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ORIGINAL RESEARCH

Biological Activity of Natural Formulation with Propolis, Lavender and Thyme Oil on *Candida* Species

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Abstract
Objective: *Candida* strains have been frequently associated with nosocomial infections in recent years. On the other hand, the public prefers natural resources rather than synthetic materials. Therefore, it is essential to research the combined effects of natural products. This study aimed to investigate the antifungal and antibiofilm effectiveness of essential oils and propolis-containing samples on *Candida* species and the toxicity of samples.
Materials-Methods: Three different samples were prepared with propolis, lavender oil, and thyme oil. Gas Chromatography-mass Spectrometry (GC/MS) GC/MS was used to determine organic compounds in samples. The antifungal effects of a natural product mix were tested against *C. albicans*, *C. krusei*, and *C. parapsilosis* by disk diffusion method. MIC and MFC tests of the mixture were performed against a standard such as Nystatin. Antibiofilm tests were performed with the microplate system, taking into account the detected concentrations, and finally, the cytotoxicity test was investigated in the L929 cell line with the WST-1 kit.
Results: *C. albicans* was determined as the most sensitive species (MIC50: 90 ppm, MIC90: 750 ppm MFC: 3120 ppm). In the antibiofilm tests, it was determined that the sample was more effective on *C. albicans*, and it inhibited biofilm formation. Also, it was determined that the IC50 value was 5052 ppm.
Conclusion: The natural product mixture, which has a scientifically proven antifungal effect, has a feature that has high added value and contributes to the economy. However, of course, it has to investigate by further studies. The findings of this study may lead to new therapeutic approaches with standardized natural products in the future.
Keywords: Antifungal, Antibiofilm, *Candida*, Propolis, Lavender Oil, Thyme Oil.

INTRODUCTION

Candida species are widely distributed in nature. They are mostly commensal fungi but can also be opportunistic pathogens in human microbiota.[1] Also, *Candida* is the most common causative agent among fungi that cause disease in humans. Candidiasis develops with the excessive proliferation of *Candida* species in the natural flora of humans. The most important reason for that is the inappropriate use of antibiotics and the weakening of the immune system.[2,3] Some of the most common *Candida* species that cause disease are *C. albicans*, *C. krusei*, and *C. parapsilosis*. *C. albicans* is a fungus often isolated from skin, gastrointestinal tract, and esophagus infections. Skin and mucous infections of *C. albicans* include thrush, *Candida* esophagitis, non-oesophageal gastrointestinal candidiasis, *Candida* vaginitis, and cutaneous candidiasis. *C. krusei* is associated with systemic candidiasis and is mainly found in immunosuppressive individuals. Further, *C. krusei* treatment is challenging because it has resistance to many antifungal drugs. Mucocutaneous infections caused by *C. parapsilosis* in cases of immune deficiency can also be chronic in humans.[4]
All *Candida* strains have been frequently associated with nosocomial infections in recent years. In order of prevalence, they are in the top five in invasive yeast infections worldwide.[5] For the ability to switch

from commensal to pathogen, properties such as having a wide range of virulence factors and the ability to change morphology and create biofilms are among the essential characteristics of *Candida* species. Biofilm formation provides highly resistant infections in the host due to their structural nature. *Candia* biofilms are resistant to antimicrobial therapy. Therefore, available therapeutic agents are not sufficient to eliminate *Candida* biofilms.[6] Therefore increased research for new compounds to show the anti-*Candida* effect are essential.[1] On the other hand, consumers prefer natural resources over synthetic materials in food and alternative treatment processes.[7] Therefore, taking advantage of the combined effects of natural products prepared differently is essential. Because, in this way, it is possible to produce powerful natural antifungal agents' alternative treatment methods are also being developed by using these natural extracts directly.[8]
Among these natural products can be the best choice is propolis. Propolis is a resinous substance obtained by collecting from leaves, shoots, and buds of different plant sources and adding enzymes and beeswax by honey bees. Although the propolis content varies depending on the region, climatic conditions, and the collection season, the essential and aromatic oil ratio generally does not exceed 10%. This 10% slice contains phenolic substances, flavonoids, and terpenoids.[9] Due to this special propolis content, it is used as an antimicrobial, antifungal, and antioxidant agent.[10] Combining this activity of propolis with various natural products can create more positive effects. In this case, lavender and thyme, belonging to the *Lamiaceae* family, are critical medicinal plants cultured worldwide due to their high-quality essential oils. The essential oils of these plants have been used for therapeutic and cosmetic purposes for centuries. In addition, it has been the reason for its use in industry and agricultural applications due to the biologically active components. Propolis, lavender, and thyme extracts are also an important branch of natural resources. All of them are products brought to the literature as natural preservatives and antifungal agents.[11] However, when it comes to a natural product, it is wrong to say that it is good in any case. It does not make sense unless it is determined that natural mixtures do not cause toxic effects and if they are not antagonistic to each other. For this reason, cell viability tests are essential. Therefore, standardization of natural products is difficult. For this reason, the microbial inhibition concentration of each natural product should be determined separately. The aim of this study is to investigate the antifungal and antibiofilm efficacy and cell viability of samples containing herbal oil and propolis on *Candida* species.

