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The biological activities of anise and fennel essential oils against indoor opportunistic fungi

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ABSTRACT

Aims: This study investigated the antifungal activities of essential oils (EOs) extracted from anise (*Pimpinella anisum*) and fennel (*Foeniculum vulgare*) against indoor opportunistic fungi (*Penicillium frequentans*, *Aspergillus flavus*, *Aspergillus niger* and *Chaetomium globosum*) isolated from an apartment wall surfaces. The antioxidant activity of extracted EOs was examined.
Methodology and results: The components of EOs were identified and quantified using gas chromatography (GC-MS). Antifungal activity, minimum inhibitory concentration (MIC) and killing potential assays of EOs were performed. EOs extracted from anise and fennel showed considerable antifungal activities against the four indoor opportunistic fungi. EO from fennel showed high antifungal activity against *A. niger* and *C. globosum*, while EO from anise showed high antifungal activity against *P. frequentans* and *A. flavus*. EOs from anise and fennel showed the same MIC values (5-8 mg/mL). EOs from anise and fennel showed high killing potential after 72 h against the indoor opportunistic fungi. The highest killing potential was against *P. frequentans* and the lowest killing potential was against *C. globosum*. Moreover, EOs from anise and fennel showed high antioxidant activities with scavenging activity after 90 min of 89.39% and 90.2%, respectively.
Conclusion, significance and impact of study: EOs extracted from anise and fennel could be used as natural antifungal agents against indoor opportunistic fungi.

Keywords: Anise, antimicrobial, antioxidant, essential oils, fennel, indoor opportunistic fungi

INTRODUCTION

Essential oils (EOs) are volatile and complex natural compounds that are mainly composed of terpenes (sesquiterpenes and monoterpenes) and some other non-terpene compounds, such as oxygenated compounds of terpenes, including phenols, oxides, ethers, ketones, aldehydes, esters and sulphur- or nitrogen-containing molecules (Ahmed *et al*., 2019; Shaaban, 2020). In the Middle Ages, Arabs developed a steam or hydro-distillation method to obtain EOs from spices, herbs, and medicinal plants. EOs have broad-spectrum antiseptic activity (fungicidal, bactericidal and virucidal) and medicinal properties, so they have been used as analgesic, anti-inflammatory, antimicrobial, spasmolytic, sedative and local anaesthesia agents (Atif *et al*., 2020). Furthermore, EOs have been widely used in cosmetics as fragrances, as well as pharmaceuticals, food preservation and the food industry as flavoring agents (Hussain *et al*., 2010). Traditionally, spices are used to modify and enhance the color and flavor of different beverages and foods, as well as for their antimicrobial and antioxidant properties.

Fennel, anise, caraway, and coriander are major seed spices that belong to the family Apiaceae (Balbino *et al*., 2021). Seed spices contain abundant active molecules responsible for plants' necessary functions, such as cellular defense mechanisms. These active molecules and their synergetic activities play a major role in the application of spices in phytotherapy and gastronomy. Sayed-Ahmad *et al*. (2017) reported the usage of seed spices from the Apiaceae family in traditional medicine to treat and prevent multiple diseases of the endocrine and digestive systems due to their ability to relieve flatulence and facilitate digestion. In addition, the antimicrobial, hypolipidemic, hepatoprotective, hypoglycaemic and

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anticancer activities of seed spices from the Apiaceae family were reported by many authors (Balbino *et al*., 2021).

Shaaban (2020) reported that the high levels of EOs in members of the Apiaceae family are responsible for their gustatory properties. The composition and content of EOs depend on the species. The antioxidant activity of seed spices from the Apiaceae family is a result of high concentrations of phenolic compounds such as tannins and flavonoids in the seeds. The nutritional and phytoceutical values of seeds from Apiaceae, as well as their applications, are related to the high content of non-volatile fatty oils and other lipophilic compounds, such as sterols and pigments present in the seeds (Pachuau *et al*., 2019).

People spend 80-90% of their time inside houses and indoor environments with daily inhalation of 10 m^3 of air and the presence of microbial air pollutants in these spaces can cause severe diseases. Microbial air pollutants (bioaerosols), including fungi, bacteria, and viruses, contribute to about 5-34% of indoor air pollution (Awad *et al*., 2018). The growth of opportunistic fungi in houses is challenging in the house construction industry. Fungi have mycelia and release spores into the air through reproduction. They can grow on various natural and industrial materials, such as wood, paint, paper, leather, plaster, stones, concrete and fabrics, with a low water content of 12-15%. Some types of fungal genera, such as *Penicillium* and *Aspergillus*, are known to be drought resistant. In addition, fungi can adapt to changes in temperature better than other forms of life (Chunduri, 2014).

