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Evaluation of In-vitro Anticandidal Activity of 99 Different Commercial Plant Extract, Fixed and Essential Oils against Vaginal *Candida albicans* Isolates

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ABSTRACT

Plant extracts (PE), fixed oils (FO) and essential oils (EO) are used in traditional medicine to treat various diseases. This study evaluated the anticandidal activity of 100 different commercially available PEs, FOs, and EOs against 19 *Candida albicans* vaginal isolates and *C. albicans* ATCC 10231. It was determined that 20 EOs and FOs had anticandidal activity. *Piper nigrum* FO, pine turpentine EO, pine tar EO, and *Eugenia caryophyllata* EO showed the highest anticandidal activity. The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) values of these FO and EOs were between 0.125 and 2 µL mL^{-1}. The volatile components of these FOs and EOs were determined by GC-MS analysis. There were six components in *E. caryophyllata* EO, 38 in Pine turpentine EO, 39 in *P. nigrum* FO, and 119 in Pine tar EO. In conclusion, this FOs and EOs can be used to treat vulvovaginal candidiasis.

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99 Farklı Ticari Bitki Ekstrakt, Sabit ve Uçucu Yağın Vajinal *Candida albicans* İzolatlarına Karşı In-vitro Antikandidal Aktivitesinin Değerlendirilmesi

ÖZET

Bitki ekstrakt (PE), sabit yağları (FO) ve esansiyel yağları (EO) çeşitli rahatsızlıkların tedavisinde geleneksel tıpta kullanılmaktadır. Bu nedenle bu çalışmada ticari olarak satışı bulunan 99 adet PE, FO ve EO'nun 19 *Candida albicans* vajinal izolatına ve *C. albicans* ATCC 10231 standart kültürüne karşı antikandidal aktivitesi değerlendirilmiştir. 20 adet PE, FO ve EO'nun antikandidal aktiviteye sahip olduğu belirlendi. En yüksek antikandidal aktiviteyi ise *Piper nigrum* FO, pine turpentine EO, pine tar EO ve *Eugenia caryophyllata* EO gösterdiği belirlenmiştir. Bu FO ve EO'ların minimum inhibitör konsantrasyonu (MİK) ve minimum fungisidal konsantrasyonu (MFC) değerlerinin ise en düşük 0.125 µL mL^{-1} ve en yüksek ise 2 µL mL^{-1} olduğu belirlenmiştir. Bu yağların uçucu bileşenleri ise GC-MS analizi ile belirlenmiştir. Karanfil yağında 6 bileşen, çam terebentin yağında 38 bileşen, karabiber yağında 39 bileşen ve çam katranın da ise 119 bileşen tanımlanmıştır. Sonuç olarak yeni antifungal bileşenlerin belirlenmesine katkı sağlayacak bitki esansiyel yağları belirlenmiştir.

Mikrobiyoloji

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INTRODUCTION

Candida species are commensal members of the human microbiota. They can colonize the mucosal surfaces of the oral cavity, vagina, skin, scalp, and nails. (Gonçalves et al., 2016; Tsega & Mekonnen, 2019; Permana et al., 2021). All women can carry *Candida* in the vagina without causing infection. *Candida* can cause vulvovaginal candidiasis (VVC) in various conditions that impair host immunity (Gonçalves et al., 2016; Ghaddar et al., 2020). VVC is

the most common fungal disease affecting the genital tract of women all over the world and is considered an important public health problem. The clinical symptoms of VVC are not specific. The most common clinical manifestations are vaginal pain, vulvar and vaginal erythema, and fissures with vulvar itching, burning, and irritation leading to dyspareunia and dysuria. The morbidity of VVC is the biggest problem, and it is not associated with mortality. It causes pain and suffering, and changes in self-anxiety and job performance, especially in women. Most importantly, it affects their sexual and emotional relationships and creates mental problems. The treatment of VVC is crucial, considering all these adverse effects. In addition, if not treated, many different complications may occur, such as pelvic inflammatory disease, pelvic abscess, infertility, menstrual disorders, ectopic pregnancy, and spontaneous abortion (Gonçalves et al., 2016, Tsega & Mekonnen, 2019). VVC is more critical, especially during pregnancy, because VVC has been reported to cause preterm birth, infant mortality, and invasive neonatal candidiasis in pregnant women (Tsega & Mekonnen, 2019).

Antimicrobial agents are critical in reducing the global burden of infectious diseases. Today, however, the effectiveness of antibiotics is decreasing due to the development of resistant pathogenic microorganisms. This resistance to antimicrobial agents severely threatens public health and all kinds of antibiotics. The incidence of antimicrobial resistance, including drugs of last resort used in treating infectious diseases, is increasing worldwide. Therefore, alternative antimicrobial strategies are urgently needed, reassessing the therapeutic use of older drugs such as herbs and plant-based products (Mandal & Mandal, 2011; CDC, 2022).

Throughout history, plants have been used in traditional medicine for therapeutic purposes. It was used to treat infectious diseases even when there was no knowledge about microorganisms. Plant extracts (PE) and essential oils (EO) have many impressive properties, including antiviral, antidiabetic, spasmolytic, and antioxidant activity. It has immunomodulatory, psychotropic, and expectorant effects and cancer-suppressive activities. Considering the unpleasant side effects of synthetic drugs used today, such as nephrotoxicity or ototoxicity, the use of plants with lower toxicity in treating diseases makes it even more attractive (Lang & Buchbauer, 2012; Ibišević et al., 2020). Due to resistance to synthetic antimicrobial agents, we must focus on developing alternative treatment protocols, especially with natural sources such as plants (Nalbantbaşi & Gölcü, 2009; Lang & Buchbauer, 2012; Kola-Mustapha et al., 2021).

This study aimed to determine PE, plant fixed oil (FO), and EOs that have activity similar to or higher than fluconazole and amphotericin B antifungal drugs against the vaginal *Candida albicans* ((C. P. Robin), Berkhout 1923) isolates, an opportunistic pathogen.

MATERIAL and METHOD

Culture

Nineteen vaginal *C. albicans* isolates were obtained from the Istanbul Zeynep Kamil Gynecology and Pediatrics Training and Research Hospital Microbiology Laboratory (Turkey) in 2019. 19 vaginal *C. albicans* isolates and *C. albicans* ATCC 10231 standards were checked on HiCrome™ Candida Differential Agar (M1297A, Himedia, India).

Plant Extracts and Essential Oils

Ninety-nine different PEs, FOs, and EOs were obtained in the markets and online shopping in Turkey. Information about the provided PEs, FOs and EOs is given in Table 1.

Method

Inoculum Preparation

To determine the antifungal activity, firstly, the isolates were resuscitated. For this purpose, 19 vaginal *C. albicans* isolates and *C. albicans* (ATCC 10231) cultures were inoculated into Sabouraud Dextrose Agar plates, then plates incubated at 36 ± 2°C for 18-24 hours. Revived isolates were then adjusted to 0.5 McFarland (1 - 5 x 10^6 cells mL^{-1}) cell density with physiological saline (PS, 0.85% NaCl) using a McFarland densitometer.