MATERIALS AND METHODS

Microorganisms

Candida albicans and *C. krusei*, which are clinical isolates from previous studies and were obtained from the culture collection of Duzce University Traditional and Complementary Medicine Applied and Research Centre. Also, C. *parapsilosis* ATCC 22019 was used in the study.

Chemicals and materials

RPMI-1640 medium containing %1 streptomycin and penicillin 10% inactivated fetal bovine serum (all purchased from Sigma) was used for the L929 mouse fibroblast cell line. WST-1 (2-(4-iodophenyl) -3- (4-nitrophenyl) - 5- (2,4-disulphophenyl) -2H-tetrazolium) cell proliferation assay kit (Takara Bio Inc) was used for the viability test. Potato dextrose agar (PDA), sabouraud dextrose agar (SDA) and, sabouraud dextrose broth (SDB), Mueller Hinton agar (MHA) were purchased from Merck for antifungal tests and fungal growth. PEG400 (Polyethylene glycol 400 from Sigma) was obtained from Merck. The ethanolic propolis extract, lavender oil (*Lavandula intermedia*), and thyme oil (*Origanum onites*) were purchased from Duzce University Beekeeping Center and Traditional and Complementary Medicine Applied and Research Centre at Duzce University.

Preparation of natural products

First, ethanol in commercially available propolis was removed with the help of an evaporator, and 0.455 grams of pure propolis was used. Propolis dissolved in 3.46 grams of PEG-400, and sample 1 was obtained. To obtain sample 2, 0.455 grams of commercially available lavender oil was added to sample 1 and mixed homogeneously. To obtain sample 3, 0.455 grams of commercially available thyme oil was added to sample 2. All obtained samples were stored at 4 ^{o}C.

Gas chromatography/mass spectroscopy (GC/MS) analysis of essential oils

Gas Chromatography-mass Spectrometry (GC/MS) GC/MS was used to determine organic compounds. The GC/MS instrument consisted of an Agilent 7890 gas chromatograph with an on-column auto-injector and Agilent mass selective 5975c MS detector. The GC column was HP-innowax column (60 m, 250 µm, 0.25 µm). The GC/MS was operated under the following conditions; an ion source, transfer line

temperature 60-260°C; carrier gas He (≥99.99% purity); flow rate 1 mL/min. 5 μL of the sample solution was injected into GC using splitless injection mode. The GC oven temperature was the program as follows: 100°C for 0 min, then the temperature increased by 1°C/min to 150°C for 0 min, and then with an isothermal hold at 240°C for 10 min. Agilent software was used for data characterization. The compounds were identified with their retention time, and the mass spectra were compared with internal reference library data.

Anti-*Candida* effect of samples

Disk diffusion test was used to compare the anti-*Candida* effects of all three samples.[12] *Candida* cultures in PDA medium were prepared the day before and then adjusted to a turbidity of 0.5 on the McFarland scale in saline. Yeast solutions spread on MHA. Test samples were absorbed into blank disks (Bioanalyase, blank disk, 6mm) and placed on MHA. Test plates were incubated at 35° C for 48 hours. Nystatin (Bioanalyase, NY 100U disk) was used as a positive control. Each sample was tested in triplicate. After then, the sample with the biggest inhibition zone according to the disk diffusion method was chosen for further research.

