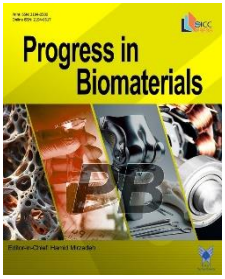

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An in Vitro Investigation into the Antifungal Properties of Electrospun Polyethylene Oxide Nanofibers Loaded with Extracted Lipids Against Dermatophytes

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Abstract

Dermatophytic infections caused by *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* remain a global health concern, especially with rising antifungal resistance. Developing biobased alternatives and novel delivery systems offers promising therapeutic potential.

In this research, fatty acids were produced by cultivating *Yarrowia lipolytica* (ATCC 18942) on oil pressing pulp. The extracted fatty acids identified by GC-MS and FT-IR as primarily oleic acid, palmitic acid, linoleic acid, and stearic acid were incorporated into polyethylene oxide (PEO) nanofibers through electrospinning (15 kV, 15 cm, 1 mL/h). The nanofibers were characterized using SEM and FT-IR. Their antifungal activity was assessed against *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* via microbroth dilution, pour plate, and agar well diffusion assays.

The minimum inhibitory concentrations (MICs) of PEO/extracted lipid nanofibers were 21 µg/ml for *M. canis* and *T. rubrum*, and 44 µg/ml for *T. mentagrophytes*. The minimum fungicidal concentrations (MFCs) were 42, 42, and 88 µg/ml, respectively.

These findings suggest that naturally derived fatty acids could serve as effective, low-risk alternatives for managing drug-resistant dermatophytosis.

Keywords: *Yarrowia lipolytica*; Fatty acids Nanofibers; *Microsporum canis*; *Trichophyton rubrum*; *Trichophyton mentagrophytes*

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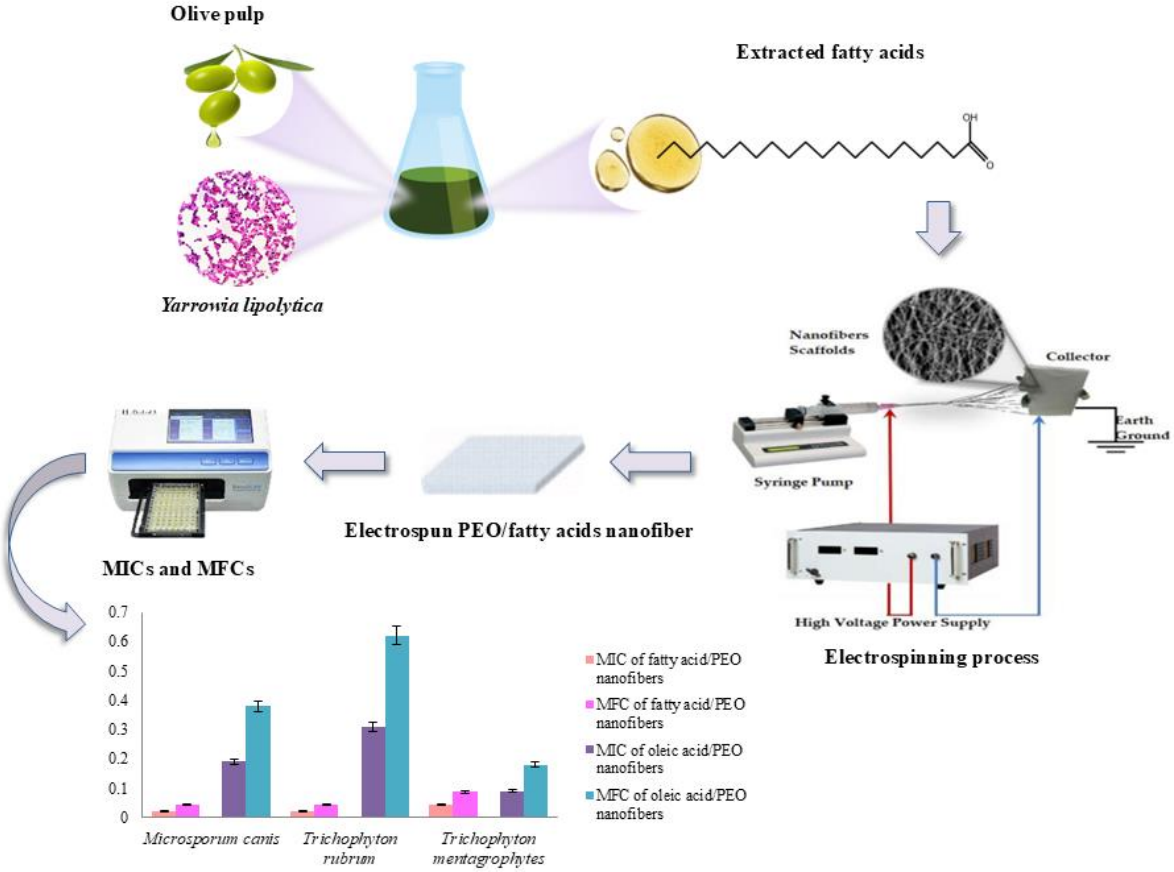


1. Introduction

Superficial fungal infections are among the most common infections affecting humans worldwide. Although these infections occur globally, they are predominantly concentrated in tropical regions, mainly due to the hot and humid climate, which promotes fungal growth [1]. Fungal spores, including keratinophilic dermatophyte fungi, are ubiquitous in soils around the world. However, their abundance and distribution vary depending on environmental factors and the availability of nutrients essential for their survival and growth. Fungal diseases in humans and animals are significant global health concerns influenced by various social, economic, host-specific, and climatic factors. Exposure to infectious fungal spores can lead to skin infections such as dermatophytosis, which can be transmitted from soil to humans [2].

Dermatophytes, a group of keratinophilic fungi, infect keratinized tissues such as skin, nails, and hair and typically thrive in warm, moist environments [3] . Dermatophytosis is one of the most prevalent fungal infections affecting both humans and animals worldwide. Millions of individuals suffer from dermatophyte infections annually, with approximately 20%–25% of the global population affected [2]. Epidemiological studies across Asia show that the most common dermatophyte species are *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*. Different forms of dermatophytosis are named based on the anatomical site affected, including tinea capitis (scalp), tinea barbae (face), tinea manuum (hands), tinea unguium (nails), tinea cruris (groin), and tinea pedis (feet), the latter also known as athlete's foot [1, 4, 5].

Conventional antifungal agents for dermatophytosis treatment include clotrimazole, ketoconazole, fluconazole, amphotericin B, terbinafine (TBF), and flucytosine; however, many patients seek alternative or complementary therapies. Over the past decade, dermatophyte infections have increased globally, partly driven by emerging antifungal resistance and changing epidemiological patterns[3].

The increase in drug-resistant pathogenic fungi and adverse effects associated with antifungal drugs have accentuated the urgent need to develop novel therapeutics capable of overcoming resistance [3]. While these infections are rarely life-threatening, affected

individuals may suffer from lowered self-esteem due to physical discomfort, recurrent infections, prolonged treatment periods, and treatment failures [6]. Accordingly, researchers are investigating natural, biodegradable compounds with antifungal properties.

Lipids are widely present in nature and perform important biological roles, including antimicrobial activity. Simple natural lipids such as fatty acids exhibit broad-spectrum antimicrobial effects against gram-positive and gram-negative bacteria, viruses, yeasts, fungi, and parasites that infect the skin and mucous membranes of humans and animals. Consequently, fatty acids have garnered interest as pharmaceutical agents that can substitute or complement traditional antibiotics. Fatty acids also demonstrate potential as antifungal agents against fungi that infect plants, humans, animals, and contaminate foods. In vitro studies have shown that fatty acids possess both fungistatic and fungicidal effects across a wide range of fungal pathogens, making them promising candidates for therapeutic and industrial applications [7]. Among fungal pathogens, *Candida* species and dermatophytes (*Epidermophyton*, *Trichophyton*, and *Microsporum*) are particularly significant.

Due to the limited efficacy and potential toxicity of conventional antifungal drugs, new treatment approaches are being explored. The synergistic use of natural compounds may reduce toxicity and serve as alternative therapies. Another key area of development involves novel antifungal formulations, with growing interest in nanoscale carriers that improve drug delivery and efficacy [8, 9].

Single-cell oils, produced by oleaginous microorganisms, can contain lipids comprising 20% to 80% of their dry biomass [10]. *Yarrowia lipolytica* is a notable oleaginous yeast capable of utilizing triglycerides and fatty acids as extracellular carbon sources [11,12]. Various oil pulps, byproducts generated during oil pressing, serve as substrates for lipase and fatty acid production. These oil pulps are effective inducers due to their residual oil content and are regarded as abundant, inexpensive, and renewable feedstocks that help reduce environmental impact [13]. Substrates such as oil pressing pulps from sesame, olive, and sunflower seeds have been successfully employed for fatty acid production by *Yarrowia lipolytica* [14].