Determination of Anticandidal Activity by Agar Well Diffusion Method

The antifungal activity of 99 commercial PE, FO, and EOs against *C. albicans* was evaluated using the agar well diffusion method. Amphotericin B and fluconazole antifungal discs were used as positive controls. Within 15 minutes of the inoculum suspension preparation, the suspension was applied with a sterile cotton swab to the dried surface of the Mueller-Hinton Agar + 2% Glucose + 0.5 µg mL^{-1} Methylene Blue Agar (Himedia, India) plate. Afterwards, within 15 minutes, wells with a diameter of 6 mm were drilled with a cork borer set, and 20 µL of PE, FO, and EOs were added to the wells. Zone diameters were measured after incubation at 36 ± 2°C for 24 hours. The study was carried out in 3 parallels. All data are given as mean (X) ± standard deviation (Sx) using the Minitab 17.0 program.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration

As a result of agar well diffusion, four effective oils were selected. The minimum inhibitory concentration

Table 1. Plant extracts, fixed and essential oils.
Tablo 1. Bitki ekstrakt, sabit ve esansiyel yağlar

No	Ingredients	Type	Produced By	No	Ingredients	Type	Produced By
1	*Urtica sp.*	FO	A	*51*	*Calendula officinalis* flower	FO	T
2	*Apricot kernel*	FO	A	*52*	*Petroselinum crispum* seed	FO	T
3	*Citrus lemon*	EO	A	*53*	*Chamomilla recutita*	FO	T
4	*Vitis vinifera* seed	FO	A	*54*	Black garlic *(Allium sativum)*	FO	T
5	Taurus mint oil (*Mentha pulegium* oil, eucalyptus oil, orange oil)	EO	B	*55*	*Cananga odorata*	EO	U
6	*Aesculus hippocastanum*	FO	L	*56*	*Pinus palustris*	EO	V
7	*Nigella sativa*	FO	C	*57*	Pine tar	FO	J
8	Black garlic oil (*Allium sativum* bulb oil %50 + Sunflower seed oil %50)	FO	M	*58*	*Amygdalus amara*	FO	K
9	*Aesculus hippocastanum*	EO	N	*59*	*Salvia triloba*	EO	K
10	*Carthamus tinctorious*	FO	D	*60*	*Rubus idaeus*	PE	K
11	Pine turpentine	EO	D	*61*	*Aloe vera*	EO	K
12	*Prunus amygdalus dulcis*	FO	D	*62*	*Pimpinella anisum*	FO	K
13	Adiyaman mint (*Mentha pulegium*, orange oil, lemon oil)	EO	O	*63*	*Juniperus communis*	EO	K
14	Anise	FO	F	*64*	*Carthamus tinctorius*	FO	K
15	*Nigella sativa*	FO	E	*65*	*Persea gratissima*	FO	K
16	*Alpinia sp.*	EO	F	*66*	*Calendula officinalis*	EO	K
17	*Daucus carota sativa*	FO	E	*67*	*Citrus bergamia*	EO	K
18	*Cucurbita pepo* seed	FO	E	*68*	*Salvia rosmarinus*	EO	K
19	*Lavandula stoechas*	EO	F	*69*	*Triticum sp.*	FO	K
20	*Prunus armeniaca* kernel	FO	E	*70*	*Juglans sp.*	FO	K
21	*Actinidia deliciosa*	FO	F	*71*	*Pinus sylvestris*	EO	K
22	*Sesamum indicum* seed	FO	E	*72*	*Melaleuca alternifolia*	EO	K
23	*Prunus persica* kernel	FO	E	*73*	*Menthe-chn oil*	EO	K
24	*Persea sp.*	FO	P	*74*	*Nigella sativa*	FO	K
25	*Triticum sp.*	FO	P	*75*	*Primula elatior*	EO	K
26	*Juglans sp.*	FO	P	*76*	*Laurus sp.* (semen)	FO	K
27	*Cucurbita pepo* seed	FO	P	*77*	*Laurus sp.*	EO	K
28	*Prunus armeniaca* kernel	FO	P	*78*	*Ocimum basilicum*	EO	K
29	*Sesamum indicum* seed	FO	P	*79*	*Rosa sp.*	FO	K
30	Chili oil (*Capsicum annuum* oleum, *Helianthus annuus* seed oleum)	FO	G	*80*	*Sinapis sp.*	EO	K
31	Jasmine oil (*Jasminum officinale* flower oil, propylene glycol)	EO	G	*81*	*Daucus carotae*	FO	K
32	Patchouli oil (*Pogostemon cablin* leaf oil, *Helianthus annuus* seed oil)	EO	G	*82*	*Cocos nucifera*	EO	K
33	*Sinapsis alba*	FO	R	*83*	*Elaegnus angustifolia*	EO	K
34	*Carthamus tinctorius*	FO	H	*84*	*Centaurium minus*	EO	K
35	*Calendula officinalis* flower	EO	H	*85*	*Lavandula cariensis*	EO	K
36	*Citrus bergamia*	EO	H	*86*	*Eugenia caryophyllata*	EO	K
37	*Ocimum basilicum*	EO	H	*87*	*Lavandula angustifolia*	EO	K
38	*Cucurbita pepo* seed	FO	H	*89*	*Anemone apennina*	EO	K
39	*Lavandula cariensis*	FO	H	*90*	*Eucalyptus sp.*	EO	K
40	*Piper nigrum*	FO	H	*91*	*Olea europea*	FO	K
41	*Prunus armeniaca* kernel	FO	H	*92*	*Cedrus sp.*	EO	K
42	*Citrus lemon*	EO	H	*93*	*Allium cepa*	FO	K
43	*Punica granatum* seed	FO	H	*94*	*Cinnamomum sp.*	EO	K
44	*Momordica chantia* fruit	PE	H	*95*	*Amygdalus dulcus*	FO	K
45	*Petroselinum sativum*	EO	H	*96*	*Vitis vinifera*	FO	K
46	Balsam	EO	H	*97*	*Jasminum sp.*	EO	K
47	*Cedrus libani*	EO	H	*98*	*Cananga oderata*	EO	K
48	Pine turpentine oil (Turpentine, Tocopheryl acetate)	EO	I	*99*	*Lilium candidum*	EO	K
49	*Ricinus communis* seed, tocopheryl acetate)	EO	I	*100*	*Zingiber officinale*	EO	K
50	*Foeniculum vulga re*	FO	S				

was achieved by microdilution as specified in NCCLS M27-A2. 0.5 McFarland cell suspension was diluted 1:1000 with RPMI 1640 medium with 0.165 M MOPS with 0.2% glucose (Himedia, India) and 3% DMSO, and the final cell density was 0.5 - 2.5 x 10^3 cells mL^{-1}. Six different concentrations (4, 2, 1, 0.5, 0.25, and 0.1 µL mL^{-1}) of PE, FO, and EOs were prepared in RPMI 1640 medium. 100 µL of RPMI 1640 medium containing 2x PE, FO, and EOs was added to each well of the U-bottom microplates. Then, 100 µL of the suspension containing 1:1000 diluted cell suspension was added to the wells containing 2x PE, FO, and EOs. The final volume in each well was ensured to be 200 µL. RPMI 1640 medium containing 4 µL mL^{-1} PE, FO, and EO was used as the negative control, and RPMI 1640 medium containing only culture was used as the positive control. Microplates were evaluated at 660 nm in a microplate reader (Thermo Multiscan FC) after 24 hours and 48 hours of incubation at 37°C. The study was carried out in 3 parallels. The first well without growth was determined as the MIC value.

The minimum fungicidal concentration (MFC) value was determined after incubation at the appropriate temperature and time by planting with the drip planting method from the defined MIC value and the subsequent three wells. The MFC MIC^{-1} ratio was used to interpret the activity of the PE, FO, and EOs (Gatsing et al., 2009; Snoussi et al., 2018; Mseddi et al., 2020).

Determination of Essential Oil Volatile Component Composition

GS-MS determined the volatile components of four oils. Volatile compounds were analyzed using a gas chromatograph 7890 A connected to an MSD 5975 C (Agilent Technologies) series mass spectrometer. CP WAX 52 was determined using a CB capillary column (50 m x 0.25 mm ID, df:0.2 µm). The carrier gas is helium at a 1.2 mL min^{-1} flow rate. The temperature schedule for the GC is 60°C initial temperature, and after 2 minutes, it is increased to 220°C with a temperature increase of 2°C min^{-1}. After reaching 220°C, the temperature was held constant for 20 min. 100 µL of sample is dissolved in 1 ml of hexane, and 1 µL is the injection volume. The injector temperature is 240°C, and the detector temperature is 250°C. The mass spectrometer was operated in electron impact mode at 70 eV. Integrations were made with MSDCHEM software.