Based on isolation and culturing methods, about 100 species of indoor fungi have been described (Flannigan *et al*., 2016). Martin-Sanchez *et al*. (2021) estimated that there are 1000 to more than 7000 indoor fungal species using another technique called internal transcribed spacer (rDNA ITS) barcode sequencing.

Asthma, respiratory illnesses, headaches, hypersensitivity, rhinitis and other nosocomial infections, as well as allergy difficulties, might develop as a result of prolonged exposure to infectious bioaerosols (Hassan *et al.*, 2021).

The development of "sick building syndrome" (SBS) and the biodegradation of natural and synthetic materials are caused by indoor opportunistic fungi. SBS is defined as a medical condition where humans inside a building suffer from different nonspecific symptoms upon inhalation, such as chest pain, headache, and sinusitis, for no apparent reason (Prenafeta-Boldú *et al*., 2022). The aim of the present study was to investigate the antifungal activity of natural EOs extracted from the seeds of two spices belonging to the Apiaceae family: anise (*Pimpinella anisum*) and fennel (*Foeniculum vulgare*). In addition, the antioxidant activity and killing potential of the extracted EOs were studied.

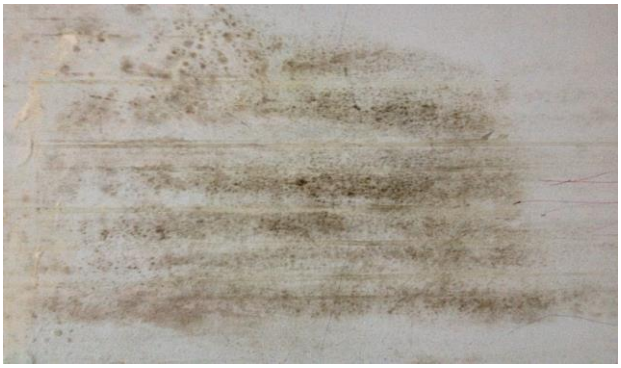

Figure 1: The infected wall with opportunistic fungi.

MATERIALS AND METHODS

Fungal strains

The indoor opportunistic fungi used during this study were isolated from infected walls inside an apartment ("12.42'31°15 N and "44.41'0°30 E) in Alexandria, Egypt, as shown in Figure 1. Fungal isolates were obtained using sterile cotton-tipped swabs and transferred to the laboratory. The swabs were streaked on the surface of Petri dishes containing malt extract-agar (MA) medium. The inoculated Petri dishes were incubated at 28 °C for 7-10 days. After incubation, single fungal isolates were obtained, purified, and kept for further experiments.

Morphological identification of isolated opportunistic fungi

The isolated opportunistic fungi were identified using morphological characteristics such as colony diameter, color of conidia, extracellular exudates, pigmentation, and color of reverse mycelium. The microscopic features of fungal isolates were also examined, including the conidial heads, fruiting bodies, degree of sporulation and homogeneity characteristics of conidiogenous cells. This was done using an optical light microscope (10x90 Olympus CH40) (Barron, 1968; Ainsworth, 1971; Ellis, 1971; 1976; Pitt, 1985; Klich and Pitt, 1992).

Extraction of anise and fennel EOs

Anise (*P. anisum*) and fennel (*F. vulgare)* seeds were used for the extraction of EOs. Seeds were purchased from a local market and extraction was performed using the hydro-distillation method with a Clevenger apparatus. 100 g of anise and fennel seeds were gently crushed separately, mixed with 600 mL of distilled water and then introduced to the Clevenger apparatus, which had previously been prepared for distillation. The distillation was carried out for 3 h and the distillate was transferred to a separation funnel and allowed to settle down for 24 h until two phases of different densities became visible. The upper layer representing EOs was withdrawn and held in tightened vials at 4 °C for further laboratory analysis.

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Chemical identification of extracted EOs

The anise and fennel EOs' components were identified and quantified using gas chromatography (GC-MS; Shimadzu GC-MS-QP2010 Plus). The carrier gas was helium at a flow rate of 13 mL/min to identify the main chemical compounds in the extracted EOs. The mass spectrometer was set to scan 12.00-500.00 *m/z*. Samples were injected with a splitting ratio of 5.0, and the injector temperature was set to 280 °C. The total flow rate was 13.1 mL/min, and the column flow rate was 1.69 mL/min. The column oven temperature was 50 °C and the hold time was 2-5 min. The total run time was approximately 30 min.

Antifungal activity study

To establish growth curves, organisms were transferred aseptically from stock slants into 9 mL broth and incubated at 30 °C. Antifungal activities of the two EOs against isolated opportunistic fungi were determined using the drop diffusion method (Hili *et al*., 1997). A drop (20 µL) of extracted essential oils was placed separately in the center of a Petri dish containing potato-dextrose agar (PDA). Petri dishes were incubated at 30 °C for 48 h. After incubation, the diameter of the inhibition zone was measured in mm. Three replicates of Petri dishes were used for each EO.