Determination of minimal inhibitory (MIC) and fungicidal (MFC) concentrations

The sample with the most inhibition zones from the disk diffusion test was considered the most effective. Then MIC and MFC concentrations of the most effective sample were determined for all three *Candida* species by the modified broth dilution method.[1,13] For MIC, a product dilution and SDB medium were added in the dose range of 100000 to 20 ppm for a total of 120 µl per well of the 96 well plate. Each yeast (at 0.5-2.5x 103 cell/ml) was inoculated into the medium containing the medium and test product and then incubated at 35° C for 48 hours. For MFC, 50 µl of non-growth MIC value and above concentrations were added to the SDA. Colonies were counted and compared with control. MFC was defined as the lowest dose that killed more than 99.9% of inoculated *Candida* cells.[1,14] Experiments were conducted three times for each strain on different days.

Antibiofilm assay

The effect of the most effective sample on biofilm formation was investigated. 40 µl SDB, 80 µl yeast (prepared with 0.5 Mcfarland turbidity), and 80 µl different doses of product or water were added to each well of 96 well plate. After 24 hours of incubation at 35°C, the absorbance value (OD) was read at 630 nm by using a microplate reader (Biotek BT 800, USA). AfAfterwardthe contents of all wells were poured and washed three times with distilled water. After drying at room temperature, 125 µl 0.4% crystal violet was added to each well and waited 30 minutes. Then the dye was poured, washed three times, and left to dry again at room temperature. 100 µl of 95% ethanol was added to remove the cells forming biofilms and trap the dye and transferred it into a clean well. OD measured at 490 nm. The ratio of OD value at 490 nm and 630 nm (= OD 490/OD630) was used to assess biofilm formation. Results were evaluated.[1,15] All experiments were performed three times.

WST-1 assay

The cytotoxicity of the active sample was determined in the L929 mouse fibroblasts cell line by the WST-1 cell viability test. Ten different dilutions of the sample between 90 and 50000 ppm were performed. When the cells reached the appropriate concentration, they were inoculated into 96-well culture dishes. It was added 5x104 cells per well and serial dilutions and incubated at 37°C (in a 5% CO2 atmosphere) for 24 hours. After incubation, 10 µl of WST-1 solution was added to each well and incubated for 4 hours. At the end of the incubation, each well's absorbance value (OD) was read at 490 nm wavelength and 630 nm reference range using the microplate reader. All assays were performed three times, and the viability (%) was calculated as the following equation.

Viability (%) = (OD in herbal sample group/OD in control group)×100

Statistical analysis

Inhibition data are given as mean (± SD). Data are given as mean (± SD). The statistically significant differences of each group compared to the control group were compared with Mann Whitney U using SPSS 15.0.

The percentage of cell inhibition x log of the concentration was recorded, and their IC50 and respective confidence intervals (IC 95%) were recorded from non-linear regression. Analyzes and graphs were elaborated using the GraphPad Prism version 9.0 software (San Diego, CA, USA).

RESULTS

GC-MS analysis of essential oils of thyme and lavender used in samples

By GC MS analysis of commercially available lavender and thyme oil, preliminary information was obtained about its chemical components. Microbiological studies were carried out on the products that were brought together in line with the

information obtained. Linalool, linalyl acetate, 1,8-cineole, and camphor accounting for 40.11, 25.23, 6.13, and 5.88%, respectively, were also characterized as major chemical compounds in lavender essential oils. Also, the thyme essential oil was characterized by high amounts of Carvacrol (57.65%) and linalool (15.64%) (Table 1).

Antifungal effects of samples

In the antifungal effect test performed using the disc diffusion test, the effects of lavender oil, thyme oil, and propolis extract (sample 3) combined in equal mass ratios, as well as the effects of only propolis extract (sample 1) and propolis extract-lavender oil (sample 2) mixture were also examined and compared.