RESULTS

Agar Well Diffusion

The data in this study were obtained by sequential application of different techniques to evaluate the anticandidal activities of 99 PE, FO, and EO against 19 vaginal *C. albicans* isolates and *C. albicans* ATCC 10231 standard cultures. The first step is determining whether EOs have antifungal activity against *C. albicans*. For this purpose, the agar well diffusion method was used, and it was determined that 79 PE, FO, and EO did not have anticandidal activity. On the other hand, 20 FOs and EOs were determined to have anticandidal activity. Inhibition zone diameters of PE, FO, and EOs with anticandidal activity are given in Tables 2 and 3.

Tables 2 and 3 show that the inhibition zones of PE, FO, and EOs against the tested isolates vary. It was determined that *P. anisum* FO (62) and *C. bergamia* EO (67) gave inhibition zones of 7 - 10 mm only against the V1c isolate. *L. stoechas* EO (19), *J. officinale* EO (31), and *A. vera* EO (61) did not have antifungal activity against 16 isolates. On the other hand, pine turpentine EO (48), pine tar EO (57), and *E. caryophyllata* EO (86) were determined to have anticandidal activity against all isolates. It was determined that *C. lemon* EO (42) and *Cinnamomum sp.* EO (94) had anticandidal activity against 19 isolates, and *C. bergamia* EO (36) and *O. basilicum* EO (78) had anticandidal activity against 18 isolates. Therefore, *L. stoechas* EO (19), *J. officinale* EO (31), *A. vera* EO (61), *P. anisum* FO (62), and *C. bergamia* EO (67) inhibited the growth of a very limited number of clinical isolates. *C. lemon* EO (3), Taurus mint EO (5), *N. sativa* FO (7), pine turpentine EO (11), *N. sativa* FO (15), *P. nigrum* FO (40), *C. lemon* EO (42), *O. basilicum* EO (78) (excluding V8c isolate), *E. angustifolia* EO (83) and *Lavandula angustifolia* EO (87) yielded a zone diameter of 10 - 20 mm. When these ten EOs were compared with reference antifungal agents, it was determined that they had limited effects on the growth of clinical isolates.

Fluconazole and amphotericin B were used as positive controls. The efficacy of antifungal agents was confirmed by *C. albicans* ATCC 10231. Reference values for clinical *Candida* isolates are given in Table 4. As seen in Tables 2 and 3, it was determined that clinical isolates formed inhibition zones between 15 - 25 mm and 30 - 42 mm against amphotericin B and fluconazole, respectively. In short, it was determined that all tested clinical isolates were susceptible to two antifungal agents. *C. bergamia* EO (36), pine turpentine EO (48), pine tar FO (57), *E. caryophyllata* EO (86), and *Cinnamomum sp.* EO (94) gave a ≥20 mm inhibition zone against tested all isolates. As a result, they formed a zone of inhibition as much as antifungal agents.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC)

In the second step of the study, two FOs and two EOs were selected, which were determined to have high

anticandidal activity by the agar well diffusion method. MIC values of these four selected oils against *C. albicans* isolates were evaluated using the microplate method. The MIC values of the selected two FOs and two EOs are given in Table 5. It was determined that MIC values vary between 0.5 and 2 µL mL^{-1} for pine turpentine EO (48) and *P. nigrum* FO (40). It was determined that the MIC value of *E. caryophyllata* EO (86) was 0.25 µL mL^{-1} for all isolates. The MIC value of pine tar FO was determined to be 0.125 µL mL^{-1} for all isolates.

This study determined the MIC value of the first well without growth. The MFC value was determined after incubation at the appropriate temperature and time by planting with the drip planting method from the well determined as the MIC value and the next three wells. MFC values of the four selected FOs and EOs are given in Table 5. In line with Table 5, it was determined that MFC values vary between 2 µL mL^{-1} for pine turpentine EO (48) and *P. nigrum* FO (40). It was determined that the MFC value of pine turpentine EO (48) and *E. caryophyllata* EO (86) (except for one isolate) was 1 µL mL^{-1} for all *C. albicans* isolates. The MFC value of pine tar FO was determined to be <0.125 µL mL^{-1} for all isolates.

Table 2. Inhibition zone diameters (mm) of PEs, FOs, and EOs against vaginal C. albicans isolates using the agar well diffusion assays.

Tablo 2. Agar kuyusu difüzyon analizi vajinal C. albicans izolatlarına karşı PE'lerin, FO'ların ve EO'ların inhibisyon zon çapları (mm).

No	Isolates									
	V1c	V2b	V3c	V4c	V5c	V6c	V7c	V8c	V9c	V10c
3	6.00 ± 0.01*	12.25 ± 0.62	6.00 ± 0.01	12.67 ± 0.71	11.72 ± 1.56	9.00 ± 0.40	11.28 ± 0.72	12.92 ± 0.59	10.31 ± 0.73	9.30 ± 0.65
5	10.49 ± 0.78	10.85 ± 0.59	9.77 ± 1.18	11.04 ± 0.74	10.87 ± 0.68	10.49 ± 0.77	10.19 ± 0.68	10.35 ± 0.73	9.45 ± 0.69	6.00 ± 0.01
7	10.49 ± 0.33	8.23 ±0.46	7.19 ± 0.22	8.54 ± 0.53	8.08 ± 0.96	10.93 ± 0.74	8.78 ± 0.74	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
11	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	8.36 ± 0.40	6.00 ± 0.01	11.57 ± 0.31	6.00 ± 0.01
15	13.82 ± 0.66	11.30 ± 0.50	9.39 ± 0.97	6.00 ± 0.01	6.00 ± 0.01	12.26 ± 1.07	6.00 ± 0.01	9.63 ± 0.96	12.22 ± 1.61	9.85 ± 0.83
19	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	8.54 ± 1.02	9.02 ± 0.73	7.93 ± 0.84	6.00 ± 0.01	6.00 ± 0.01
31	6.00 ± 0.01	7.43 ± 0.35	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	7.81 ± 1.40	6.00 ± 0.01	8.73 ± 0.46	6.00 ± 0.01
36	17.69 ± 0.65	18.07 ± 1.78	11.77 ± 0.74	19.65 ±2.28	6.00 ± 0.01	21.24 ± 1.99	13.84 ± 0.92	20.36 ± 2.26	9.34 ± 1.63	12.28 ± 1.29
40	10.02 ± 0.38	11.39 ± 1.75	6.00 ± 0.01	6.00 ± 0.01	8.78 ± 0.76	8.57 ± 0.74	11.86 ± 1.34	10.60 ± 1.07	7.91 ± 0.98	6.00 ± 0.01
42	11.52 ± 1.05	9.94 ± 1.72	10.02 ± 1.66	11.23 ± 0.78	10.72 ± 0.83	11.02 ± 1.07	16.33 ± 2.71	17.57 ± 0.64	14.08 ± 0.91	12.86 ± 1.29
48	26.88 ± 0.70	19.54 ± 0.91	18.14 ± 1.69	22.70 ± 2.44	23.47 ± 0.66	29.01 ± 1.99	35.36 ± 5.15	34.75 ± 2.66	26.02 ± 2.74	16.55 ± 0.84
57	20.97 ± 0.94	15.92 ± 1.60	15.64 ± 1.77	17.27 ± 2.47	14.98 ± 1.13	16.30 ± 3.33	19.45 ± 0.65	25.11 ± 1.26	18.32 ± 0.53	9.70 ± 0.95
61	7.94 ± 0.67	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	9.61 ± 0.75	6.00 ± 0.01	16.99 ± 0.62	6.00 ± 0.01	10.12 ± 1.29
62	7.50 ± 0.77	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
67	9.11 ± 1.86	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
78	11.19 ± 1.12	12.78 ± 2.04	11.25 ± 1.02	9.58 ± 1.96	9.84 ± 0.89	6.00 ± 0.01	9.44 ± 0.77	23.21 ± 1.14	14.23 ± 1.92	9.75 ± 0.25
83	10.10 ± 1.50	8.46 ± 0.45	6.00 ± 0.01	7.02 ± 0.87	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	8.18 ± 0.77	6.00 ± 0.01
86	30.68 ± 4.92	24.25 ± 3.40	23.00 ± 1.67	19.78 ± 3.23	21.09 ± 3.59	21.08 ± 1.38	18.32 ± 0.99	27.38 ± 2.02	17.55 ± 2.26	25.08 ± 3.64
87	10.77 ± 1.01	10.24 ±1.20	9.56 ± 1.06	6.00 ± 0.01	9.43 ± 1.28	6.00 ± 0.01	9.27 ± 1.05	8.39 ± 0.69	6.00 ± 0.01	10.36 ± 1.40
94	30.11 ± 1.36	24.00 ± 2.07	15.16 ± 2.19	17.84 ± 1.02	24.18 ± 2.25	21.51 ± 1.89	31.89 ± 1.96	25.98 ± 1.51	20.09 ± 1.32	16.71 ± 0.80
*FLU***	38.70 ± 1.95	35.59 ± 0.15	41.22 ± 0.22	35.20 ± 0.37	34.09 ± 1.80	39.60 ± 2.54	37.15 ± 1.16	26.23 ± 0.56	34.42 ± 0.46	31.74 ± 1.26
*AMP****	20.33 ± 0.43	19.35 ±0.63	22.41 ± 0.12	21.39 ± 0.65	21.98 ± 0.86	21.61 ± 0.91	20.32 ± 0.45	19.61 ± 0.33	18.06 ± 0.25	15.10 ± 0.01

