of 6.4 mg/mL, the oil exhibited an antimicrobial effect on clinical isolates of MRSA, *S. epidermidis*, and *C. albicans*. In contrast, our research demonstrated even more significant results: the growth of the reference strain *C. albicans* ATCC 24433 was inhibited at a concentration of 0.625 mg/mL, while all clinical isolates of *Candida* were inhibited at concentrations ≤5 mg/mL. The study by Bacic et al. (2021) examined the chemical composition and antimicrobial effects of *H. italicum* essential oil from Bosnia and Herzegovina. A total of 41 components were identified, with the main constituents being *α*-pinene, neryl acetate, *γ*-curcumene, and *β*-selinene. When tested against the reference strain of *C. albicans* ATCC 10231 using the disc diffusion method, the essential oil exhibited weak anti-*Candida* activity. Zheljazkov et al. (2022) presented the chemical composition and antimicrobial potential of essential oils from the species *H. arenarium* (L.) Moench and *H. italicum*. They tested *H. italicum* essential oils derived from plants introduced from Bosnia, Corsica, and France. The main constituents identified were neryl acetate (4.04–14.87%) and *β*-himachalene (9.9–10.99%). The results obtained using the disk diffusion method demonstrated moderate antifungal activity against *C. krusei* and *C. tropicalis*, with the highest activity observed against the Gram-positive bacteria *S. aureus*. The previously reported data on the antifungal properties of immortelle essential oil in the literature prompted us to investigate its synergistic potential and antibiofilm effect against various clinical isolates of *Candida* species.

Synergistic potential

In our study, we investigated the interaction between the essential oil of *H. italicum* and the commercial antifungal agent (**Tab. 3**). In this test, both the reference strain and two representatives of human clinical *Candida* isolates were evaluated. A synergistic effect was observed between immortelle oil and nystatin against the standard strain of *C. albicans* ATCC 24433, with an FIC index of 0.187. This type of interaction was also observed against isolate C_7 (*C. krusei*), with an FIC index of 0.25, as well as against isolate C_9 (*C. albicans*). Notably, no antagonistic interactions were detected between the tested substances. It is important to highlight that the MIC values indicating growth inhibition showed a significant decrease. This suggests that similar antifungal effects can be achieved with lower doses of antifungal agents, demonstrating the cooperative interaction between the tested substances.

According to the existing literature, several studies highlight the synergistic potential of essential oils, both as standalone treatments and in combination with established antifungal agents (Orchard et al., 2019; Soulaimani et al., 2021; Blanc et al., 2023). The study conducted by Stojanović-Radić et al. (2020) has also demonstrated a synergistic effect of *Inula helenium* L. oil (Asteraceae) with nystatin against clinical isolates of *Candida*. This may

Table 3. Interaction between the essential oil and nystatin against clinical isolates of *Candida* spp. and the reference strain of *C. albicans* ATCC 24433

Combinations	***Candida albicans* ATCC 24433**			***Candida krusei* C_7**			***Candida albicans* C_9**		
	MIC alone	MIC combined	FIC	MIC alone	MIC combined	FIC	MIC alone	MIC combined	FIC
EO+N									
immortelle	0.625	0.039	0.062	0.625	0.078	0.125	1.25	0.312	0.25
nystatin	0.004	0.0005	0.125	0.004	0.0005	0.125	0.004	0.001	0.25
***FICI Interaction**			**0.187 Syn**			**0.25 Syn**			**0.5 Syn**

MIC – Minimum inhibitory concentration (mg/mL); FIC – Fractional inhibitory concentration; FICI – Fractional inhibitory concentration index; Syn – synergism

be a consequence of *I. helenium* oil's chemical composition, where sesquiterpenes dominate. However, we did not find any available studies made to directly compare our results on the interaction between a commercial sample of immortelle EO and the antifungal agent nystatin against human *Candida* isolates. The results of our study demonstrated that immortelle oil significantly inhibited the growth of planktonic *Candida* cells, both as a standalone treatment and in combination with nystatin. These findings underscore the importance of exploring the potential synergism of essential oils, as such research could contribute to developing new strategies for treating fungal infections.

Effect of the immortelle oil on biofilm production

We investigated the biofilm-forming ability of *Candida* species and evaluated the potential of *H. italicum* essential oil to inhibit biofilm formation. The results are presented in **Figs. 1** and **2**. A significant reduction in biofilm formation was observed after treatment with *H. italicum* essential oil at both MIC and half-MIC concentrations for isolates C_1, C_4, C_5, and C_{11}. The inhibition percentage was approximately 60% or lower, demonstrating the strong efficacy of immortelle oil against clinical human *Candida* isolates. The reference strain *C. albicans* ATCC 24433 also exhibited high sensitivity to the tested essential oil, significantly reducing biofilm formation by over 60%. Moderate reduction efficiency was observed with isolates C_2 and C_8, where the inhibition percentage was around 40% or slightly lower, depending on the agent concentration and the specific *Candida* isolate.