It has been determined that the first planned triple mixture is more effective. The results were determined by measuring the zone diameters formed by the impregnated discs at that point. The highest inhibition sites were detected at S3. Considering CLSI guidelines, all three *Candida* strains appeared to be sensitive to Nystatin used as a positive control in the study (Table 2).

Table 1. GC/MS analysis results of examined essential oils

Entry	RT (min)	Compound Name	Thyme Oil % of Total	RT (min)	Compound Name	Lavender oil % of Total
1	9.504	Alfa-pinene	0.34	9.749	Alfa-pinene	0.43
2	16.908	Beta-myrcene	0.92	11.563	Camphene	0.35
3	17.851	Alfa-terpinene	0.89	17.403	Beta-myrcene	0.94
4	23.196	Gama-terpinene	3.13	19.830	Limonene	1.10
5	25.458	Orto-cymene	3.31	20.794	1,8-cineole	6.13
6	56.725	Linalool	15.64	23.132	Beta-ocimene	1.35
7	59.672	Beta-caryophyllene	1.57	24.676	Trans-beta-ocimene	2.30
8	61.478	Terpinene-4-ol	1.77	51.765	Camphor	5.88
9	72.054	Borneol	3.13	58.154	Linalool	40.11
10	74.680	Beta-bisabolene	3.78	58.915	Linalyl acetate	25.23
11	109.955	Thymol	2.97	61.080	Trans-caryophyllene	1.33
12	110.881	Carvacrol	57.65	63.651	Lavandulyl acetate	1.51
13	-	-		73.166	Borneol	4.43
14	-	-		89.433	Geraniol	0.61
15	-	-		107.194	Triacetin	0.77
16	-	-		111.139	Levamenol	0.42

The inhibition region of S3 was significantly larger than the positive control disk. Sample 3, the most effective formulation, was used for further experiments. MIC and MFC tests were performed for sample 3, and the lowest effective doses were determined. In addition, it was determined that the most sensitive species was *C. albicans* (Table 3).

Table 2. Zone diameters of formulations for *Candida* spp.

Yeast	Zone Diameter (mm) (±SD)			
	Formulation-1	Formulation-2	Formulation-3	Nystatin (100µg)
C. albicans	10 (±0.57)	13 (±1)	>30	25
C. krusei	8 (±0.57)	10 (±0)	30 (±0.57)	20
C. parapsilosis	R*	R*	17 (±0.57)	15

**Resistant*

Table 3. MIC and MFC tests for Sample 3

Yeast	Sample 3				
	MIC_{50}, ppm	MIC_{90}, ppm	MFC, ppm	NY (MIC), ppm	NY (MFC), ppm
C. albicans	90	750	3120	4	8
C. krusei	370	1500	6250	8	16
C. parapsilosis	180	1500	6250	8	16

NY:Nystatin

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According to the MIC assay, it was determined that *C. albicans* was more sensitive than other *Candida* species for cidal concentration (Figure 1). The inhibition rates of S3 against *Candida* strains depending on the concentration are given in Figure 1. Each concentration showed a significant difference for *C. albicans* compared to the control group (p<0.05). There was no significant inhibition against *C. krusei* at 90 ppm and 180 ppm, while 90 ppm of S3 against *C. parapsilosis* was not significant (p>0.05).

Antibiofilm effects of samples

Since S3 was selected as the most effective sample in previous tests, only the antibiofilm effect of S3 was investigated. The biofilm ability of each strain was also tested in control groups not treated with S3 for each bacteria. It was observed that *C. parapsilosis* produced weak biofilms, while *C. albicans* and *C. krusei* produced strong biofilms. Therefore, the strain most susceptible to biofilm formation against S3 was *C. parapsilosis*. It was determined that the biofilm formation ability of *C. krusei* was stronger and more resistant to S3 treatment groups. 75 ppm of S3 almost eliminated the formation of biofilms for *C. albicans* and *C. parapsilosis* while inhibiting more than 75 ppm for *C. krusei.* Inhibition rates of all concentrations against *C. albicans* and *C. parapsilosis* were statistically significant compared to control groups (p<0.05). 9 ppm of S3 was not a significant difference against *C. krusei* compared to the untreated control group. The inhibition rates of different concentrations of S3 on biofilm formation against *Candida* strains are given in Figure 2.