*Results are given as X + Sx **FLU: Flucanozole (25 mcg) (SD232-5CT, Himedia, India), ***AMP: Amphotericin B (20 IU, 21.19 mcg, Bioanalyse, Turkey)

Table 3. Inhibition zone diameters (mm) of PEs, FOs, and EOs against vaginal C. albicans isolates using the agar well diffusion assays.

Tablo 3. Agar kuyusu difüzyon analizi vajinal C. albicans izolatlarına karşı PE'lerin, FO'ların ve EO'ların inhibisyon zon çapları (mm).

No	Isolates									
	V12c	V13c	V14b	V15c	V16b	V17c	V18c	V19c	V20c	10231
3	6.00 ± 0.01*	9.42 ± 0.29	10.33 ± 0.48	10.11 ± 1.22	11.15 ± 2.05	9.67 ± 0.43	6.00 ± 0.01	8.75 ± 0.43	8.67 ± 0.78	6.00 ± 0.01
5	6.00 ± 0.01	9.81 ± 0.68	10.44 ± 1.17	11.18 ± 2.22	10.18 ± 0.53	10.79 ± 1.11	9.86 ± 0.42	6.00 ± 0.01	8.84 ± 0.65	6.00 ± 0.01
7	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	9.84 ± 0.88
11	10.65 ± 0.92	10.69 ± 0.49	9.33 ± 0.71	6.00 ± 0.01	6.00 ± 0.01	9.01 ± 0.54	9.18 ± 0.45	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
15	6.00 ± 0.01	9.68 ± 0.77	10.62 ± 0.50	8.75 ± 0.59	10.93 ± 0.68	8.62 ± 0.11	9.48 ± 0.31	6.00 ± 0.01	10.38 ± 0.45	11.72 ± 0.99
19	7.23 ± 1.05	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
31	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
36	18.98 ± 2.88	10.93 ± 0.93	12.64 ± 2.55	16.87 ± 3.77	19.17 ± 3.42	11.91 ± 0.46	6.00 ± 0.01	13.57 ± 1.08	9.93 ± 0.43	18.03 ± 2.97
40	12.81 ± 0.71	7.56 ± 1.23	6.00 ± 0.01	6.00 ± 0.01	11.09 ± 0.81	9.86 ± 0.59	9.97 ± 0.20	8.02 ± 0.30	10.17 ± 0.63	9.17 ± 0.64
42	18.91 ± 0.95	17.92 ± 4.04	14.70 ± 0.78	19.91 ± 6.22	15.32 ± 0.58	12.81 ± 0.42	15.80 ± 0.72	13.14 ± 0.80	6.00 ± 0.01	11.66 ± 1.08
48	38.49 ± 2.96	34.23 ± 4.31	36.23 ± 2.58	38.23 ± 2.60	38.60 ± 2.22	33.27 ± 0.96	34.16 ± 2.12	16.78 ± 2.45	27.55 ± 0.82	18.79 ± 1.44
57	21.61 ± 1.93	16.88 ± 1.45	19.81 ± 1.65	21.53 ± 0.41	19.98 ± 2.91	16.06 ± 1.57	20.90 ± 1.74	15.30 ± 0.18	23.06 ± 0.91	9.61 ± 1.65
61	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	9.48 ± 0.49	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
62	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
67	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
78	15.68 ± 1.23	16.19 ± 0.97	11.84 ± 0.61	14.60 ± 0.89	16.13 ± 1.09	12.52 ± 0.78	11.14 ± 0.60	10.48 ± 1.47	6.00 ± 0.01	11.94 ± 1.06
83	11.35 ± 1.37	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
86	23.37 ± 1.85	23.15 ± 3.86	16.99 ± 0.59	18.30 ± 0.70	18.38 ± 1.29	15.43 ± 0.83	18.19 ± 1.51	18.27 ± 1.28	19.66 ± 0.38	13.83 ± 1.71
87	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	12.62 ± 1.99
94	32.98 ±1.96	22.41 ± 3.24	21.08 ± 2.34	15.08 ± 1.13	18.00 ± 0.67	13.47 ± 0.93	15.44 ± 1.37	13.82 ± 0.87	6.00 ± 0.01	14.33 ± 1.33
*FLU***	30.03 ± 0.91	42.03 ± 0.69	41.88 ± 0.58	26.65 ± 0.90	39.79 ± 1.21	42.17 ± 1.92	33.67 ± 0.28	38.39 ± 0.76	35.30 ± 0.77	26.83 ± 1.16
*AMP***	22.12 ± 0.56	24.87 ± 1.06	23.73 ± 0.30	22.93 ± 0.39	22.89 ± 0.45	23.47 ± 0.53	22.48 ± 0.25	22.80 ± 0.38	19.72 ± 0.28	16.54 ± 0.26

*Results are given as X + Sx. **FLU: Flucanozole (25 mcg) (SD232-5CT, Himedia, India), ***AMP: Amphotericin B (20 IU, 21.19 mcg, Bioanalyse, Turkey)

Table 4. Inhibition zones in mm against clinical isolates and evaluation of reference antifungal agents

Tablo 4. Klinik izolatlara karşı mm cinsinden inhibisyon bölgeleri ve referans antifungal ajanların değerlendirilmesi

Antifungal agents	*Resistant*	*Susceptible - Dose dependent*	*Sensitive*
Fluconazole 25mcg	14	15-18	19
Amphotericin B 10 mcg	9	10-14	15

The MFC MIC^{-1} ratio used to interpret the activity of the essential oil was determined and given in Table 5. The MFC MIC^{-1} ratio is considered fungistatic when the MFC MIC^{-1} ratio is >4, and fungicidal agents when the MFC MIC^{-1} ratio is ≤4 (Gatsing et al. 2009; Snoussi et al. 2018; Mseddi et al. 2020). Therefore, as can be seen in Table 5, it was determined that four selected essential oils were fungicidal agents.