Nanofibers are broadly defined as fibers with diameters below 100 nm and are characterized by a high surface-area-to-volume ratio, making them advantageous for various technological applications. Their large surface area increases the rate of chemical reactions and enhances interactions with biological systems. Furthermore, their low density, high surface-to-volume ratio, and small pore size render them suitable for wide-ranging uses. Electrospinning is considered the most effective method for fabricating micro- and nanofibers on an industrial scale. This technique converts polymer solutions into continuous fibers with controlled morphology [15–20].

The present study aims to produce fatty acids using *Yarrowia lipolytica* cultured in a medium containing oil pressing pulps as a carbon source. A major challenge in microbial lipid production is the high cost of raw materials such as glucose; therefore, utilizing inexpensive, underutilized, and readily available oil pressing pulps presents a cost-effective alternative strategy. The fatty acids extracted from these microbial lipids were fabricated into electrospun nanofibers to evaluate their antifungal effects against common dermatophyte species.

2. Material and Methods

2.1. Strains and cultivation conditions

Yarrowia lipolytica (ATCC 18942) was obtained from the Iranian Biological Resources Center, Iran. The yeast strain was cultured on potato dextrose agar (PDA) and incubated at 25 °C in an incubator (Memmert, Germany) until full growth was achieved, then maintained at 4 °C [21]. Dermatophyte strains, including *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Microsporum canis*, were sourced from Pasargad Laboratory and the Pasteur Institute of Iran. These strains were subcultured on dermatophyte test medium (DTM) and Sabouraud dextrose agar (SDA) for maintenance. All chemicals and culture media were purchased from Merck (Germany).

2.2. Slide Culture technique

The slide culture technique was employed to examine the intact mycelium of *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*. Initially, a U-shaped sterile glass tube was placed on a sterile petri dish to serve as a support, and a sterile glass slide was carefully positioned atop the tube. A 1×1 cm block of Sabouraud dextrose agar (SDA) was aseptically transferred onto the glass slide using a sterile scalpel. The dermatophyte inoculum was then applied to all four sides of the agar block using a sterile wire needle. A sterile coverslip was gently placed over the agar block to ensure firm adherence. Approximately 7–8 ml of sterile distilled water was added to the petri dish to maintain humidity, and the dish was covered.

The plates were incubated at 28 °C, placed within a clean plastic basket to avoid contamination. Cultures were monitored regularly for fungal growth, conidia formation, and moisture maintenance.
Once sufficient fungal structures formed, the coverslip was carefully removed using sterile forceps and mounted onto a second slide containing a drop of lactophenol cotton blue (LPCB). The agar block was excised using a flame-sterilized wire needle. Another drop of LPCB was added to the slide, then covered with a fresh coverslip. The prepared slide was examined under a microscope using a 40× objective lens. To preserve the preparation, edges of the slides were sealed with nail polish and stored at room temperature. All steps were performed under a laminar flow hood to maintain aseptic conditions [22]. This procedure was repeated for each dermatophyte species tested.

2.3. Preparation of inoculum

PDA (Potato Dextrose Agar) was used to prepare fresh cultures and activate the yeast. The yeast strain was incubated at 25 °C for 24 hours. For pre-cultures, single colonies from PDA plates were selected to inoculate 100 ml flasks containing 20 ml of culture medium composed of 15 g glucose, 1 g peptone, 0.5 g yeast extract, 1 g KH_2PO_4, and 0.5 g $MgSO_4 \cdot 7H_2O$ per liter of distilled water. Cultures were incubated at 25 °C and 200 rpm for 24 hours in a shaker incubator (Iran-Khodsaz, Iran) [23]. These components were selected to provide a balanced nutrient composition, in which glucose serves as a carbon and energy source, peptone and yeast extract supply essential nitrogen, amino acids, and growth factors, while KH_2PO_4 and $MgSO_4 \cdot 7H_2O$ act as buffering and mineral supplements to support optimal yeast growth.

Preparation of lipid production medium
Ingredients of the production medium included 30 g oily pulp (collected from oil pressing shops in Tehran, Iran), 1 g peptone, 1 g yeast extract, 1.5 g $MgSO_4$. $7H_2O$, 7 g K_2HPO_4, and 2 g Na_2HPO_4 are added in 1 liter distilled water and sterilized by autoclave (ecotec CS 21, Iran Tolid; Iran) [24]. The Neubauer counting chamber was used to create 10^7 cells/ml of pre-inoculum standard applying to inoculate into the production medium [25].

2.4. Lipid extraction

Five milliliters of culture medium containing *Yarrowia lipolytica* after 72 hours of incubation were transferred under sterile conditions to a 15 ml falcon tube and centrifuged at 1753 × g for 10 minutes at 4 °C using a refrigerated centrifuge (KS 3-30, Sigma, Germany). The resulting pellet was washed once and resuspended in 7 ml of a 1M hydrochloric acid (HCl), then heated to boiling to disrupt the yeast cells. The Erlenmeyer flask was immediately placed in chilled water after boiling. Subsequently, 30 ml of chloroform-methanol (3:2, v:v) solution was added, and the mixture was incubated at 37 °C for approximately 1 hour in a shaker incubator. The final extraction mixture was transferred into a separatory funnel and allowed to separate for 1 hour, resulting in the formation of organic and aqueous phases. The organic phase was collected and dried in an oven at 80 °C until a constant weight was reached. The lipid content was determined gravimetrically by measuring the weight difference of the Erlenmeyer flask before and after drying, representing the amount of fat produced [26].

2.5. Characterization of fatty acids

2.5.1. *Gas chromatography-mass spectrometry (GC-MS)*

GC-MS was employed to determine the fatty acid profile of Extracted lipids from olive oil pulp (ELOP). The extracted lipids were dissolved in benzene, and 5% methanolic HCl (prepared by mixing 95 ml chilled methanol with 5 ml acetyl chloride) was added. The mixture was shaken thoroughly and refluxed for two hours to methylate the fatty acids, producing fatty acid methyl esters (FAMEs). After reflux, a 5% NaCl aqueous solution was added, and FAMEs were extracted into hexane. The hexane layer was washed with a 2% $KHCO_3$ solution to remove impurities and then dried over anhydrous Na_2SO_4. One microliter of the FAME sample was injected into an Agilent 7890B gas chromatograph equipped with a VF-5ms column (30 m × 0.25 mm × 0.50 µm) and a flame ionization detector (FID). The oven temperature was programmed from 130 °C to 180 °C, increasing at 3 °C/min. Identification of fatty acids was performed by comparing the mass spectra with the Wiley Registry® 10th Edition and NIST 2014 Mass Spectral libraries, as well as authentic fatty acid standards [27].

2.5.2. *Fourier Transform Infrared Spectroscopy (FT-IR)*

The types of microbial lipid bonds were investigated by FT-IR (Spectrum RXI, Perkin Elmer; USA) [24].

2.6. Nanofibers and electrospinning

Polyethylene oxide (PEO) with a molecular weight of 900,000 Da was purchased from Sigma-Aldrich. To prepare a 3% (w/v) PEO solution, 0.75 g of PEO powder

was placed in a volumetric flask and volume was adjusted to 25 ml with distilled water. The solution was stirred gently on a magnetic stirrer at low speed for 24 hours at room temperature to obtain a homogeneous solution.

Based on minimum inhibitory concentration (MIC) values obtained from microbroth dilution assays of the ELOP and oleic acid against dermatophytes, a 35% concentration was selected for preparing the lipid solutions. To prepare 35% (w/v) extracted lipid solution from olive oil pulp, 8.75 g of lipid powder was dissolved in a 25 ml volumetric flask and volume was brought to the mark with appropriate solvent. Similarly, 8.75 ml of oleic acid was placed into a 25 ml volumetric flask and volume adjusted to 25 ml to prepare a 35% (v/v) oleic acid solution.

The extracted lipid solution was mixed with the 3% PEO solution at various volume ratios of 80:20, 70:30, 60:40, and 50:50 (lipids:PEO, v/v) . Then, 0.5 ml of Tween 80 was added as an emulsifier to each mixture. The solutions were stirred on a magnetic stirrer at 200 rpm for 24 hours at room temperature to ensure complete homogenization. The same procedure was followed to prepare oleic acid-PEO mixtures at similar ratios.