Cytotoxicity of sample-3

Cytotoxicity assays were performed at ten different concentrations, and the results were given in % viability. According to the results of the cell viability test conducted with the WST-1 kit, and IC50 value of the S3 is given in Figure 3. IC50 has been defined as the dose that kills half of the cells in the medium.

Data are presented as the mean ± standard error of the mean or as half-maximal inhibitory concentration (IC50) values. The 95% confidence intervals were obtained through nonlinear regression. Results are expressed as mean±S.E. Data was analyzed using GraphPad Prism version 9.0 software (San Diego, CA, USA). The percentage viability data varied with concentration. As the concentration increased, the vitality decreased. The best viability was determined at a dose of 90 ppm. Cell viability was about 90% at 180, 370, and 750 ppm concentrations. After 3120 ppm of S3, viability was severely reduced. The IC50 value at the 95% confidence interval is between 4238 and 6018. According to the analysis results, the IC50 value was 5052 ppm.

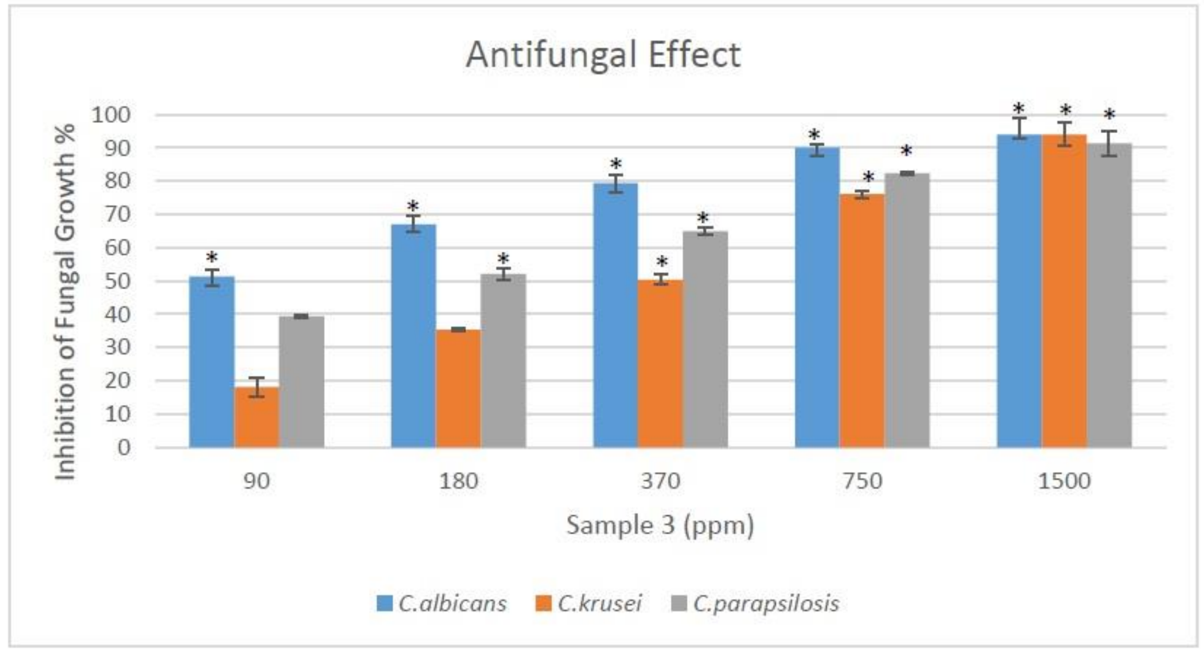

Figure 1. The effect of sample 3 on fungal growth: The normal growth of the control group for each yeast (untreated *Candida* strains) was considered 100%. The inhibition rates were calculated for each concentration. Since the positive control showed 100% inhibition against all yeasts after 16 ppm, it was not included in the graph.