The literature emphasizes the notable ability of natural products from various plant species to reduce biofilm formation (Butassi et al., 2021; Karpiński et al., 2021). For instance, Butassi et al. (2021) provided a comprehensive literature review (covering the period from 2017 to May 2021) on essential oils, propolis, plant extracts, algae, lichens, microbial compounds, and nanosystems containing natural products. The review discusses their *in vitro* and *in vivo* potential to modulate fungal biofilms. These findings demonstrated that various plant secondary metabolites and their compounds exhibit significant activity inhibiting biofilm formation and reducing mature biofilm structures against *Candida* isolates. In the review study by Karpiński et al. (2021) 69 plant-derived compounds from plants belonging to various families were shown activity against *Candida* biofilms. Among these compounds, monoterpenes were the most prevalent, followed by sesquiterpene lactones and sesquiterpenes. Since our commercial sample of *H. italicum* contains the main compounds that fall into the sesquiterpene

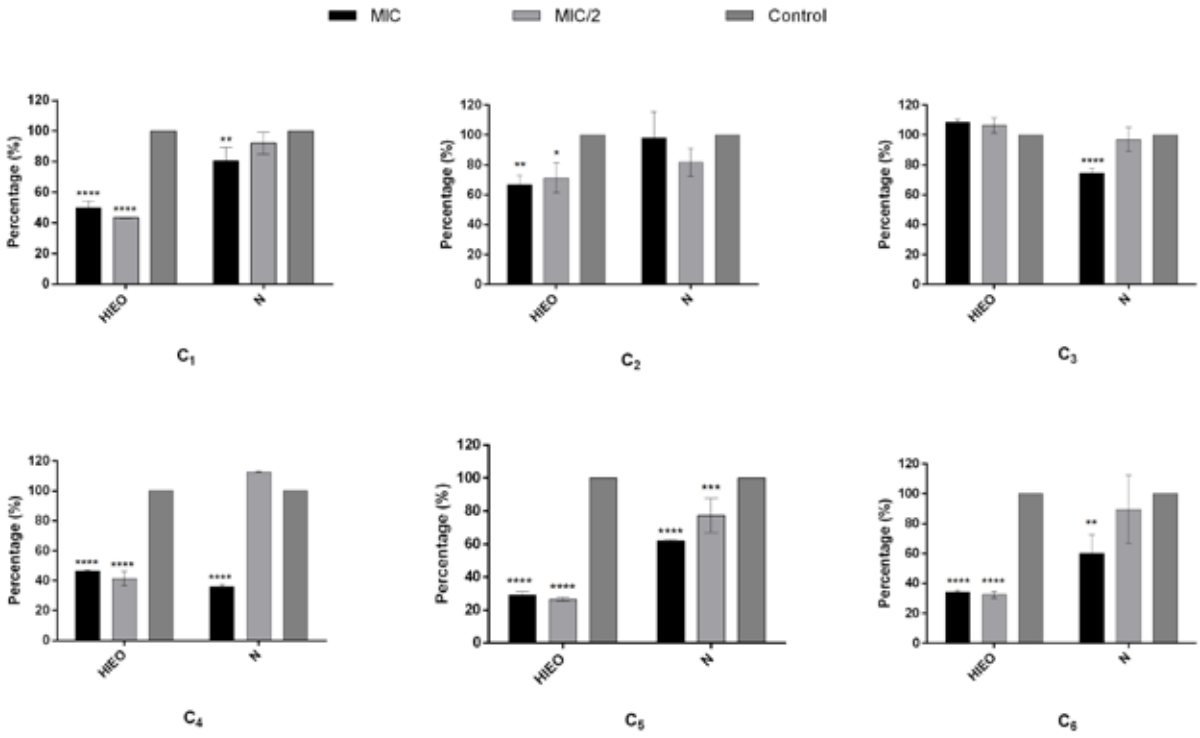

Fig. 1. Effects of the immortelle oil on *Candida* biofilm production. *Helichrysum italicum* essential oil (HIEO); nystatin (N). Statistical analysis used by ANOVA with Tuckey Post hoc test ****p<0.0001; **p<0.001; **p<0.01; *p<0.05 vs. control