Electrospinning was carried out using an electrospinning apparatus (Fanavaran Nano Meghias Company, Iran). The prepared solutions were loaded into 5 ml syringes fitted with 18-gauge needles and mounted on the apparatus's infusion pump. The positive electrode was connected to the needle, and the negative electrode was attached to the collector. Upon applying a sufficiently high voltage, a Taylor cone formed at the needle tip, and nanofibers were collected on aluminum foil positioned on the collector. Electrospinning parameters were optimized by varying the voltage from 15 to 24 kV, the needle-to-collector distance between 15 and 20 cm, and the flow rate from 0.5 to 1.5 mL/h. The formation and morphology of nanofibers from each solution were examined separately. Electrospinning was performed for several hours to obtain nanofibers with the desired thickness. Finally, the electrospun nanofibers were dried at room temperature for 24 hours to remove residual solvent [29].

2.7. Cross-link electrospun nanofibers

Electrospun PEO/ELOP nanofibers are water-soluble and exhibit low mechanical strength. To enhance their mechanical stability and durability in aqueous environments, chemical crosslinking was performed. Glutaraldehyde (GTA) vapor (25% aqueous solution) was employed to induce covalent cross-links within the nanofiber matrix. The nanofibers were exposed to GTA vapor in a vacuum desiccator containing 5 ml of the solution at room temperature (pH ≈ 7.0) for two hours. After crosslinking, the samples were transferred to a vacuum oven and dried at 40 °C for 24 hours to completely remove residual vapors and unreacted aldehyde groups. These controlled conditions were selected to ensure sufficient cross-linking without compromising the nanofiber morphology.

2.8. Characterization of nanofibers

2.8.1. *FT-IR*

FT-IR (Spectrum RXI, Perkin Elmer; USA) was used to record the IR spectrum of prepared nanofibers.

2.8.2. *Scanning Electron Microscope (SEM)*

A scanning electron microscope (TESCAN VEGA//XMU, Czech Republic) was used to examine the morphology of the nanofibers. A small portion of the nanofibers was fixed onto a slide. The nanofibers were coated with a thin layer of silver using a desk sputter coater (Nanostructure Coating Model DSR1, Iran) to improve electrical conductivity. Energy-dispersive X-ray spectroscopy (EDS) was also conducted during electron microscopy to perform chemical analysis, identifying the elements present and their relative amounts.

2.8.3. ELOP release profile

Phosphate-buffered saline (PBS) was used as the medium for the release study. At 37 °C, a known amount of PEO/ELOP nanofibers was added to 50 ml of PBS. At predetermined time intervals, 1 ml of the PBS solution was withdrawn and immediately replaced with an equal volume of fresh PBS to maintain a constant volume. The amount of released compound in the collected solution was quantified using a UV–Vis spectrophotometer at 470 nm. All experiments were conducted in triplicate.

2.9. Antifungal susceptibility testing of treatments in vitro

2.9.1. *Standard suspension of dermatophytes*

Cultures were incubated at 28 °C for 7 to 14 days. The spore suspension was prepared by adding sterile physiological saline containing 0.1% Tween 80, followed by gently scraping the surface of the culture medium using a sterile Pasteur pipette. The spore concentration, consisting of a mixture of microconidia and macroconidia, was determined using a Neubauer counting chamber and adjusted to 10^7 spores/ml for

Trichophyton species and 10^6 spores/ml for Microsporum species, in accordance with the Clinical and Laboratory Standards Institute (CLSI) M38-A protocol. Standardized dermatophyte suspensions were used throughout all stages of the experiments [30].

2.9.2. *Pour plate*

In the pour plate method, 0.5 mg/ml of the prepared treatment was added to an empty sterile petri dish, which was then covered with molten and cooled Sabouraud dextrose agar (SDA) under sterile conditions. The plate was gently rotated to ensure thorough mixing of the treatment and medium, and the agar was allowed to solidify. Subsequently, 10 µl of the standardized dermatophyte suspension was inoculated in the center of the plate and incubated. After 14 days of incubation, the diameters of the growing colonies were measured and compared [31]. This procedure was repeated for each dermatophyte species and treatment, with positive and negative controls included separately.

2.9.3. *Agar well diffusion assay*

A well was punched in the center of a Sabouraud dextrose agar (SDA) plate (without antibiotics) and filled with 0.5 mg/ml of the treatment. The dermatophyte inoculum was streaked on the plate at a distance of approximately 1.5 cm from the central well. Plates were incubated at 28 °C for 14 days. This procedure was repeated for each dermatophyte species and treatment, with positive and negative controls included separately for comparison [31].

2.9.4. *MIC determination by microbroth dilution (CLSI method)*

The microbroth dilution assay was performed to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. A 96-well microtiter plate was used for the assay. Stock solutions were prepared, including Clotrimazole (positive control), standard suspensions of *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*, PEO/fatty acid nanofiber solution, and PEO solution.

- Column 1 contained 150 µl of culture medium only (media control).
- Column 2 contained 50 µl of culture medium and 50 µl of fungal suspension (negative control).
- Column 3 contained 50 µl of culture medium, 50 µl of fungal suspension, and 50 µl of Clotrimazole (positive control).
- Column 4 contained 50 µl of culture medium, 50 µl of fungal suspension, and 50 µl of PEO solution (PEO control).

Serial dilutions of the treatment solution were prepared from column 5 to the last column. In column 5, 50 µl of culture medium and 50 µl of treatment solution were added. A 50 µl aliquot from the most concentrated well was transferred to the next well, and this step was repeated across subsequent columns, removing 50 µl from the last well to perform serial dilutions. Finally, 50 µl of the standard fungal suspension was added to each well (Table 1) . The microplate was incubated at 28 °C for 72 hours.

After incubation, the optical density of each well was measured using an ELISA microplate reader (BioTek, USA).

To determine the MFC, 10 µl aliquots from wells showing no visible fungal growth in the MIC assay were plated onto SDA (Sabouraud dextrose agar) in three-well plates. Plates were incubated at 28 °C and inspected periodically. The lowest concentration of treatment that prevented fungal growth was recorded as the MFC.
Each assay was performed separately for every dermatophyte species, and all treatments were compared with positive and negative controls.

3. Results

3.1. Slide culture

The slide culture microscopic images of unbroken mycelium of *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* are shown in Figure 1 and Figure 2.

3.2. GC-MS and FT-IR

The GC-MS and FT-IR analyses (Figure 3) confirmed that *Yarrowia lipolytica* produced several fatty acids, including palmitic acid, oleic acid, linoleic acid, and stearic acid, when cultivated in a production medium containing olive oil pressing pulp (Table 1). The total microbial lipid yield under these conditions was quantified as 4.07 mg/ml.

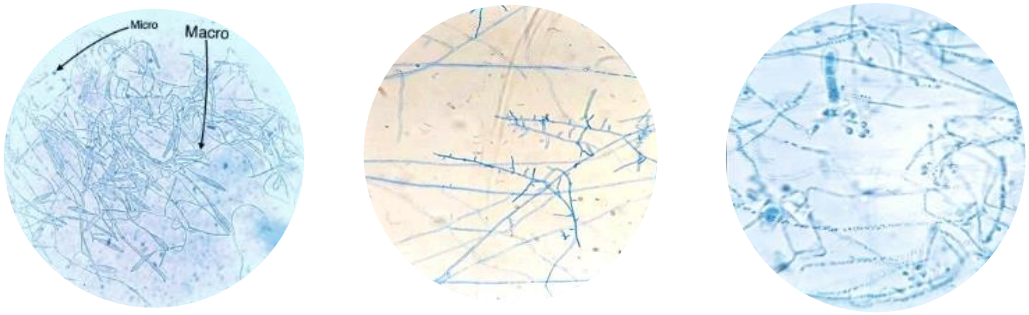
In the GC-MS chromatogram, distinct peaks appeared at retention times corresponding to specific fatty acids. Palmitic acid was detected at 8.81 minutes, oleic acid at 17.46 minutes, linoleic acid at 22.62 minutes, and stearic acid at 24.17 minutes. These results indicate that fatty acids were successfully extracted and converted to their methyl esters. Complementary FT-IR spectral analysis supported the GC-MS findings. A strong absorption band at 2922.34 cm^{-1} corresponded to the R–COOH stretch typical of carboxylic acids.

Table 1. Layout of the 96-well plate for MIC assay.