Minimum inhibitory concentration (MIC) assay

A stock solution of the extracted EOs was prepared by diluting 0.3 g EOs in a 20 mL dimethylsulfoxide (DMSO) solution. Malt-yeast-glucose-peptone (MYGP) broth was prepared as a culture broth. A stock solution was then added to the culture broth to produce final concentrations of 10-500 µg/mL and then the volume of each concentration was increased to 2.0 mL using MYGP. Next, each concentration was added to a flask containing 50 mL of MYGP with the addition of 200 µL of fungal spore suspension in the same flask.

The samples were incubated for 72 h at 30 °C. After the incubation, the samples were measured using a spectrophotometer at 700 nm. Control samples were incubated under the same conditions. The minimal inhibitory concentration (MIC) was defined as the lowest concentration that resulted in a reduction of >90% in the observed absorbance.

Killing potential assay

The fungal killing potential of extracted EOs was studied according to the methodology described by Alviano *et al*. (2008) with some modifications. Accordingly, 1.2 mL aliquots of each fungal strain culture, grown for 48 h in MYGP broth at 30 °C, were added to 0.8 mL of each EO (40 mg/mL) to obtain a final concentration of 16 mg/mL. After the addition, 1 mL aliquots of each system were collected at 12 h intervals for up to 3 days and added to the same volume of sterile physiological saline solution (0.85% NaCl) to count the viable cells. The killing kinetics curve for each strain with each EO was obtained by comparing the fungal population at the beginning of the experiment (time = 0 min) and that at each 12 h interval after the addition of the EOs.

Antioxidant activity of anise and fennel EOs

The antioxidant activity of EOs extracted from anise and fennel was investigated in terms of free radical scavenging activity using a radical solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The efficiency for scavenging DPPH was quantitatively evaluated by the ability of EOs to reduce and decolorize the purple-colored DPPH solution to yellow (Sanchez-Moreno, 2002). In brief, 100 µL of different serial concentrations (1000 to 10000 ppm) of anise and fennel EOs were mixed separately with an equal volume of prepared DPPH (60 µM in absolute methanol).

The mixtures were vortexed and allowed to stand for 30 min in the dark at 25 °C. The absorbance of the resulting solution was measured at 517 nm (OD517) against a blank (methanol). L-ascorbic acid (0.1%) was used as a reference antioxidant (positive control) and control was set up by mixing 100 µL of distilled water with 100 µL of DPPH. The experiments were performed in triplicate. The scavenging activity against DPPH was calculated as a percentage based on the control reading using the following:

$$\text{Reduction (\%)} = [A_0 - A_1/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

RESULTS AND DISCUSSION

Morphological identification of isolated opportunistic fungi

The purified opportunistic fungal isolates were subjected to morphological and microscopic identification. Four main fungal species were identified: *P. frequentans*, *A. flavus*, *A. niger* and *C. globosum* (Figures 2 and 3). *Penicillium frequentans* showed rapid growth, green color with a yellowish border and a valvate appearance, and the backside of the colony was reddish with a yellowish border on potato-dextrose agar (PDA) medium. On Czapek Dox medium, this species showed rapid growth and white colour with a velvet appearance and the backside of the colony was a greenish cream color (Pitt, 1985).

The conidia spores of *A. flavus* had a thick mycelial mat that could be seen with unaided eyes and had a size of 3 to 6 µm. The conidiophores are rough in texture, coreless and originate from the hyphal threads. The thread-like tiny hyphae occur as septate branches and form the mycelium. There is hyaline in each septate of the branched hyphae. The uniseriate and biseriate phialides originate from the conidiophores (Klich and Pitt, 1992).