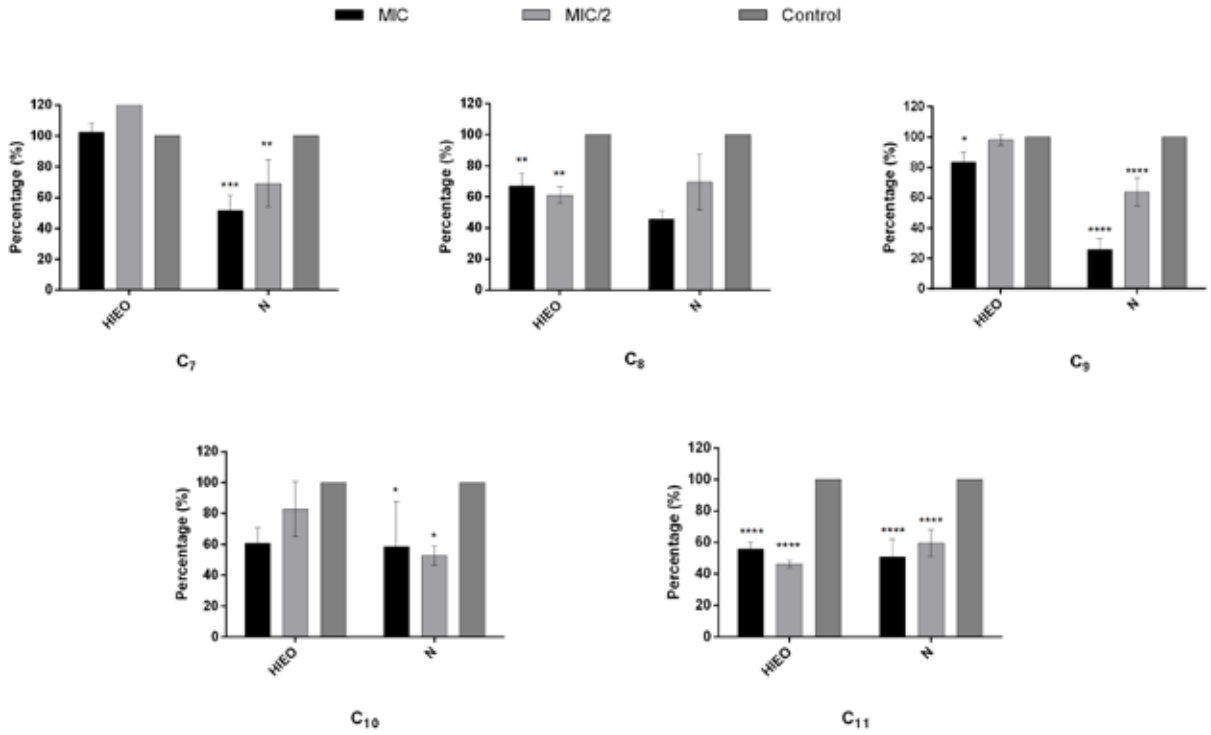

Fig. 2. Effects of the immortelle oil on Candida biofilm production. Helichrysum italicum essential oil (HIEO); nystatin (N). Statistical analysis used by ANOVA with Tuckey Post hoc test ****p<0.0001; **p<0.001; **p<0.01; *p<0.05 vs. control

and monoterpenes category, these components likely contribute to the significant antibiofilm activity observed in our research (**Figs. 1** and **2**). In their study, Manoharan et al. (2017) screened 83 essential oils for their effectiveness against *C. albicans* biofilm formation, including the essential oil of *H. coriaceum* (DC.) Harv. The results showed the biofilm reduction by immortelle essential oil, which contains *β*-caryophyllene - a sesquiterpene compound known for its bioactive properties (Manoharan et al., 2017). To date, we have not found relevant literature to directly compare our findings on the antibiofilm effects of immortelle essential oil against clinical isolates of *Candida*, the causative agent of candidosis.

Given the limited relevant literature on the antifungal potential of immortelle oil (*H. italicum*) on planktonic and biofilm forms of the genus *Candida*, our study provides a valuable contribution to ongoing and future research in this field.

Conclusion

Our research demonstrated a potent anti-*Candida* effect of the tested essential oil from the *Helichrysum* genus. In this study, we analyzed the chemical composition of *H. italicum* essential oil, identifying a total of 52 compounds. The most abundant constituents were neryl acetate, *α*-pinene, *γ*-curcumen, and *β*-selinene. Immortelle essential oil, evaluated using the microdilution method, exhibited a significant inhibitory effect on tested strains of the genus *Candida*. Additionally, the oil was examined for potential synergistic interactions with nystatin on selected clinical *Candida* isolates, with the results confirming a notable synergistic potential. The potential to reduce *Candida* biofilm formation after treatment with immortelle oil at MIC and half-MIC concentrations was also investigated. Significant antibiofilm activity was observed in most treated strains when compared to the control group.

The data obtained suggest that immortelle oil, which effectively inhibits both planktonic and biofilm-forming *Candida* cells, holds promise as a potential alternative agent treatment for candidosis. Our research provides a strong foundation for further testing on clinical strains responsible for candidosis, particularly given the rising incidence of recurrent infections and growing resistance to current antifungal treatments.

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