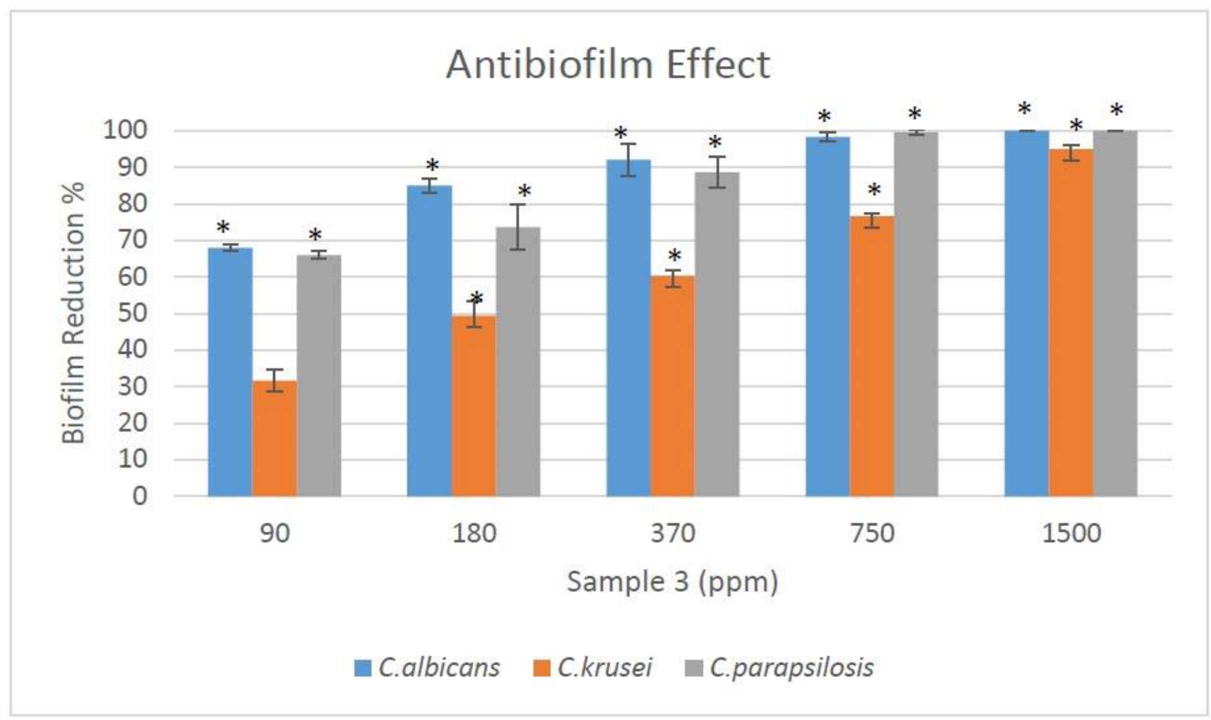

Figure 2. Antibiofilm effect of sample 3: The normal growth of the control group for each yeast (untreated *Candida* strains) was considered 100%. The inhibition rates were calculated for each concentration. Since the positive control showed 100% inhibition against all yeasts after 16 ppm, it was not included in the graph.

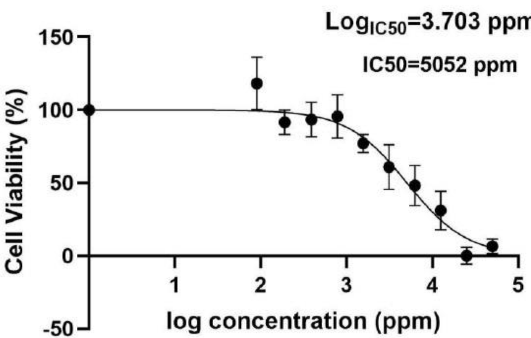

Figure 3. IC50 value of sample 3

DISCUSSION

The use of natural products in microbial treatments is becoming more popular day by day. Nowadays, cures containing very different mixtures of natural products are becoming widespread. Natural ingredients contain quite a lot of phenolic components. Phenolic components, on the other hand, are beneficial for microbial treatment.