Volatile Composition of *Piper nigrum* FO (40), Pine Turpentine EO (48), Pine Tar FO (57), and *Eugenia caryophyllata* EO (86)

Volatile composition of *P. nigrum* FO (40), pine turpentine EO (48), pine tar FO (57), and *E.*

caryophyllata EO (86) were identified by GC-MS. Volatile compositions are given in Table 6. *P. nigrum* FO (40) was composed of ten main compounds, and these compounds are 38.376% dehydroabietic acid, 10.152% trans beta-caryophyllene, 9.615% delta-3-carene, 6.772% benzenemethanol, 5.836% limonene, 4.289% beta pinene, 3,578% alpha pinene, 2.9399% propylene glycol, 2.796% delta-elemene and 2.069% 1,2,3,-propanetriol, triacetate. Pine turpentine EO (48) was determined that it consisted of 5 main compounds: 73.261% alpha pinene, 12.111% beta pinene, 3.338% limonene, 3.152% camphene and 3.123% delta-3-carene. Volatile component analysis of pine tar FO (57); the first four main compounds were determined as 9.915% limonene, 7.65% alpha pinene, 5.365% delta-3-carene and 4.269% alpha-terpineol. *E. caryophyllata* EO (86) consists of 3 main compounds: 91.01% eugenol, 5.720% caryophyllene, and 1.814% propylene glycol. These main compounds are responsible for possible anticandidal activity in all EOs and FOs.

Table 5. MIC, MFC value, and MFC MIC-1 ratio (µL mL-1) of four selected FO and EO.
Tablo 5. Seçilen dört FO ve EO'nun MIC, MFC değeri ve MFC MIC-1 oranı (µL mL-1).

Isolates	*Piper nigrum* FO (40)			Pine turpentine EO (48)			Pine tar FO (57)			*Eugenia caryophyllata* EO (86)		
	MIC	MFC	MFC MIC^{-1}	MIC	MFC	MFC MIC^{-1}	MIC	MFC	MFC MIC^{-1}	MIC	MFC	MFC MIC^{-1}
V1c	1	2	2	2	2	1	<0.125	<0.125	<0.125	0.5	1	2
V2b	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V3c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V4c	1	2	2	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V5c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V6c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V7c	2	2	1	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V8c	1	2	2	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V9c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V10c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V12b	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V13c	1	2	2	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V14b	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V15c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V16b	2	2	1	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V17c	1	2	2	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V18c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V19c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V20c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
10231	2	2	1	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2

Table 6. Volatile component composition of P. nigrum FO (40), pine turpentine EO (48), pine tar FO (57), and E. caryophyllata EO (86)
Tablo 6. P. nigrum FO (40), çam terebentin EO (48), çam katranı FO (57) ve E. caryophyllata EO'nun (86) uçucu bileşen bileşimi

Compounds	*Piper nigrum FO (40)*		*Pine turpentine EO (48)*		*Pine tar FO (57)*		*Eugenia caryophyllata EO (86)*	
	RT	*Abundance %*	*RT*	*Abundance %*	*RT*	*Abundance %*	*RT*	*Abundance %*
(4aR, 6R, 8aR)-4-acetyl-1,1,3,6-tetramethyl-4a,5,6,7,8,8a-hexahydro-1H-2-benzopyran	-	-	-	-	92.992	0.769	-	-
1,1'-biphenyl, bis(1-methylethyl)-	-	-	-	-	84.819 86.947	2.143 0.414	-	-
1,2,3,-Propanetriol, triacetate	56.221	2.069	-	-	-	-	-	-
1,3,8-p-Menthatiene	-	-	-	-	14.450	0.038	-	-
1,3-Cyclohexadinene, 1,3,5,5-tetramethyl-$$ 1,3,5,5-tetramethy-1,3-cyclohexadiene	-	-	30.227	0.296	9.237	1.866	-	-
1,3-Dioxolane, 4-methy-2-phenyl	44.381	0.189	-	-	-	-	-	-
1,3-Pentadiene, 1,1-diphenyl- (Z)-	84.597	1.924	-	-	-	-	-	-
1,4.a.beta.-dimethyl-7-isopropyl-1,2,3,4,4a,9,10,10a.alpha.-octahdrophenanthrene	-	-	-	-	70.234	2.351	-	-
1,4a.beta-dimethyl-7-isopropyl-2,3,4,4a,9,10-hecahydrophenanthrene	-	-	-	-	72.193	1.866	-	-

-1,7-exo-trimethylenebicyclo[3.2.1]octane	-	-	48.076	0.021	-	-	-	-
1-[2-(trimethylsilyl)ethynyl]-3-phenylcyclohexene	-	-	-	-	73.751 79.166	0.467 0.511	-	-
10,11-deihydrobenzo[k]fluoranthene	-	-	-	-	85.02	1.046	-	-
1-Methoxy-4-(1'-methylethyl)cyclohexa-1,4-diene	-	-	-	-	50.75	0.400	-	-
1-tert-butyl-1-(naphtyl-1)-1-silacyclobutane	-	-	-	-	71.971	0.345	-	-
2-(2'-thienyl)-6-phenylpropenenitrile	72.442	1.360	-	-	-	-	-	-
2-(3-isopropyl-4-methoxyphenyl)-6-methylpyridazin-3(2H)-one	-	-	-	-	66.322 64.575	0.424 0.226	-	-
2,3-dimethyl-2-cyclopenten-1-one	-	-	-	-	28.039	0.180	-	-
2,3-Xylenol	-	-	-	-	56.44	0.377	-	-
2,4,6-octatriene, 2,6-d,methyl-	-	-	-	-	20.237	0.808	-	-
2,5-bis(dimethylchlorosilyl)furan	-	-	-	-	88.657	0.513	-	-
2,5-Dimethyl-4-(2,5-dimethylphenyl)pyridine	-	-	-	-	95.253	0.763	-	-
2-acetylfuran	-	-	-	-	26.287	0.094	-	-
2-butanone, 3,3-dimethyl-	-	-	-	-	27.684	0.095	-	-
2-Cyclopenten-1-one, 3,4-dimethyl-	-	-	-	-	24.829	0.025	-	-
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy	-	-	-	-	47.279	0.175	-	-
2-cyclopenten-1-one, 3-methyl	-	-	-	-	26.919	0.121	-	-
2-Pyridineacetonitrile, .alpha.-(4-methoxyphenyl)-	-	-	-	-	83.175	0.251	-	-
2-trimethylsiloxymethyl-4-trimethylsiloxy-1-penten-3-yne	-	-	-	-	65.183	0.337	-	-
3,5-dimethyl-cyclopentenolone	-	-	-	-	42.149	0.140	-	-
3,6-nonadien-1,9-dicarboxylic acid, 5,5-dimethyl-, dimethylester	-	-	-	-	59.709	0.320	-	-
3-ethyl-2-methyl-2H-naphtho[2,3-b]pyran-5,10-dione	-	-	-	-	74.645	0.487	-	-
4,14-dimethyl[2.2]metacyclophane	68.507	0.939	-	-	-	-	-	-
4b,8-dimethyl-2-isopropylphenanthrene, 4b,5,6,7,8,8a,9,10-octohydro-	-	-	-	-	72.62	3.765	-	-
4-Terpineol	-	-	31.498	0.098	31.555	1.112	-	-
5,8-dihydroxy-3-methyl-1,2-dihydro-9,10-antraquinone	-	-	-	-	71.088	0.873	-	-
5.beta.-Podocarpa-8,11,13-tiren-16-oic acid, methyl ester	-	-	-	-	88.947	0.480	-	-
5-acetyl-4,7-dimethoxy-6-hydroxybenzofuran	-	-	-	-	93.641	0.498	-	-
7H-Cyclohepta[b]maohtalen-7-one, 8,9-dihydro-9,9-dimethyl	74.364	0.520	-	-	-	-	-	-
7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	-	-	-	-	10.516	0.072	-	-
7-phenyl-3,3,5-trimethyl-1,2-dihydro-3H-pyrazolo/3,4-d/pyridazin-4(5H)-one	-	-	-	-	65.748	0.691	-	-
Acetic acid	-	-	-	-	23.962	0.258	-	-
Alloocimene	-	-	-	-	6.846	1.041	-	-
Alpha pinene	6.587	3.578	6.766	73.261	6.641	7.650	-	-
Alpha terpinene	-	-	-	-	10.618	0.866	-	-
Alpha terpineol	-	-	36.757	0.231	38.862	4.269	-	-
Alpha terpinolene	-	-	14.667	0.104	14.725	2.621	-	-
Alpha-copaene	-	-	23.434	0.023	25.305	0.028	-	-
Alpha-Cubebene	23.422	0.083	-	-	-	-	-	-
Alpha-Humulene	34.889	0.614	-	-	-	-	34.894	0.462
Alpha-iso-methyl ionone	-	-	-	-	-	-	-	-
Alpha-Longipinene	-	-	-	-	-	-	-	-
Alpha-Muurolene	27.304	0.026	-	-	38.025	0.213	-	-
Alpha-pinene oxide	-	-	19.094	0.203	-	-	-	-
Alpha-Selinene	37.816	0.130	-	-	-	-	-	-
Amorphene	-	-	36.011	0.121	-	-	-	-
Azacyclotridecan-2-one, 1-(3-aminopropyl)	-	-	-	-	76.852	1.115	-	-
Azulene	-	-	-	-	38.772	0.159	-	-
Benzene, 1-(1,1-dimethylethyl)-4-ethyl-	-	-	-	-	20.745	0.047	-	-
Benzene, 1,1'methylenebis[4-methyl-	-	-	-	-	69.351	0.343	-	-
Benzene, 1,3-dimethyl-	-	-	-	-	9.445	1.292	-	-
Benzene, 1-ethenyl-4-methyl	-	-	-	-	18.363	0.398	-	-
Benzene, 1-ethyl-3-methyl	-	-	-	-	13.821	0.033	-	-
Benzene, 1-ethyl-3-methyl-	-	-	-	-	12.332	0.506	-	-
Benzene, 1-ethyl-4-(1-methylethyl)	-	-	-	-	17.132	0.199	-	-
Benzene, 1-methyl-2-(1-methyl-2-propenyl)-	-	-	-	-	24.344	0.150	-	-
Benzene, 1-methyl-3-(1-methylethyl)-	-	-	-	-	14.186	2.110	-	-
Benzene, 1-methyl-4-(1-methylethyl)-	-	-	-	-	25.163	0.045	-	-
Benzene, 1-metyl—2-(1-methyl-2-propenyl)-	-	-	-	-	22.463	0.785	-	-
Benzene, 4-ethyl-1,2-dimethyl-	-	-	-	-	16.701	0.193	-	-
Benzene, diethylmethy	-	-	-	-	20.989	0.086	-	-
Benzene,1,2,3-trimethyl	-	-	-	-	17.29	0.673	-	-
Benzenemethanol	46.351	6.772	-	-	-	-	-	-
Beta ocimene	-	-	-	-	19.11	2.105	-	-
Beta pinene	8.447	4.289	8.519	12.111	8.485	0.899	-	-
Beta terpineol	-	-	-	-	33.159	0.366	-	-
Beta-Bisabolene	38.172	0.067	-	-	-	-	-	-
Beta-citronellol	-	-	-	-	-	-	-	-