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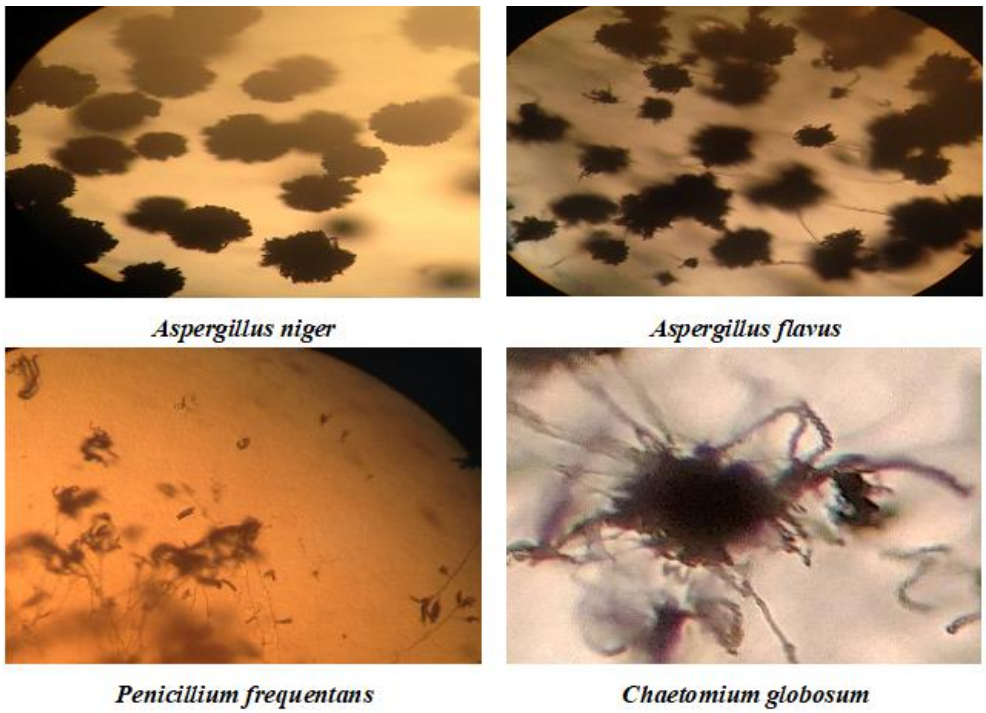

Figure 3: Microscopic appearance of the four opportunistic fungal isolates.

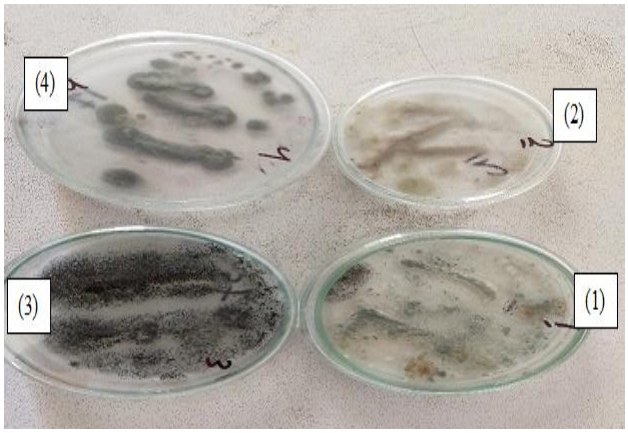

Figure 2: The isolated opportunistic fungi, *P. frequentans* (1), *A. flavus* (2), *A. niger* (3) and *C. globosum* (4).

Aspergillus niger consists of smooth and colorless conidiophores and spores. The conidial heads of the organism are globose and dark brown and have been shown to divide into several columns as it ages. *Aspergillus niger* produces dark or dark brown spores from conidial heads (biseriate) (Klich and Pitt, 1992). *Chaetomium globosum* has a high growth rate with a cottony texture and white color colonies. With the maturation of the colonies, the colony surface color changes to olive, while the color on the backside transforms from brown, red or tan into black. The hyphae are brownish, septate and contain hyaline.

There are ascospores, asci and perithecia as well. The perithecia are huge, flask-shaped, surrounded by a tall spine, brown to black in color and brittle. Furthermore, perithecia have ostioles (rounded apertures) that contain ascospores and asci. The inside of the ascospore is brown, unicellular and clavate to cylindrical (Ellis, 1971; 1976; Prokhorov and Linnik, 2011).

Chemical composition of EOs

In Egypt, fennel and anise have been widely used for their medicinal properties for centuries. In the present study, the constituents of EOs extracted from fennel and anise seeds were chemically identified using GC-MS. The results of GC-MS are represented in Figure 4 for fennel and Figure 5 for anise. The most abundant identified constituents are expressed as an area percentage (%) along with their retention times (RTs). The obtained constituents from GC-MS analysis were identified using the WILEY library, which provides information about various compounds based on their RTs and GC-MS response.

Based on the GC-MS response, the amount of obtained compounds can be calculated as a percentage (%) as reported in the literature (Alam *et al*., 2019). Calculating these compounds' masses using the WILEY library is impossible. Therefore, the amount of identified compounds is presented as a percentage (%) along with their RTs. The representative GC-MS chromatograms of fennel and anise EOs are shown in Figures 4 and 5, respectively.