It might be more effective when the combined antimicrobial effects of natural products are used together on pathogenic microorganisms. Because it is already known that medicinal plants have some pharmacologically important features via their component. In their combined use, the amount of phenolic components increases, and they turn into more effective antifungal agents due to the variety of phenolic components contained in the product.[16]

In our study, Carvacrol (%57.65), Linalool (%15.64) in thyme oil, and Linalool (%40.11), linalyl acetate (%25.23) in lavender oil were found highly. Also, there are many minor ingredients such as gamma-terpinene, orto-cymene, beta-caryophyllene, borneol, beta-bisabolene, thymol in thyme oil, and alfa-pinene, camphene, beta-myrcene, limonene, beta-ocimene, trans-beta-ocimene, lavandulyl acetate, in lavender oil. Yılmaz[17] indicated that major components were Linalool (24.97-2.52-43.86-39.43 %), linalyl acetate (3.4-0.29-9.37-15.76 %), eucalyptol (33.81-43.81-18.47-12.08 %), camphor (13.12-15.91-8.72-9.21 %), and alpha-terpineol (2.84-2.47-1.28-3.86 %) in different parts of (stem, leaf, flower, and mix) *Lavandula x intermedia* Emeric cultivated in Türkiye (Bismil-Diyarbakır). In another study, Linalool (36.801%–28.486%) and Linalyl acetate (33.087%–4.648%) were found in Super A and Grey Hedge cultivar of *L. x intermedia* in Türkiye (Western Anatolia).[18] Some previous studies determined similar Linalool, linalyl acetate, camphor, and eucalyptol values as dominant components in L. intermedia cultivated in Southeast

Spain[19] and Italy[20]. In the chemical profile of the thyme essential oil, Carvacrol and p-Cymene were found as the major components, with an abundance of 74.749% and 9.464, respectively (Table 1). Similarly, it was observed that the oil from samples collected from Türkiye showed mainly Carvacrol, Linalool, and p-Cymene.[21,22] Differences in plant essential oil content are due to genetic factors and cultivation practices. They even differ depending on the period of development during which the plants are harvested and depending on it.[23] Additionally, the propolis used in this study was collected from Duzce (in Türkiye). The propolis is native to that region and is rich in phenolic components of more than 100mg/g for each component, such as quercetin, p-coumaric acid, ferulic acid, trans-cinnamic acid, benzoic acid, emodin, hesperidin, gallic acid, rosemarinic acid.[24]

The samples created in the study are based on propolis. Because it has already known that propolis is an effective natural bee product to control *Candida* infection.[25] In this study, it was aimed to increase its effect with other effective natural ingredients. The thyme and lavender essential oils are herbal products thought to have antifungal effects.[26,27] In previous studies, Lavender oil has been shown to have anti-candidal effects on vulvovaginal candidiasis isolates.[28] It has also been shown to have fungistatic and fungicidal activity against oropharyngeal and vaginal *Candida* strains.[29] In this study, the antifungal efficiency was investigated for three different samples. However, the most effective formula in this study was S3 contains thyme oil. S3 was considered the most effective formula because the zone diameter was the largest. The largest zone diameter may not mean the most effective, but on the other hand, natural products do not work with specific receptor-like antimicrobials. It was interpreted as causing more cell inhibition because essential oils directly change the cell composition of microorganisms. Hence, it has been thought that the antifungal effects of thyme essential oil stand out. Thyme oil is considered a more effective natural product than the other two natural products. There are many studies in which thyme oil has an antifungal effect on clinical *Candida* strains isolated from different body parts, such as skin, urine, wound, and vagina. Due to the biological activity of thyme essential oil, its industrial and medical use has come to the fore in recent years.[30] Zhang et al.[31] reported that MIC was 65 ppm, MFC was 100 ppm for thymol, while MIC was 120 ppm, and MFC was 140 ppm for carvacrol. Also, some combinations of natural products create a stronger antimicrobial effect via their combined effect. It is thought that; these combinations can be a natural therapeutic agent for candidal mycoses, among the important fungal infections today. Considering the chemical composition of essential oils and propolis, except for the major components, there are other components in trace amounts. It is thought that these minor components may be effective due to their combined interaction with other components.[32] In general, phenolic components disrupt the lipids and hydrophobic structure found in the cell membrane of microorganisms, making them more permeable. The amount of these phenolic components determines the MIC and MFC values. In addition to the bacteriostatic effect, the product's concentration and duration of action determine the bactericidal effect. In another study, it has been reported that the kinetic curves of *Litsea cubeba* oil (antibacterial) are 6250 ppm, and the cells are completely destroyed.[33] They also reported that the delay of *E. coli* cells could prolong the phase growth to about 12 hours it was killed at 1250 ppm within 2 hours. In this way, it can be tried that the factors determining the activity of natural mixtures are functional groups with active components in the composition and their synergistic interactions.[34]