Beta-elemene	30.704	0.334	-	-	-	-	-	-
Beta-phellandrene	-	-	-	-	11.645	0.430	-	-
Beta-Selinene	37.519	0.336	-	-	-	-	-	-
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	-	-	-	-	10.128	0.133	-	-
Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-	-	-	26.612	0.069	-	-	-	-
Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	-	-	-	-	24.548	0.026	-	-
Borneol	-	-	36.903	0.349	36.97	2.059	-	-
Bornyl acetate	-	-	-	-	30.198	0.345	-	-
Butane 2,2-dimethyl-	12.667	0.068	-	-	-	-	-	-
Cadinene	-	-	39.738	0.146	-	-	-	-
Calarene	-	-	29.707	0.038	-	-	-	-
Camphene	-	-	7.5 21.763	3.152 0.013	7.51	1.382	-	-
Camphor	-	-	-	-	26.618	0.616	-	-
Carene	-	-	-	-	12.5954	0.106	-	-
Caryophyllene	-	-	-	-	-	-	30.98	5.720
Caryophyllene oxide	51.039	0.423	-	-	-	-	51.036	0.580
Copaene	-	-	25.27	0.081	-	-	-	-
Corylon	-	-	-	-	43.986	0.576	-	-
Creosol	-	-	-	-	50.306	3.670	-	-
Cycloheptane, 4-methylene-1methyl-2-(2-methy-1-propen-1-yl)-1-vinyl-	54.339	0.063	-	-	-	-	-	-
*Cycloheptane, 5-ethyldine*1-methyl-*	-	-	-	-	12.902	0.093	-	-
Cyclohexanecarboxylic acid, 1,3-dimethyl-2-[2-[3-(1-methylethyl)phenyl]ethyl]-, methyl ester, (1.alpha,2.alpha.,3.alpha.)-	-	-	-	-	81.729	0.300	-	-
Cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl	-	-	-	-	57.136	0.204	-	-
Cyclohexene, 5-methyl-3-(1-methylethyl)-, trans	-	-	-	-	13.619	0.098	-	-
Dehydroabietic acid	82.395	38.376	-	-	-	-	-	-
Dehydroabietic aldehyde	78.714	0.309	-	-	-	-	-	-
Dekta-Cadinene	-	-	-	-	39.772	0.536	-	-
DELTA 3-Carene	9.59	9.615	9.613	3.123	9.655	5.364	-	-
Delta-Cadinene	39.726	0.217	-	-	-	-	-	-
Delta-elemene	24.091	2.796	-	-	-	-	-	-
D-Frenchyl alcohol	-	-	-	-	30.561	0.930	-	-
Dimethyl 4-phenyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate	80.487	1.522	-	-	-	-	-	-
Ethanone, 1-(1,2,3,5,6,7-hexahydro-1,1,5,5-tetramethyl-s-indacen-4-yl)-	-	-	-	-	82.406	0.506	-	-
Ethyl benzene	-	-	-	-	9.006	0.101	-	-
Ethylphenol	-	-	-	-	56.65	0.830	-	-
Eugenol	-	-	-	-	60.246 64.259	0.354 0.225	60.279	91.010
Fenchone	-	-	20.216	0.034	-	-	-	-
Fenchene	-	-	-	-	7.320	0.254	-	-
Fenchyl alcohol	-	-	30.505	0.079	-	-	-	-
Furfural	-	-	-	-	24.099	0.274	-	-
Gamma Terpinene	13.052	0.140	-	-	13.103	0.300	-	-
Gamma-gurjunene	-	-	-	-	24.68	0.031	-	-
Gamma-Terpineol	-	-	-	-	62.436	0.166	-	-
Germacrene-d	-	-	30.668	0.025	-	-	-	-
Guaiacol	-	-	-	-	45.48	1.397	-	-
Indan, 2-butyl-5-hexyl	-	-	-	-	90.098	0.324	-	-
Inden	-	-	-	-	24.932	0.055	-	-
Isoborneol	-	-	-	-	-	-	-	-
Isoeugenol	-	-	-	-	68.225	1.191	68.301	0.321
Isolongifolene	-	-	29.355	0.419	-	-	-	-
Izal	-	-	-	-	53.053	0.327	-	-
Junipene	-	-	-	-	29.42	1.379	-	-
Limonene	11.247	5.836	11.279	3.338	11.365 8.16	9.915 0.317	-	-
Linalool	28.543	0.055	-	-	-	-	-	-
Longicyclene	-	-	-	-	25.614	0.190	-	-
Longipinene	-	-	23.917	0.059	-	-	-	-
Methanone,(4-nitrophenyl)(4-methylphenyl)	-	-	-	-	72.882	0.358	-	-
Methyl abietate	81.695	0.519	-	-	-	-	-	-
Muurolene	-	-	37.973	0.053	-	-	-	-
Myrtenal	-	-	32.763	0.090	-	-	-	-
Myrtenol	-	-	41.794	0.270	-	-	-	-
Naphtalene, 1-methyl-7-(1-methylethyl)	-	-	-	-	58.224	0.296	-	-
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	-	-	-	-	36.045	0.370	-	-
Naphthalene, 6-ethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-7-(1-methylethyl)-	-	-	-	-	76.274	0.332	-	-
Norbornane, 2-isopropylidene	-	-	-	-	8.013	0.951	-	-
o-Cymene	14.122	0.565	-	-	-	-	-	-
p-Cresol	-	-	-	-	57.034	0.334	-	-