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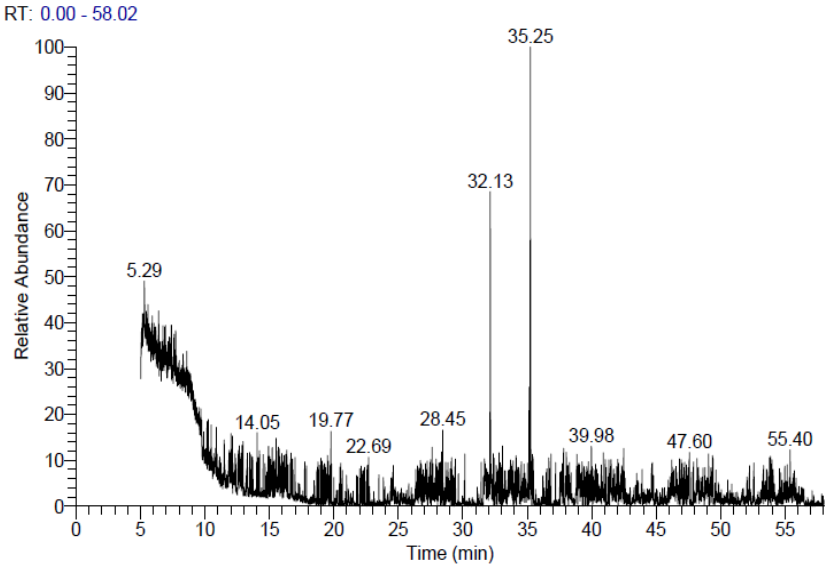

Figure 4: Gas chromatogram of essential oil extracted from fennel (*F. vulgare*) seeds.

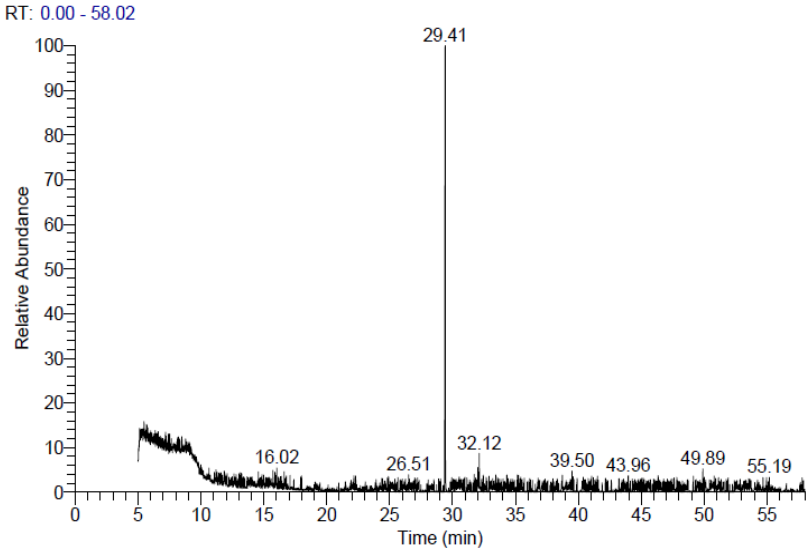

Figure 5: Gas chromatogram of extract of *P. anisum* (anise) seeds.

In total, 47 different constituents were identified in the EO extracted from fennel seeds (Figure 4). The most abundant identified constituents were ethyl 9-octadecanoate (12.70%) and hexadecanoic acid, ethyl ester (8.74%). Some other identified constituents were present in notable amounts, such as tetradecadien-4,9 ol-1 (2.88%), 10,10'-dibromo-9,9'-bianthryl (2.43%), 2-bromo-5-methoxy-N(1),N(4)bis(3'butenyl)N(1),N(4)-bis(phenylsulfonyl)-1,4-phenylenediamine (2.41%), methyl cis-3-(1,1-dichloro-2-propenyl)-2,2-dimethylcyclopropane-1-carboxylate (2.23%) and molybdenum, tetracarbonyl-bis[2-(4-butyl-4-phosphacyclohexyl)ethyl]butylamine (2.13%). The remaining identified constituents showed low to moderate amounts (1.25-1.99%). Previous studies have reported many of the same identified constituents as those in the present study but with different percentages (Alam *et al*., 2019; Abdellaoui *et al*., 2020; Ashokkumar *et al*., 2021). Viuda-Martos *et al*. (2011) and Diao *et al*. (2014) studied EOs extracted from Egyptian fennel and identified 20 constituents, and the major constituents were trans-anethole (65.59%), estragole (13.11%), limonene (8.54%) and fenchone (7.76%).

Similar constituents of fennel EO were reported by Renjie *et al*. (2010), who used supercritical fluid extraction (SFE) and simultaneous distillation-extraction (SDE) methods. The variety of identified constituents in the EO extracted from fennel could be the reason for the multiple

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Table 1: Antifungal activity of anise and fennel extracts against opportunistic fungi using the drop diffusion method.