Although sample 3 showed a high antifungal effect in the study, fungi have some escape routes from antifungal agents. The effectiveness of natural products on fungi can be eliminated by some metabolites of fungi. The biofilm formation, which is an escape for microorganisms especially in antimicrobial treatment, is common in *Candida* species.[7] The present study showed that the strain most susceptible to biofilm formation of fungal cells against sample-3 was *C. parapsilosis*. There are many reports about the antibiofilm effects of propolis, lavender oil, and thyme oil separately. In those studies, it has been shown that propolis reduces the formation of *Candida* species biofilm formation.[35] Similarly, it has been revealed by many researchers that essential oils disrupt the biofilm structure of different microorganisms.[36] Moreover, lavender and thyme oil have been shown to have antibiofilm effects on different *Candida* species, similar to this study's results.[28]

It is important that natural products are pharmacologically effective. However, on the other hand, combining natural products with no toxic effects when used together is so important. Phenol components in natural products can be toxic due to

increased doses and combined use. In vitro cytotoxicity tests used for this purpose are measurement methods performed in cell culture to evaluate substances with drug characteristics or whose toxic profile has been investigated. There are many tests applied to determine cell viability, and the WST-1 assay is one of them. It is a more sensitive method compared to the MTT test because it does not require additional thawing, and the absorbance is recorded at various times during the experiment. In this study, % viability data varied depending on concentration. As concentration increased, vitality decreased. The best viability was determined at a concentration of 90 ppm. After a dose of 3120 ppm, vitality decreased significantly. The IC50 value was determined as 5052 ppm. Tamfu et al.[37] determined the IC50 value of three different propolis, whose MIC value was 250-500 µg/mL against *Candida,* and it was found to be >100 ppm in the NIH-3 T3 fibroblast cell line. In the previous investigations, the effects of thymol, one of the major components of *T. vulgaris*, on Chinese hamster lung fibroblast (V79) cells MTT assay[38], and the effects of two thyme oils on human keratinocytes with MTT assay[39] were investigated. In addition, there is a cytotoxicity study on murine fibroblasts with 12 plant oils, including lavender oil.[40] Our study results are consistent with other studies. The concentrations required to eliminate yeasts have been shown to show no toxicity or very low toxicity.

CONCLUSION

Candida infections are the most common systemic and superficial mycoses in recent years. Moreover, *Candida* species resistant to various antifungal drugs are frequently reported. The research for natural product samples has increased due to resistance development. This is the first study in which the combined effects of propolis, lavender oil, and thyme oil were investigated by antibiofilm and antifungal assays. In this study, the efficacy of three different combinations was tested, and it was determined that the most effective sample was the S3 sample. Hence, the antifungal activity of the S3 in three different *Candida* species was investigated with other test methods, and its inhibition on biofilm formation was determined. Also, the biological activity of S3 was investigated with further experiments. It has been found that it can be as effective as an antifungal agent at appropriate concentrations. It was thought that the clinical use of these concentrations may be important because they do not show toxicity. Using lavender, thyme essential oils, and propolis combinations can be a practical and safe approach to treating *Candida* infections. But of course, it has to investigate by further studies. Especially considering the increased resistance of antimicrobial agents, the necessity to development of effective natural products is quite high. The findings of this study may lead to new therapeutic approaches with standardized natural products in the future.

Author contributions: Conceptualization: [NS, HG]; Study Design: [PAY, NS]; Investigation/Data collection: [PAY, NS, PGR, HG]; Writing: [PAY, NS, PGR]; Editing: [PAY, NS, PGR].

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