p-Cymene	-	-	14.131	0.298	14.055	0.434	-	-
p-Cymene-8-ol	-	-	44.884	0.086	-	-	-	-
p-Ethylguaiacol	-	-	-	-	53.882	2.507	-	-
Phellandrene	10.077	0.221	-	-	-	-	-	-
Phenanthrene, 3,6-dimethy	76.069	0.847	-	-	-	-	-	-
Phenol, 2,6-dimethyl-	-	-	-	-	48.177	0.153	-	-
Phenol, 2-ethyl-4,5-dimethyl-	-	-	44.656	0.023	-	-	-	-
Phenol, 2-ethyl-5-methyl	-	-	-	-	60.499	0.162	-	-
Phenol, 3,5-dimethyl-	-	-	-	-	60.922	0.537	-	-
Phenol, 3-ethyl-5-methyl	-	-	-	-	46.17	0.165	-	-
Phenyl 3-(2-methylthio)-thienyl sulfide	63.191	1.130	-	-	-	-	-	-
Pinocarveol	-	-	34.355	0.303	-	-	-	-
Podocarp-8-en-15-oic acid, 13.beta.-methyl-13-vinyl-, methyl ester	-	-	-	-	83.381	0.754	-	-
Prehnitol	-	-	-	-	22.35	0.326	-	-
Propylene glycol	31.232	2.939	-	-	-	-	31.224	1.814
Propylguaiacol	-	-	-	-	57.574	0.877	-	-
Sabinene	11.594 8.76	0.443 0.358	11.614	0.433	-	-	-	-
Sclaren	-	-	-	-	84.462	0.521	-	-
Selin-4,7(11)-diene	33.326	0.058	-	-	-	-	-	-
Simonellite	-	-	-	-	79.832	0.762	-	-
Trans Beta Caryophyllene	31.048	10.152	30.970	0.090	-	-	-	-
Trans-(+)-carveol	-	-	44.095	0.044	-	-	-	-
Trans-2-caren-4-ol	-	-	37.768	0.028	-	-	-	-
Trans-Caryophyllene	33.584	0.032	-	-	31.032	0.695	-	-
Trans-Isolimonene	-	-	-	-	13.00	0.055	-	-
Verbenol	-	-	35.683	0.820	-	-	-	-
Ylangene	-	-	41.567	0.022	-	-	-	-
Z-Citral	-	-	26.882	0.027	-	-	-	-

RT: Retentime time (min). -: compounds not detected.

DISCUSSION

Antibiotics saved countless lives around the world with the discovery of penicillin by Sir Alexander Fleming in 1928. However, along with life-saving antibiotics, life-threatening antibiotic resistance has also emerged. Therefore, the current study will offer a clue for plant-based antimicrobial discovery against the global threat of antimicrobial resistance. (Asmerom et al., 2020).

Recently, the rate of systemic fungal infections caused by *Candida* species has increased due to antifungal resistance, especially in immunocompromised hosts (AIDS) and haematological and cancer diseases. Topical and systemic antifungal agents, which are cytotoxic and fungistatic, are used in the treatment of fungal infections. Thus, the antifungal drugs used by the patient to regain his health cause more side effects. Moreover, there is information in the literature about which plant is used for which disease in traditional medicine. For this reason, there is an increasing trend towards herbs and herbal medicines as viable alternatives to synthetic antifungal drugs (Mansourian et al., 2014). Therefore, this study evaluated the anticandidal activity of commercially available plant extracts, fixed and essential oils against *C. albicans* vaginal isolates.

Clove *(Syzygium aromaticum* (L.) Merril. & Perry, syn. *Eugenia aromaticum* or *E. caryophyllata*), a member of the Myrtaceae family, is one of the oldest and most valuable spices of the Orient. It is used in medicine, especially because of its antiseptic and antimicrobial properties. It has also been determined to have antiparasitic, antiviral, antioxidant, antimutagenic, anti-inflammatory and antithrombotic properties. It is also used orally for asthma and various allergic disorders. Sesquiterpenes found in cloves have also been investigated as potential anti-carcinogenic substances (Ranasinghe et al., 2002; Pinto et al., 2009; Rana et al., 2011; Mansourian et al., 2014; Mittal et al., 2014). Clove oil contains high levels of eugenol, and it has been determined that this compound has antifungal activity (Ranasinghe et al., 2002; Mansourian et al., 2014). Various studies have examined clove oils' antifungal activity and volatile component composition (Ranasinghe et al., 2002; Pinto et al., 2009; Mansourian et al., 2014; Mittal et al., 2014). Mansourian et al. (2014) evaluated the antifungal activity of *S. aromaticum* methanol extract against 21 oral *C. albicans* isolates and *C. albicans* ATCC 10231 standard culture isolated from the mouth of patients with prosthetic stomatitis who were referred to the Faculty of Dentistry Tehran University, by agar well diffusion method. They used nystatin as a positive control and methanol as a negative control. They found that the zone of inhibition was 29.62 ± 1.28 mm for the clinical isolate, 29.67 ± 0.58 mm for the standard culture, and 28.48 ± 1.17 and 28.67 ± 0.58 mm for the positive control, respectively. In this study, it was determined that it gave an inhibition zone between 16.99 and 30.68 mm for clinical isolates and 13.83 mm for standard culture. Pinto et al. (2009) were investigated the composition and antifungal activity of EO obtained from *S. aromaticum*. They determined that EO contained 85.3% eugenol by GC-MS. They evaluated antifungal activity against four standard cultures (*C. albicans* ATCC 10231, *C. krusei* ATCC 6258, *C.*

parapsilosis ATCC 90018, and *C. tropicalis* ATCC 13803) and five clinical isolates (*C. albicans* D1, *C. albicans* D5, *C. glabrata* D10R, *C. krusei* D39, and *C. tropicalis* D42). They determined that the MIC value was 64 µL mL^{-1} (v v^{-1}) against all tested isolates except for *C. parapsilosis* ATCC 90018 (0.32-0.64 µL mL^{-1}). They determined that the MFC value was 0.64 µL mL^{-1} for *C. krusei* ATCC 6258, *C. tropicalis* ATCC 13803, *C. albicans* D5, *C. krusei* D39, and *C. tropicalis* D42, and 0.64-1.25 µL mL^{-1} for other yeasts. Yassin et al. (2020) evaluated the anticandidal activity of *S. aromaticum* ethyl acetate extract against *C. albicans, C. glabrata* and *C. tropicalis* vaginal isolates. They determined that the ethyl acetate extract gave 20.9, 14.9 and 30.7 mm inhibition zones for *C. albicans, C. glabrata* and *C. tropicalis*, respectively, and contained 58.88% eugenol. In this study, it was determined that commercial *E. caryophyllata* EO contains 91.01% eugenol, its MIC value is 0.25 µL mL^{-1}, and its MFC value is 0.5 µL mL^{-1}. This study determined the MFC MIC^{-1} value was 2, and they were accepted as fungicidal agents (Gatsing et al., 2009; Snoussi et al., 2018; Mseddi et al., 2020). MIC and MFC values were lower than in other studies due to the high eugenol content of commercial *E. caryophyllata* EO and the fact that it was an effective compound for antifungal activity. It should be noted that clove oil may have toxic or irritating properties; however, despite these harmful properties, it has a wide range of uses (Hammer et al., 1999; Mansourian et al., 2014). As a result, it was determined that the higher the eugenol, the volatile component of *E. caryophyllata* EO, the higher its antifungal activity. *E. caryophyllata* EO with high eugenol content appear as an important therapeutic alternative to antifungal drugs. These results should be determined and grounded, especially by further in vivo studies.