Column	Contents	Description
1	150 µl culture medium only	Media control
2	50 µl culture medium + 50 µl fungal suspension	Negative control
3	50 µl culture medium + 50 µl fungal suspension + 50 µl Clotrimazole	Positive control
4	50 µl culture medium + 50 µl fungal suspension + 50 µl PEO solution	PEO control
5	50 µl culture medium + 50 µl treatment solution + 50 µl fungal suspension	Highest concentration of treatment
6–11	Serial dilutions of treatment solution + fungal suspension	Dilution series
12	Lowest concentration of treatment + fungal suspension	Final dilution

Table 2. Fatty acid profile of olive oil pressing pulp fermented by Yarrowia lipolytica

Common Name	Systematic Name	Percentage%	Structural Formula
Palmitic acid	Hexadecanoic acid ethyl ester	7	$CH_3(CH_2)_{14}COOH$
Oleic acid	9-octadcenoic acid methyl ester	75	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Linoleic acid	9,12-Octadecadienoic acid	15	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
Stearic acid	Octadecanoic acid	3	$CH_3(CH_2)_{16}COOH$



(A) (B) (C)

Figure 1. Microscopic images of slide culture. (A) Microsporum canis (B) Trichophyton rubrum (C) Trichophyton mentagrophytes

Figure 2. Slide culture (A)Microsporum canis (B)Trichophyton rubrum (C)Trichophyton mentagrophytes

(A) (B) (C)

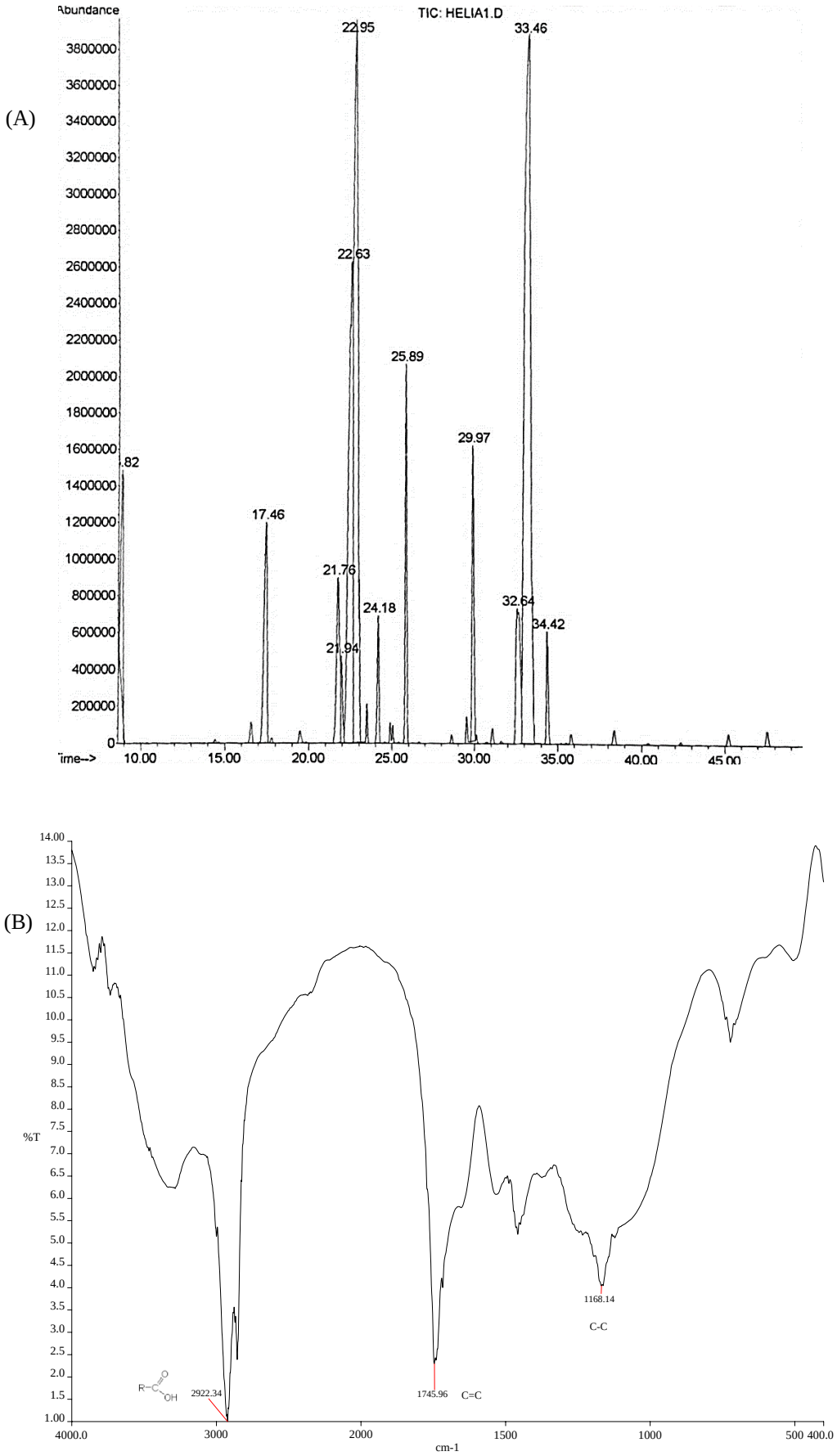


Figure 3. Characterization of lipids produced in culture medium containing olive oil pressing pulp by Yarrowia lipolytica. (A) Gas chromatography-mass spectrometry (GC-MS) chromatogram (B) Fourier Transform Infrared Spectroscopy (FT-IR) graph

The band at 1745.96 cm^{-1} was assigned to C=C double bonds in unsaturated fatty acids such as linoleic and oleic acids. Additionally, a band at 1168.14 cm^{-1} represented C–C stretching vibrations along the fatty acid carbon chain Together, the GC-MS and FT-IR data validate the presence and composition of fatty acids in the microbial lipid extracts derived from olive oil pulp. Based on these results, olive oil pressing pulp was

selected as the substrate for large-scale lipid production. Subsequent experiments were designed to evaluate the antimicrobial effects of these lipids on the dermatophytes *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Microsporum canis*. Notably, oleic acid identified in the extracts is well documented for its potent antimicrobial properties, supporting its potential role in antifungal activity.

3.3. Optimal parameters in the electrospinning process

The PEO/ELOP mixture at a 70:30 (v/v) ratio was used for nanofiber fabrication via electrospinning. At this composition, the polymer solution produced uniform nanofibers without the formation of beads or droplets, as confirmed by microscopic analysis. Optimization trials demonstrated that lower voltages (<12 kV) or shorter needle-to-collector distances (<10 cm) resulted in bead formation and non-uniform fibers, while higher voltages (>18 kV) or increased flow rates (>1.5 mL/h) caused droplet formation and jet instability. Under the optimal conditions 15 kV applied voltage, a 15 cm needle-to-collector distance, and a flow rate of 1 mL/h smooth and continuous nanofibers were consistently obtained.

3.4. Result of cross-link electrospun nanofibers

Upon crosslinking, the color of the nanofibers changed from white to yellow. Throughout the crosslinking process, the nanofiber structure remained intact, and the samples exhibited a distinctly yellow appearance, indicative of successful crosslink formation.

3.5. Results of characterization of nanofibers

3.5.1. *FT-IR results*

FT-IR spectroscopy confirmed the successful incorporation of ELOP into the PEO polymer matrix. In the wavenumber range of 2800–3400 cm^{-1}, characteristic peaks were observed in both the fatty acid nanofiber and oleic acid nanofiber spectra; these peaks, present also in the FT-IR spectrum of pure PEO (Figure 4A), are attributable to the PEO component. In the 1700–2800 cm^{-1} region, the FT-IR spectra of oleic acid nanofibers and PEO displayed similar features. However, in the spectrum of nanofibers containing ELOP, additional peaks were observed, indicating the successful incorporation of these lipids into the nanofiber structure.

Between 1900 and 2300 cm^{-1}, overlapping peaks were present across all FT-IR spectra, further confirming the dominant presence of PEO within the fiber composition. Additional peaks in the 500–1900 cm^{-1} region were associated with the lipid components present in the nanofibers.

Moreover, a comparative analysis between the FT-IR spectra of PEO/extracted lipid nanofibers (Figure 4B) and PEO/oleic acid nanofibers (Figure 4C) supports the presence of oleic acid within the ELOP.

3.5.2. *SEM images*

The SEM images presented in Figure 5 demonstrate that the nanofibers were uniform, exhibited smooth surfaces, and were free of beads or defects, indicating well-formed and high-quality fiber morphology.