Essential oil	Inhibition zone (mm)			
	Penicillium frequentans	*Aspergillus flavus*	*Aspergillus niger*	*Chaetomium globosum*
Anise	78.1	67.3	69.8	50.4
Fennel	69.5	65.3	77.1	51.0

Mean diameter of growth inhibition is expressed in mm, standard error within 10%. The maximum plate diameter was 80 mm.

therapeutic activities of fennel seeds (Bahmani *et al*., 2015). The contents of EO as well as the amount of each constituent could be different from those previously reported in the literature. The variation in the contents of EO depends on the environmental, geographical and seasonal conditions (Alam *et al*., 2019). Furthermore, the variation in the amount of each constituent depends on some planting factors, including climate, humidity, temperature, collection time, extraction methods and analysis conditions (Diao *et al*., 2014).

Regarding the chemical composition of EO extracted from anise seeds (Figure 5), a total of 46 constituents were identified using the GC-MS technique, as represented in Figure 5. The most abundant identified constituent of EO extracted from anise was 2-acetoxymethyl-1,4,5,6,7,8,9,10-octahydro-1,4-epoxybenzocyclooctene (36.14%). Furthermore, (2,2-dibenzyloxy-3-nitro-5,10,15,20-tetraphenyl-2,3-dihydroporphyrinato) copper(II), 3,5-diphenyl-3,5-(2-dimethylamino-9,10 phenanthylene) tricyclo[5.2.1.0]decane-4-one-8,9(E)-dicarboxylic acid and ethyl tridecanoate showed notable amounts of 3.90%, 3.20% and 2.90%, respectively. The other constituents showed low to moderate amounts (0.99-1.60%).

As previously mentioned in the case of fennel, the EO constituents of anise showed similarities and differences regarding components and their relative quantities from those reported in the literature (Al-wendawi *et al*., 2021). Anise seeds and fruits were mentioned in some traditional texts for the treatment of epilepsy, seizures, nightmares and melancholies. Furthermore, anise seeds are used in traditional medicine as a disinfectant, analgesic for migraines and diuretic (Sun *et al*., 2019). It has been reported that anethole is the most important constituent of anise EO, which is used in different industries, such as the pharmaceutical, flavoring, perfumery and food industries. Anise EO also contains estragole, methylchavicol, eugenol and anisaldehyde as active ingredients (Cifti *et al*., 2005).

Picon *et al*. (2010) and Sun *et al*. (2019) reported that trans-anethole is the major constituent of anise oil (75-90%), while other constituents include lipids (fatty acids, stigmasterol, betaarmyrin and their salts), coumarins (bergapten, umbelliprenine, scopoletin and umbelliferone), carbohydrates, flavonoids (glycosides, flavonol, rutin, isovitexin flavone and isoorientin) and proteins. The EOs extracted from anise show some beneficial medicinal impacts on the kidneys since aqueous anise extract can have a corrective effect against nephrotoxicity caused by lead (Amina *et al*., 2016). In addition, it was suggested that anise EO could be used in the management of depression and treatment of depressed patients with irritable bowel syndrome (Mosaffa-Jahromi *et al*., 2017) since it has an antidepressant-like activity similar to that of fluoxetine (Shahamat *et al*., 2015).

Table 2: Minimum inhibitory concentration (MIC) of anise and fennel EOs against opportunistic fungi.

Fungi	MIC of EO (mg/mL)	
	Anise	Fennel
Penicillium frequentans	5	6
Aspergillus flavus	7	7
Aspergillus niger	7	7
Chaetomium globosum	8	8

Antifungal activity of EOs

Using the drop diffusion method, the antifungal activity of EOs extracted from anise and fennel was examined against the four isolated opportunistic fungi (Table 1). Anise and fennel EOs exhibited considerable antifungal activity against all tested fungal strains. The EO from fennel was more strongly antifungal than the EO from anise against *A. niger* and *C. globosum*, while the EO from anise was more strongly antifungal against *P. frequentans* and *A. flavus*, as shown in Table 1. In addition, EOs extracted from anise and fennel showed the same MIC values (5-8 mg/mL) for antifungal activity, as shown in Table 2. The drop diffusion method results and MIC measurement indicated the activity of these EOs against the tested opportunistic fungal strains.

In agreement with our results, Felšöciová *et al*. (2015) reported high antifungal activity of anise EO against different species of *Penicillium*. Anise seeds are rich in phytochemical contents with high antimicrobial activity and can be used in pharmaceutical applications and health supplements (Bagdassarian *et al*., 2013). Kosalec *et al*. (2005) reported strong antifungal action of EO extracted from anise in lower concentrations against four dermatophyte species (*Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum gypseum* and *M. canis*), as well as antimycotic activity against five *Candida* species (*Candida albicans*, *C. tropicalis*, *C. Krusei*, *C. pseudotropicalis* and *C. parapsilosis*). They attributed anise EO's antifungal and antimycotic effects to components such as anisaldehyde, *trans*-anethole, estragole and anisketone or synergism.