Pinus sp. species belonging to the Pinaceae family is the most dominant conifer tree in Turkey, and there are five species in Turkey: *P. brutia* Ten., *P. nigra* Arn., *P. sylvestris* L., *P. pinea* L. and *P. halepensis* Mill. The term "turpentine essential oil", which refers to the so-called "terpene oil", "turpentine spirits", "pine terpene", "gum turpentine", "turpentine from Bordeaux" or "pine oleoresin", is obtained by hydrodistillation of gum pine. Due to its pleasant smell, turpentine is used in several industries (pharmaceuticals, perfumes, and other chemicals industries such as household cleaning products, paints, pesticides, rubber, varnishes, etc.). Turpentine is used for the traditional treatment of respiratory system and urinary tract diseases, back pain, and stomach and dermatological conditions (Gülçin et al., 2003; Tümen & Reunanen, 2010). Hassan and Amjid (2009) determined the volatile components of the EO of *P. roxburghaii* stems collected from the Lahore region of Punjab province by GC-MS. They identified 17 components in the EO of *P. roxburghaii* stems. The main component in the EO is α-pinene (41.9%), followed by 3-carene (16.3%), caryophyllene (12.3%), p-cymene (1.9%), terpinenol (1.8%), limonene (1.7%), borneol acetate (1.1%), caryophyllene oxide (1.0%), camphene (0.9%), tepinyl acetate (0.8%), ß-phallenderene (0.7%), farnecene (0.6%), o-cymene (0.4%), butanoic acid (0.3%), 3-methyl-, 2-phenylethyl ester (0.3%), 1-terpinene- 4-ol (0.2%), farnesyle acetate (0.2%) and γ-terpinene (0.2%). They determined that *P. roxburghaii* EO has antifungal activity against *Aspergillus flavus, Aspergillus terreus*, and *Trichoderma viride*. Tümen and Reunanen (2010) identified the components obtained by hydrodistillation of turpentine oil from oleoresin samples collected from *P. sylvestris* L. trees in three different locations in Denizli (Acıpayam, Çal, and Çamlıbel), Turkey by GC-MS. They identified fifty-four components from turpentine oil, accounting for about 96.2% to 98.2% of the total. They determined that the main components of turpentine oil are α-pinene, ß-pinene, camphene, longifoline, delta-3-carene, limonene, and ß-caryophyllene. Ghaffari et al. (2019) determined the chemical composition of essential oil obtained from different aerial parts of *P. eldarica* from Tabriz, Iran, in June 2018. They determined that the chemical components of EO obtained from the needles of the tree are D-germacrene (18.17%), caryophyllene (15.42%), and γ-terpinene (12.96%), and ß-pinene (10.62%). They determined that the chemical components of EO obtained from the bark are limonene (16.99%), caryophyllene oxide (13.22%), and drimenol (13.2%). Finally, they stated that the chemical components of EO obtained from pollen are α-pinene (25.64%) and limonene (19.94%). In total, 83 components were characterized in EOs using GC-MS analysis; mainly, they found sesquiterpene hydrocarbons in needle EOs and monoterpene hydrocarbons in pollen and bark EOs. They determined that ß-Pinene, ß-myrcene, limonene, and caryophyllene were present in all three plant parts. They determined that the MIC value of *P. eldarica* bark against *C. albicans* of EO was 125 µg mL^{-1}. Kurti et al. (2019) identified 112 compounds in their chemical profile of total and fragmented EOs of *P. heldreichii, P. peuce, P. mugo, P. nigra*, and *P. sylvestris*. In this study, 38 components of pine turpentine EO (48) and 117 components of pine tar FO (57) were identified. The main components of pine turpentine EO (48) were determined to be α-pinene (73.261%), ß-pinene (12.111%), limonene (3.338%), camphene (3.152%), and delta-3-carene (3.123%). The main components of pine tar FO were found to be limonene (9.915%), α-pinene (7.65%), delta-3-carene (5.364%), and α-terpineol (4.269%). Compared to the literature, the main components in the studies are similar, but their ratios and number of compounds

vary. This is due to the difference in species and the differences in the way the EO is obtained. Therefore, different results were obtained in antifungal activity as the chemical composition changed. This study determined that the MIC value of commercial pine turpentine EO (48) varied between 0.5 - 2 µL mL^{-1}, and the MFC value varied between 1 - 2 µL mL^{-1}. MFC MIC^{-1} value was determined as 1-2, and fungicidal agents were accepted when the MFC MIC^{-1} ratio was ≤ 4. In pine tar FO (57), the MIC value was determined to be below <0.125 µL mL^{-1}. Therefore, further studies are needed.

E. angustifolia L., a member of the Elaeagnaceae family, which includes three genera and about 50 species, can grow in different habitats (such as Eurasia, Australia, North America and Malaysia). The leaves of *E. angustifolia* L. are used as tea, animal feed, and wood pulp, while the fruits are consumed fresh or as jam and beverage. Due to their high therapeutic potential bioactive content, its leaves and flowers have been used to treat various diseases such as asthma, flatulence, jaundice, nausea, stomach upsets and vomiting. Medicines were prepared and applied from *E. angustifolia* L. for treating stomach ailments (such as ulcers and stomach pain) in Turkish folk medicine. The fruit has been used as an analgesic agent to reduce pain in rheumatoid arthritis in Iran. It has been reported recently that the fruit and fruit seeds of *E. angustifolia* have muscle relaxant activity and antibacterial, anti-inflammatory, and antinociceptive effects (Incilay, 2014; Hamidpour et al., 2019). Okmen and Türkcan (2014) investigated the antimicrobial activity of methanol extracts of *E. angustifolia* plant samples collected at Muğla Sıtkı Koçman University Campus in July 2012. They reported that did not give an inhibition zone against *C. albicans* RSKK 02029. Incılay (2014) evaluated the antimicrobial activity of *E. angustifolia* flower, fruit, fruit peel, and leaf collected from the Malatya region. It was determined that the extracts from flower and leaf methanol: water (80:20) had the highest antimicrobial activity against Gram-positive bacteria, and their MIC values ranged from 62.5 to 500 µg mL^{-1}. Monjazeb Marvdashti et al. (2023) determined that the MIC and MFC values of *E. angustifolia* L. whole fruit ethanolic extract against *C. albicans* PTCC 5027 were 2 and 4 mg mL^{-1}, respectively. This study determined that *E. angustifolia* EO gave inhibition zones between 7.02 and 11.35 mm. The chemical composition, MIC, and MFC values of this EO were not determined, since the study was carried out, especially with those that gave high inhibition zones.

CONCLUSION

Considering the current and still growing problem of drug resistance, the antimicrobial properties of plant extract, fixed, and essential oils can be considered valuable resources. While an in-depth analysis of the mechanisms of action may be useful in the search for new therapeutic molecules (i.e., the only active ingredient found in the essential oil), synergistic mechanisms between the components of essential oils are known to be necessary. Consequently, as the present work is preliminary, it will lead to the discovery of bioactive compounds that will fuel the future field of plant-based antimicrobial discovery and development. In line with the results of this study, it can be concluded that important therapeutic agents are as effective as alternatives to anticandidal agents. However, safety and toxicity issues will need to be addressed if these plant extracts are used for medicinal purposes. Therefore, further research on the isolation and characterization of bioactive compounds is necessary. All the results should be evaluated together, and the basis for further in vivo studies should be established.

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Author Contributions

GÖA; conceived and designed the experiments, performed the experiments, analyzed data and SK; contributed vaginal candida isolates. GÖA and SK; writing—review, and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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