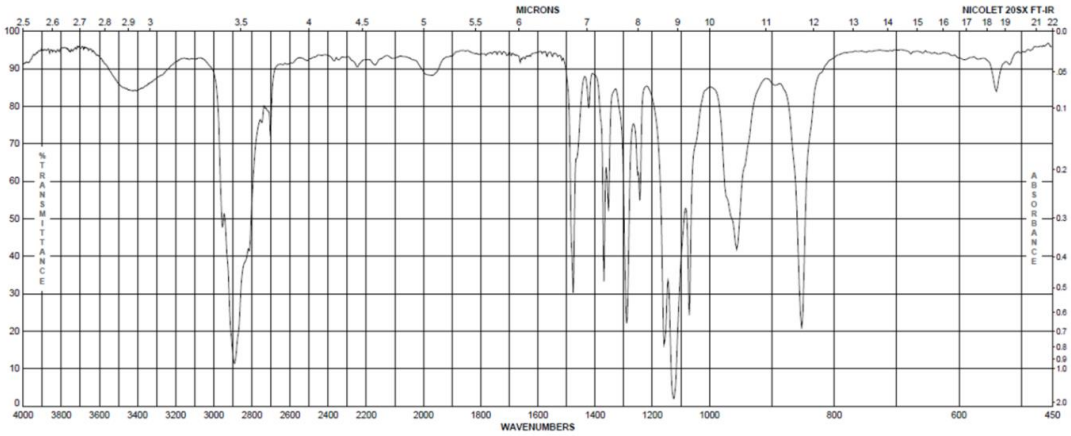
3.5.3. *Energy-dispersive X-ray spectroscopy (EDS) analysis*

Each peak in the EDS spectrum corresponds to a specific element, with sharper peaks indicating higher relative abundance. The EDS analysis of the nanofibers revealed prominently sharp peaks for carbon and oxygen, reflecting their major presence in the samples. These elements are the primary constituents of fatty acids, PEO , and oleic acid, as illustrated in the spectra shown in Figure 6. Additionally, the aluminum and silver peaks detected in the spectrum are attributed to the sample holder (aluminum substrate) and the silver coating applied to the samples for conductivity during SEM imaging, respectively.

3.5.4. *ELOP release profile*

The release profile of extracted lipids from the nanofibers was monitored over a 7-day period using UV–Vis spectrophotometry. Quantification of the released lipids was carried out based on a previously prepared calibration curve (standard curve) constructed from known concentrations of [ELOP/oleic acid] at their maximum absorption wavelength. At the end of the study, 97.34% of the extracted lipids were released from the PEO/ELOP nanofibers. Similarly, the release of oleic acid from the PEO/oleic acid nanofibers reached 95.93%. These results demonstrate a sustained and efficient release of lipid components from both nanofiber formulations.

(A)



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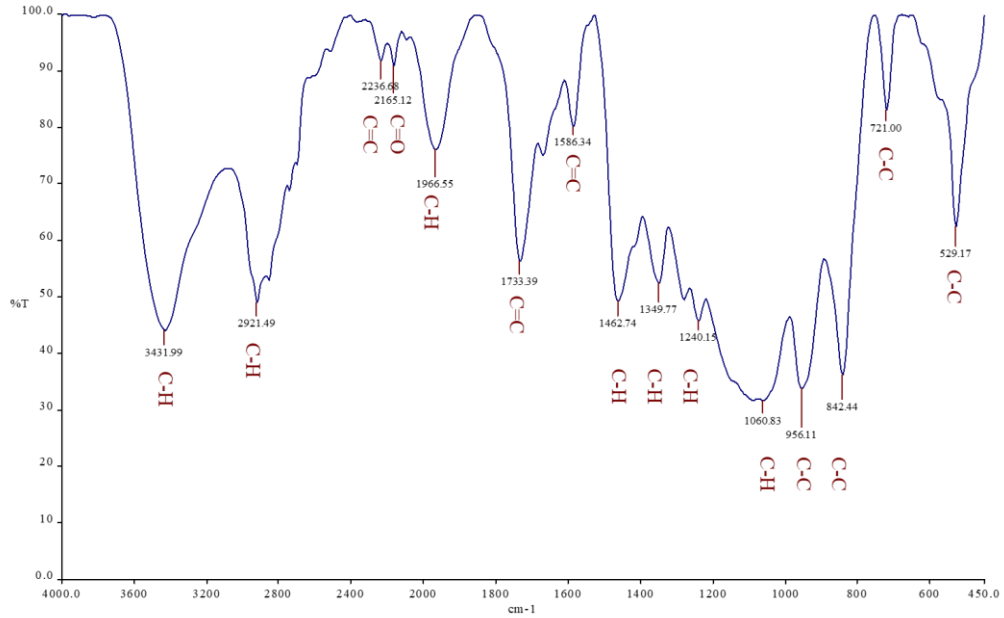

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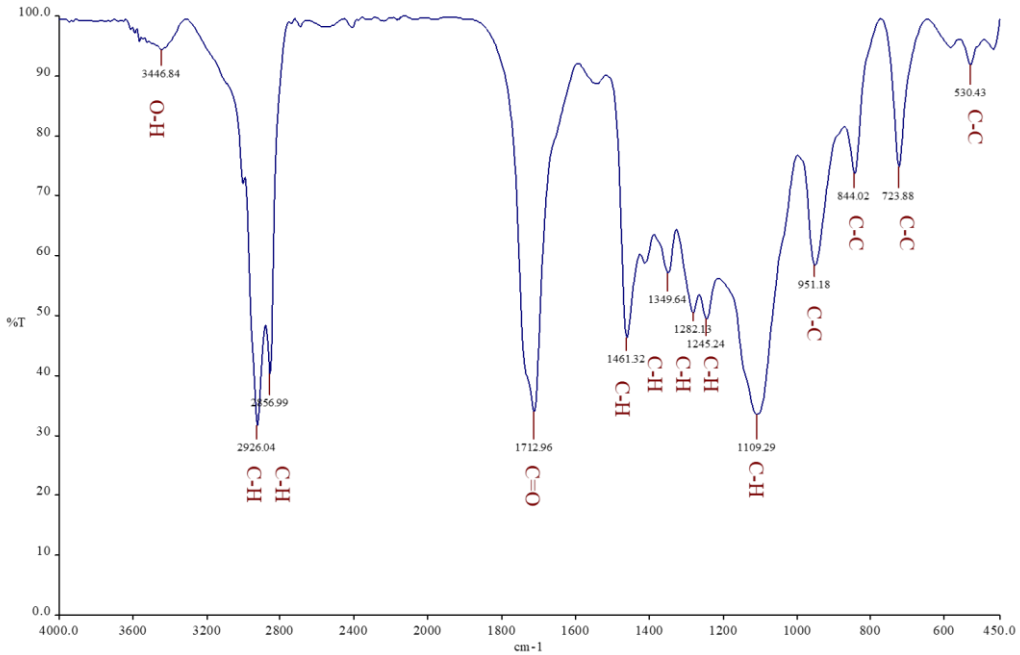


Figure 4. Fourier Transform Infrared Spectroscopy (FT-IR) spectrum. (A)PEO (B) PEO/ELOP nanofibers (C) PEO/oleic acid nanofibers

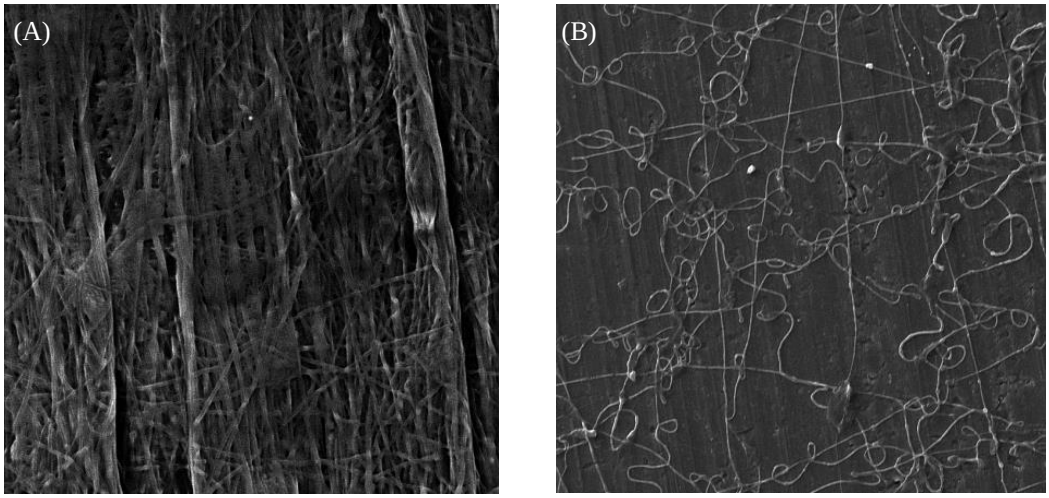


Figure 5. Scanning electron microscope (SEM) images. (A)PEO/oleic acid nanofibers, (voltage 10 Kv, view field 108.2 µm, magnification 2.00 K×, working distance 10.06 mm, and backscattered electrons (BSE) mode) (B) PEO/ELOP nanofibers (voltage 20 Kv, view field 2216.7 µm, magnification 1.00 K× , working distance 3.726 mm, and secondary electrons (SE) mode)