Different studies reported the antimicrobial activity of EO extracted from fennel, which can be a source of pharmaceutical materials used in the preparation of new

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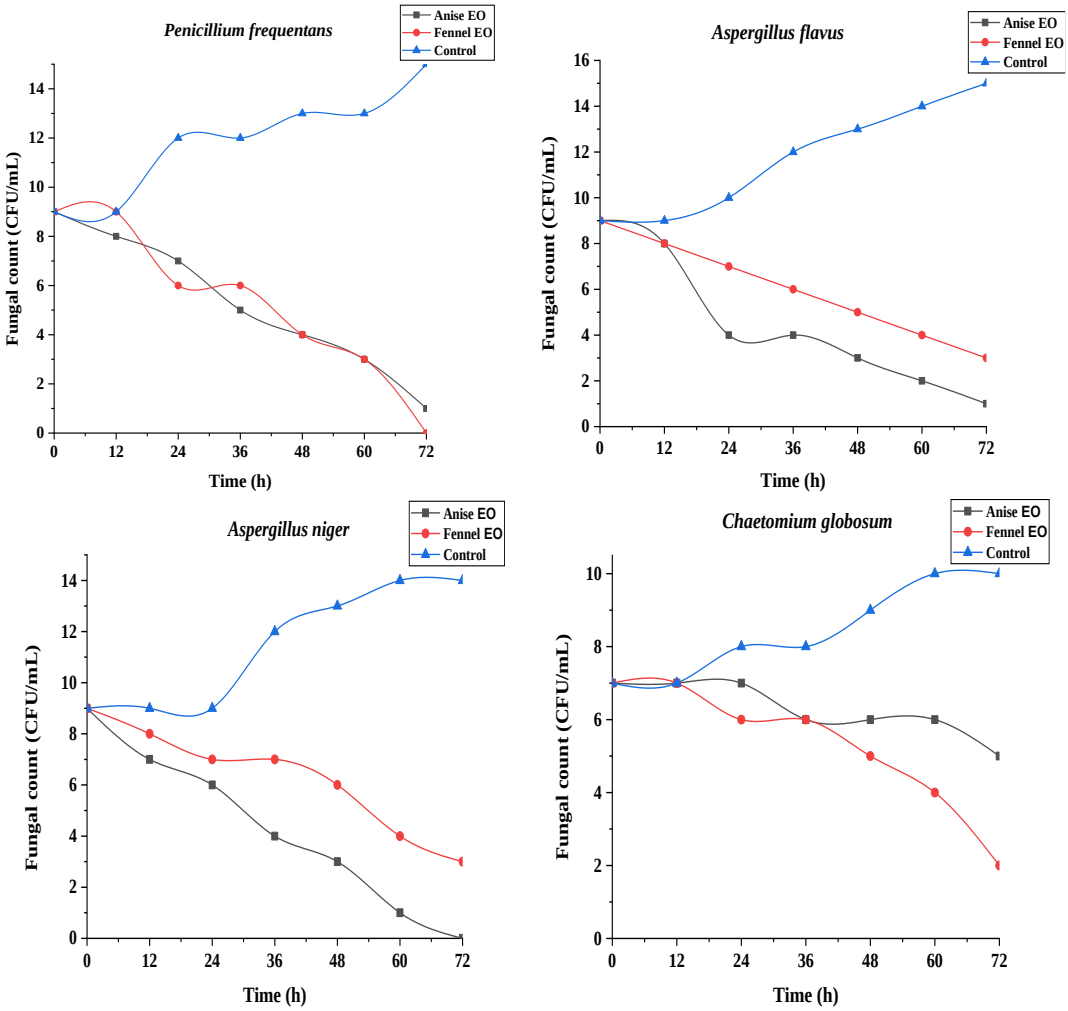

Figure 6: The killing potential of anise and fennel EOs within 72 h against opportunistic fungi.

antimicrobial and therapeutic agents (Roby *et al*., 2013). Furthermore, EOs extracted from fennel have been reported to have antifungal, antitumor and antithrombotic effects (Goswami and Chatterjee, 2014). Chen *et al*. (2020) reported the antifungal activity of EO extracted from fennel against four plant pathogenic fungal strains (*Fusarium fujikuroi*, *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum* and *Phytophthora capsici*). They attributed this effect to the rich content of *trans*-anethole and the high proportion of oxygenated components of fennel EO. EOs extracted from anise (Radaelli *et al*., 2016) and fennel (Roby *et al*., 2013) were suggested to serve as natural preservatives as an alternative to chemical preservatives in the control and inactivation of pathogenic microorganisms in commercially produced foods.