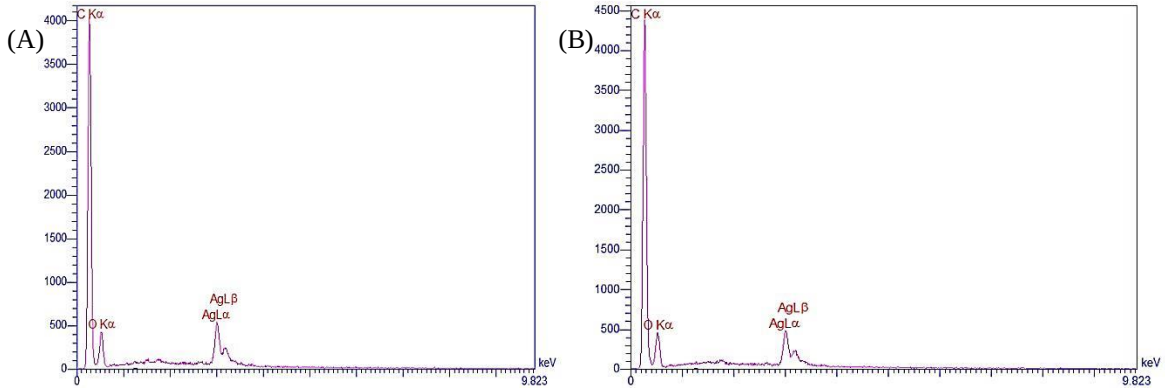


Figure 6. Energy-dispersive X-ray spectroscopy (EDS) (A) PEO/oleic acid nanofibers, (B)PEO/ELOP nanofibers

3.6. Antifungal susceptibility testing of treatments in vitro

3.6.1. *Results of the pour plate method*

Microsporum canis, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* were subjected to treatment with oleic acid, PEO/oleic acid nanofibers, ELOP, and PEO/ELOP nanofibers using the pour plate method. All treatments effectively inhibited the growth of these dermatophyte species, as demonstrated in Figures 8, 9, and 10. These findings indicate that both free and nanofiber-incorporated lipid formulations possess significant antifungal activity against key pathogenic fungi.

3.6.2 Results of agar well diffusion assay

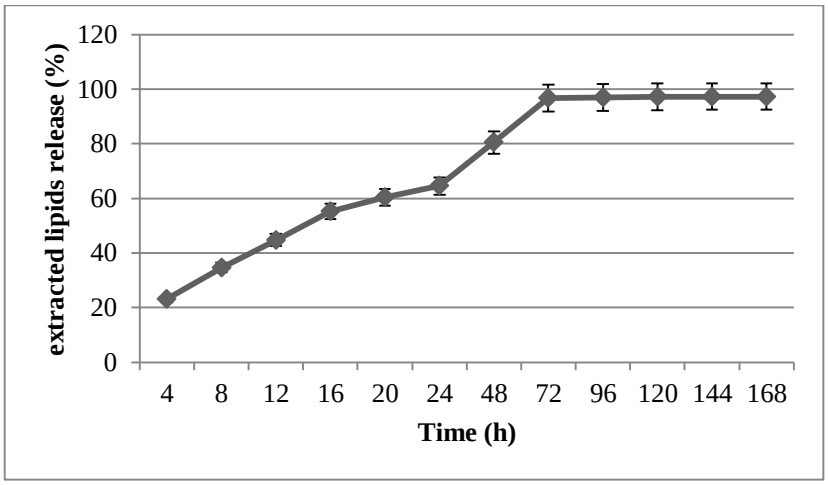
The antifungal activities of oleic acid, PEO/oleic acid nanofibers, ELOP, and PEO/ELOP nanofibers were evaluated against *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* using the agar well diffusion assay. Following fungal growth completion, the diameters of the colonies (in millimeters) on plates containing each treatment, as well as positive and negative controls, were measured using a caliper. The results are presented in Table 2 and illustrated in Figures 11 to 14.

3.6.3. Results of investigation of MIC and MFC by microbroth dilution

The MIC values of the extracted PEO/fatty acid nanofibers against *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* were determined as 21, 21, and 44 µg/ml, respectively. These MICs are higher compared to the positive control, clotrimazole, which exhibited an MIC of 4 µg/ml. Despite the relatively higher MICs, the use of natural compounds incorporated in these nanofibers offers the advantage of reduced side effects, making them a potentially safer and more suitable alternative to

conventional chemical antifungal agents. The detailed MIC and MFC values, along with representative aliquots, are presented in Tables 3 and 4 and illustrated in Figures 15 and 16. Notably, the incorporation of lipids into PEO nanofibers did not reduce their antifungal efficacy, as the MIC values remained similar to those of free lipids. Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test confirmed that the differences between the nanofiber formulations and the positive control were significant (p <0.05).

(A)



(B)

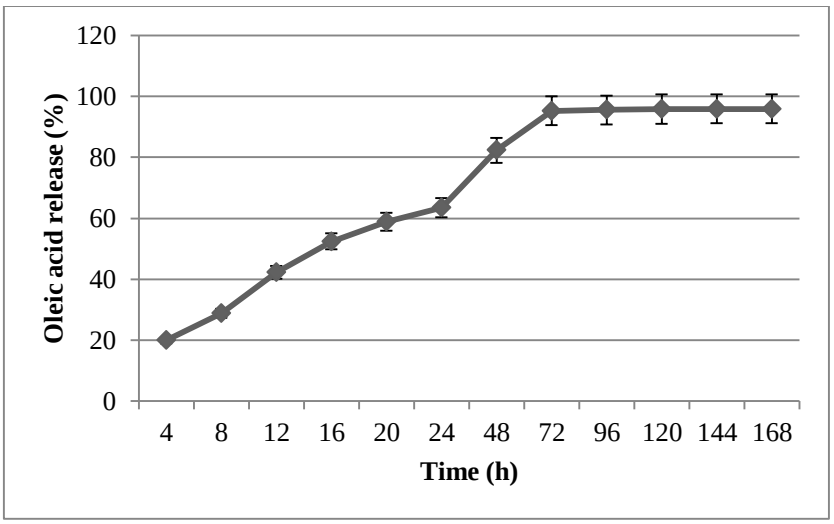


Figure 7. release profile (A) PEO/ELOP nanofibers (B) PEO/Oleic acid nanofibers

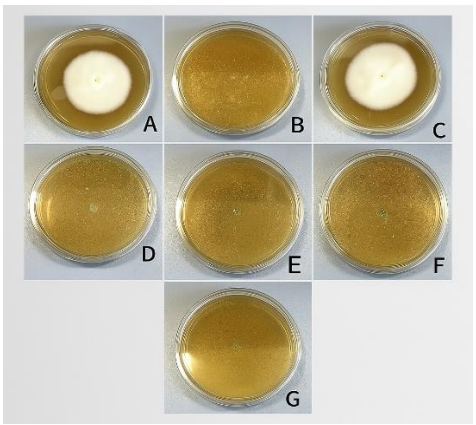


Figure 8. Antifungal activity of the treatments on Microsporum canis in pour plate method .(A)Negative control, (B) ELOP (C) PEO control (D)Oleic acid (E) PEO/ELOP nanofibers (F) PEO /oleic acid nanofibers (G)Positive control (Clotrimazole)

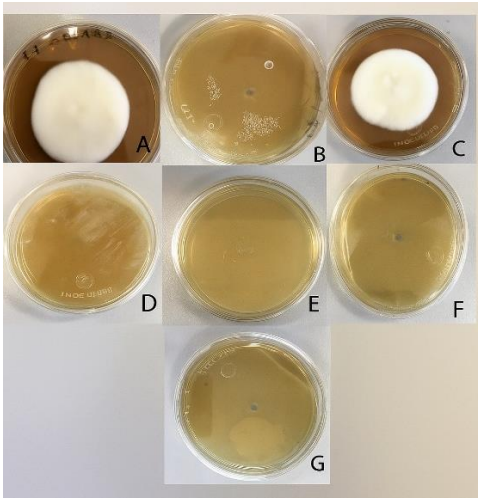


Figure 9. Antifungal activity of the treatments on Trichophyton rubrum in pour plate method. (A)Negative control (B) ELOP (C) PEO control (D)Oleic acid (E) PEO / ELOP nanofibers (F) PEO /oleic acid nanofibers (G) Positive control (Clotrimazole)

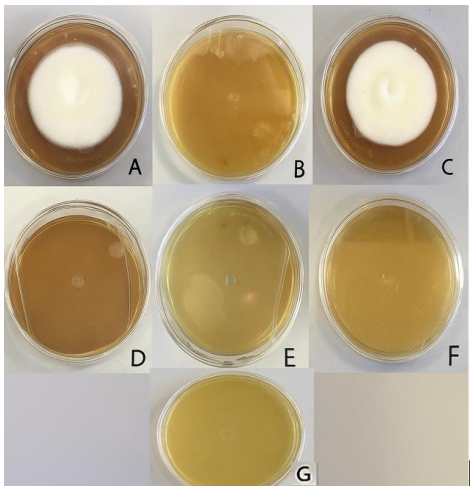


Figure 10. Antifungal activity of the treatments on Trichophyton mentagrophytes in pour plate method. (A) Negative control (B) ELOP (C) PEO control (D)Oleic acid (E) PEO / ELOP nanofibers (F) PEO /oleic acid nanofibers (G) Positive control (Clotrimazole)

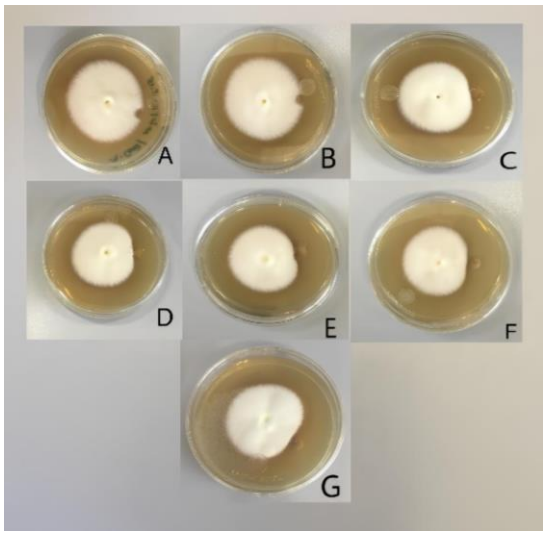


Figure 11. Antifungal activity of the treatments on Microsporum canis in agar well diffusion assay. (A)Negative control (B) PEO control (C) ELOP (D) Oleic acid (E) PEO / ELOP nanofibers (F) PEO /oleic acid nanofibers (G) Positive control (Clotrimazole)

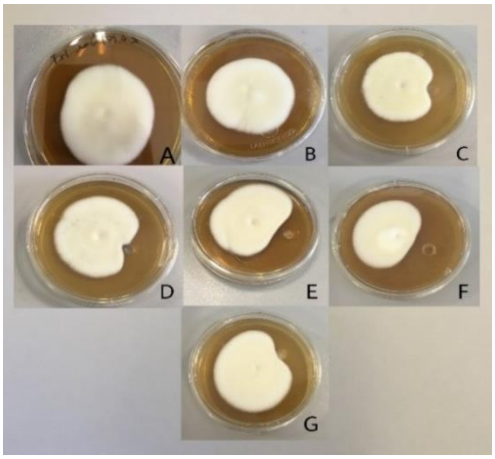


Figure 12. Antifungal activity of the treatments on Trichophyton rubrum in agar well diffusion assay. (A)Negative control (B) PEO control, (C) Positive control (Clotrimazole) (D) Oleic acid (E) PEO /extracted fatty acid nanofibers (F) ELOP (G)PEO /oleic acid nan

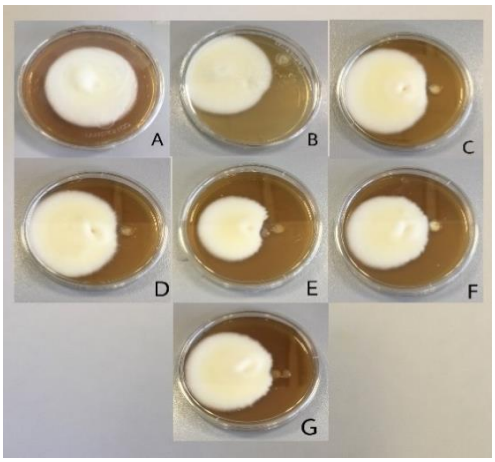


Figure 13. Antifungal activity of the treatments on Trichophyton mentagrophytes in agar well diffusion assay. (A)Negative control (B) PEO control (C) ELOP (D) Oleic acid (E)Positive control (Clotrimazole) (F) PEO / ELOP nanofibers (G) PEO /oleic acid nanofibers

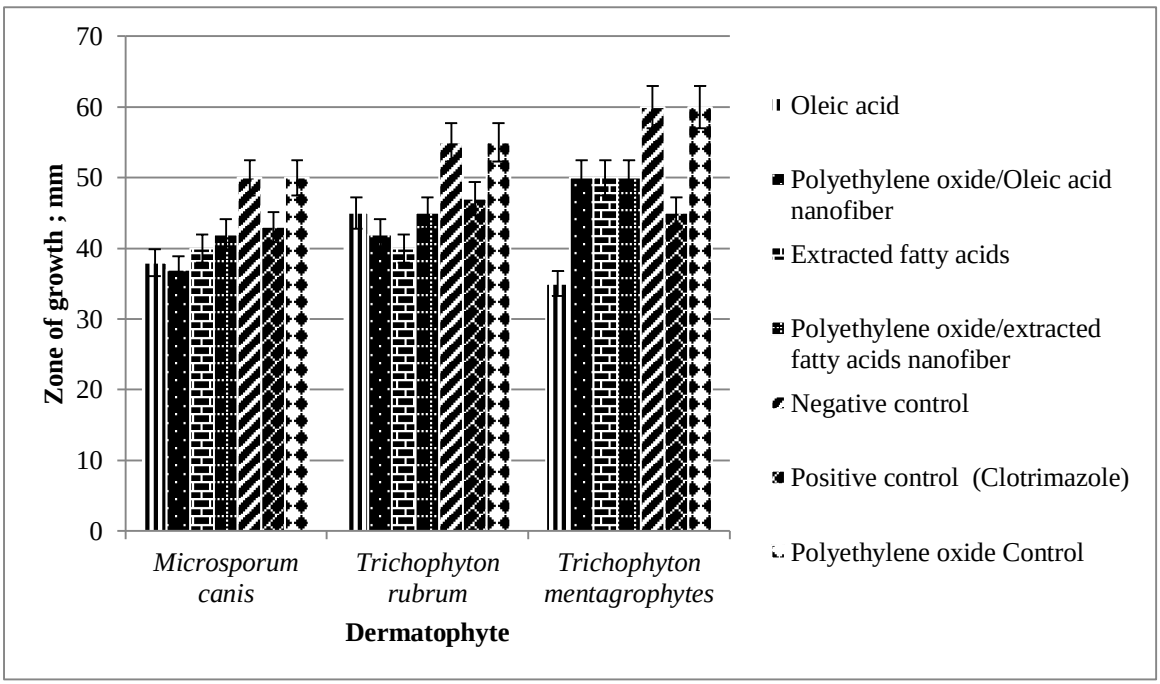


Figure 14. Inhibition zone diameters (mm) recorded in agar well diffusion assay

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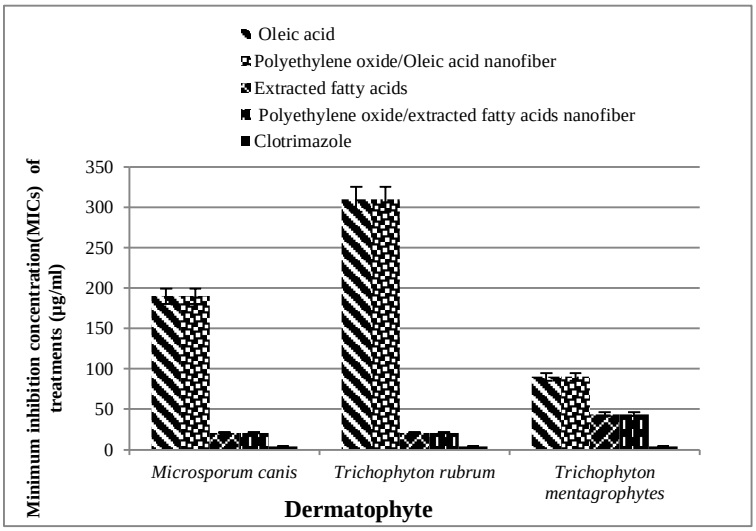

Figure 15. Minimum inhibitory concentration (MIC) values (µg/ml) of treatment against dermatophyte strains

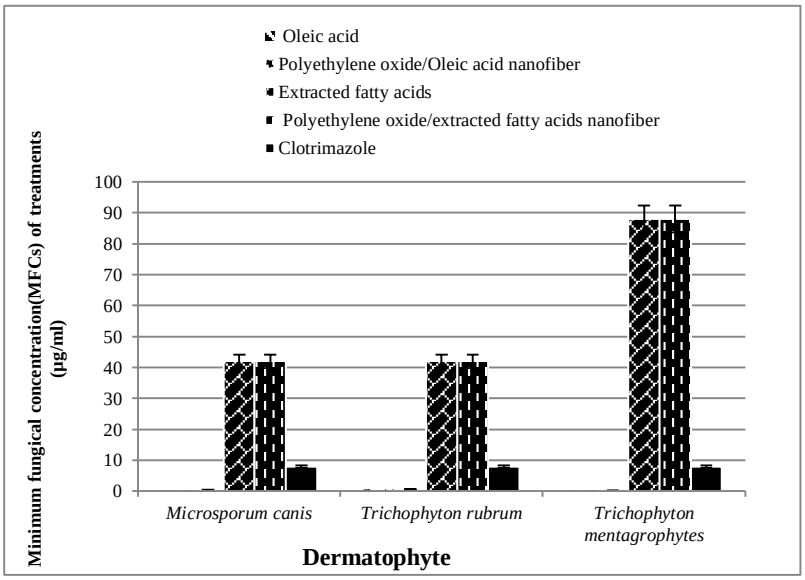

Figure 16. Minimum fungicidal concentration (MFC) values(µg/ml) of treatment against dermatophyte strains