Killing potential assay

The killing potential assay results are represented in Figure 6. The results showed that EOs from anise and fennel have great killing power against *P. frequentans*, *A. flavus* and *A. niger*. The greatest killing effect of EOs was noticed from both anise and fennel against *P. frequentans* since the fungal count reached zero after 72 h. However, a lower killing effect was noticed in the case of *C. globosum.* These results agree with the results reported by Nami *et al*. (2019) and Stan *et al*. (2021).

EOs with different concentrations extracted from natural plants and herbs have shown antifungal activity with different killing mechanisms, such as affecting the membrane/cell walls (Oro *et al*., 2015), inhibiting cell wall formation (Stan *et al.*, 2021) and affecting the membrane ionic permeability (Peixoto *et al*., 2017). Stan *et al*. (2021) reported that there are five main classes of antifungal drugs: allylamines, pyrimidines, azoles, echinocandins and polyenes. Only two of these antifungals, the echinocandins and the polyenes, are derived from natural sources: fungi and bacteria. Particularly, the mode of action targets the ergosterol metabolism process, an essential component of the fungal cell membrane and the fungal analogue of cholesterol. Researchers have

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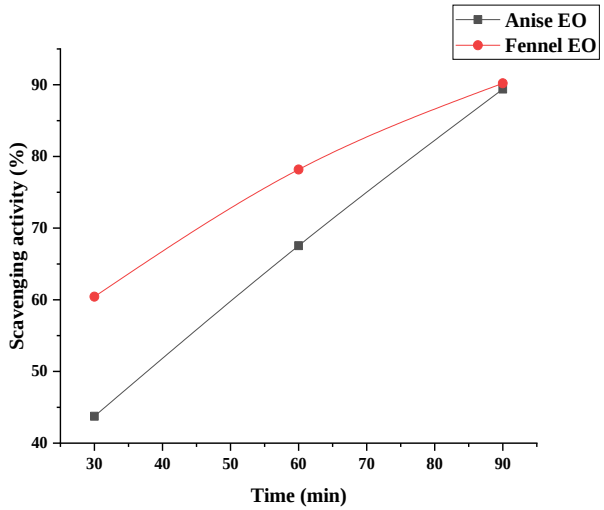

Figure 7: Antioxidant assay by fennel and anise EOs.

attempted multiple attempts to develop new antifungal agents by studying natural products with antifungal activity (Morio *et al*., 2020; Tavakkoli *et al*., 2020). Based on our results, EOs extracted from anise and fennel could be promising natural products with high antifungal activity, especially against opportunistic fungi.

Antioxidant potential of fennel and anise EOs

Roby *et al*. (2013) concluded that free radicals play a central role in the occurrence of different chronic diseases, such as cancers and cardiovascular diseases since free radicals are involved in the peroxidation process of lipids. To study the antioxidant activities of EOs extracted from fennel and anise, DPPH was used as a model of a stable lipophilic radical. Antioxidants from fennel and anise EOs react with DPPH radicals, reducing several DPPH molecules equal to the number of their available hydroxyl groups. The antioxidant activity of EOs extracted from fennel and anise was studied.

Figure 7 shows the antioxidant activity of EOs extracted from fennel and anise. The scavenging activity was measured at 30, 60 and 90 min. It was clear that the scavenging activity of EO extracted from fennel was higher than EO extracted from anise at 30 and 60 min. However, at 90 min, the scavenging activity was almost the same for both EOs extracted from fennel (90.2%) and anise (89.39%). The high antioxidant capability of fennel and anise EOs was reported in previous studies. Abdellaoui *et al*. (2017) reported that EO extracted from fennel seeds showed high antioxidant activity using DPPH free radical scavenging activity method. Furthermore, Rebey *et al*. (2018) reported the high antioxidant activity of anise seeds, revealing that EO from anise could be a novel source of natural antioxidants.

The high antioxidant activity of EOs extracted from fennel and anise could be attributed to their higher content of phenolic and hydroxlyphenolic compounds, which can scavenge DPPH through the donation of hydrogen atoms (Roby *et al*., 2013). The high antioxidant activity of fennel and anise EOs is probably the reason for its wide range of possible applications, such as antidiabetic agents (Shobha *et al*., 2013) and stabilization of crude soybean oil (Womeni *et al*., 2013). In addition, anise and fennel were reported as possible foods that could help with cancer prevention and treatment as novel anticancer natural compounds with apoptotic and antiproliferative properties (Ke *et al*., 2021).

CONCLUSION

EOs extracted from anise and fennel seeds showed remarkable antifungal activity against indoor opportunistic fungi (*P. frequentans*, *A. flavus*, *A. niger* and *C. globosum*). The MICs of anise and fennel seeds EOs were the same (5-8 mg/mL) against the four isolated indoor opportunistic fungi. Research findings suggest that these EOs could be used as natural antifungal compounds against indoor opportunistic fungi.

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