Table 3. Inhibition zone diameters (mm) recorded in agar well diffusion assay

Dermatophytes / *Treatments*	*Microsporum canis*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*
Oleic acid	38	45	35
PEO /Oleic acid nanofibers	37	42	50
ELOP	40	40	50
PEO / ELOP nanofiber	42	45	50
Negative control	50	55	60
Positive control (Clotrimazole)	43	47	45
PEO Control	50	55	60

Table 4. Minimum inhibitory concentration (MIC) values (µg/ml) of treatment against dermatophyte strains

Dermatophytes / Treatments	*Microsporum canis*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*
Oleic acid	190	310	90
PEO /Oleic acid nanofiber	190	310	90
ELOP	21	21	44
PEO / ELOP nanofiber	21	21	44
Clotrimazole	4	4	4

Table 5. Minimum fungicidal concentration (MFC) values (µg/ml) of treatment against dermatophyte strains

Dermatophytes / Treatments	*Microsporum canis*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*
Oleic acid	380	620	180
PEO /Oleic acid nanofiber	380	620	180
ELOP	42	42	88
PEO/ ELOP nanofibers	42	42	88
Clotrimazole	8	8	8

4. Discussion

Single-cell oils (SCOs) are intracellular storage lipids synthesized by oleaginous microorganisms. These microorganisms can accumulate lipids ranging from 20% to 80% of their dry cell biomass [10, 32]. *Yarrowia lipolytica* is a prominent oleaginous yeast with extensive biotechnological applications. This non-conventional, aerobic, and dimorphic yeast produces valuable metabolites, including lipases, microbial oils, and citric acid. Notably, *Y. lipolytica* is classified as generally recognized as safe (GRAS) by the FDA. It is widely distributed and frequently isolated from dairy products such as Camembert cheese, yogurt, and kefir, as well as from soy sauce, meat, and other lipid- or carbohydrate-rich environments [33, 34].

In lipid production, *Y. lipolytica* can utilize oily substrates, such as pulps remaining from oil pressing, as effective carbon sources. Additionally, this yeast serves as a model organism for studying dimorphism, producing pseudohyphae under nitrogen-deficient conditions [35]. In the present study, olive oil pressing pulps were used as the carbon source for lipid biosynthesis. GC-MS and FT-IR analyses of the produced lipids confirmed the presence of key fatty acids, including oleic, linoleic, stearic, and palmitic acids. Considering both composition and yield, olive oil pressing pulp was selected as the optimal substrate for large-scale lipid production and subsequent experiments.

Darvishi and Salmani (2018) investigated olive oil as a carbon source for *Y. lipolytica*. Their GC-MS analysis identified oleic, linoleic, stearic, and palmitic acids as predominant microbial lipids [36]. In our study, the GC-MS results similarly confirmed these fatty acids. Importantly, olive oil pressing pulp not only provided an effective carbon source but also enhanced total lipid production compared to olive oil alone. This finding underscores its potential as a cost-effective substrate for large-scale lipid biosynthesis.

Earlier, Dominguez et al. (2003) used olive and sunflower oils as carbon sources in liquid cultures of *Y. lipolytica*. They reported that lipid-rich media significantly increased both lipase activity and lipid production compared to glucose-containing controls [37]. Building on these results, this study employed olive oil pressing pulps to evaluate their efficacy in microbial lipid production and their feasibility as an economical substrate.

Human superficial infections caused by keratinophilic dermatophytes, such as *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*, are prevalent worldwide. These infections affect the skin, nails, and hair, with reported prevalence ranging from 20% to 25%. They often reduce patients' quality of life due to visible symptoms and represent a growing public health concern. This concern is heightened by increasing resistance to standard antifungal therapies. Limitations and side effects of conventional antifungals, particularly in vulnerable populations such as children, have encouraged exploration of natural products with antifungal activity.

For instance, El Gendy et al. (2016) studied 50 dermatophytosis patients and identified four dermatophyte species: *T. violaceum*, *T. mentagrophytes*, *T. rubrum*, and *M. canis*. They tested natural oils—including dill, garlic, ginger, black seed, olive, peppermint, and castor—for antifungal activity. All oils except dill showed significant inhibitory effects against the dermatophytes [38]. Fatty acids in these oils are confirmed antifungal agents and offer therapeutic potential with fewer side effects than synthetic drugs.

Oleaginous yeasts, such as *Yarrowia lipolytica*, can produce microbial lipids rich in fatty acids like oleic acid. These fatty acids exhibit antimicrobial properties, including activity against dermatophytes. Incorporating them into delivery systems, such as nanofibers, may enhance efficacy while reducing adverse effects. Overall, natural antifungal agents derived from microbial lipids or plant oils represent a promising strategy for dermatophytosis management, especially given rising drug resistance and safety concerns.

Hammer et al. (2002) evaluated tea tree oil against several pathogenic fungi, including *Epidermophyton floccosum*, *M. canis*, *M. gypseum*, *T. mentagrophytes*, *T. rubrum*, *T. tonsurans*, and various *Aspergillus* and *Penicillium* species. They reported both fungicidal and inhibitory effects [39]. These results align with the present study, which also employs natural compounds showing antifungal activity against key dermatophytes (*T. mentagrophytes*, *M. canis*, *T. rubrum*). Together, these studies confirm the potential of natural products as alternatives or adjuncts to conventional antifungals.

In 2017, Pinto et al. investigated fatty acid methyl esters (FAMEs) derived from vegetable oils. Their study demonstrated significant antifungal and antioxidant activity, including against *Paracoccidioides* species [40]. These findings support the potential application of fatty acids and their derivatives as effective agents in the treatment of fungal infections.

In 2019, Al-Ali and Al-Judaibi evaluated the antifungal activity of ethanol extracts and essential oils from *Azadirachta indica*, *Olea europaea* (olive), *Phoenix dactylifera* (Ajwa), and *Ziziphus spina-christi* against clinical isolates of *Candida albicans*, *C. tropicalis*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, and *Microsporum canis*. They determined MICs, MFCs, and kill-time. Their results showed that ethanol extracts and essential oils of *Olea europaea* and *Azadirachta indica* had antifungal activity against some fungi. Notably, the ethanol extract of *Olea europaea* showed no effect on *M. canis* [41].

In contrast, the present study demonstrated that ELOP (extracted lipids from olive oil pressing pulp) exhibits antifungal activity against *M. canis*. This discrepancy may arise from several factors. First, the type of oil or lipid fraction tested differs. Second, extraction methods vary: Al-Ali and Al-Judaibi used ethanol extracts and essential oils, while in this study, lipids were produced biologically by *Yarrowia lipolytica* cultured with olive oil pressing pulp and then chemically extracted. These methodological differences likely affected the lipid composition and bioactive compounds, resulting in varied antifungal efficacy.

Previous studies support the antifungal potential of fatty acids. Garg and Müller (1993) reported that 13 different fatty acids had significant fungicidal activity against *M. canis*, *M. gypseum*, *T. rubrum*, and *T. mentagrophytes* [42]. Similarly, El-Naghy et al. (1980) found that dermatophytes are particularly sensitive to oleic acid and palmitic acid [43]. GC-MS analysis in the present study confirmed the presence of oleic acid and palmitic acid in ELOP, consistent with these reports.

Importantly, this study extends previous findings by incorporating these fatty acids into electrospun nanofiber wound dressings. This strategy enables the development of natural, biocompatible, and side-effect-free antifungal dressings that can be applied directly to dermatophytic wounds, offering a practical and promising therapeutic approach.

Nanobiotechnology, an interdisciplinary field combining nanotechnology and biotechnology, focuses on the design, development, and application of nanomaterials and nanoscale devices. This field is expected to grow significantly in the near future [44].

Nanofibers, with their high surface area-to-volume ratio, tunable porosity, and low density, can be easily functionalized with biological molecules. These properties make them highly versatile for biomedical applications, including wound dressings, tissue engineering scaffolds, and drug delivery systems. Nanofibers can be fabricated using various techniques such as template synthesis, self-assembly, phase separation, electrospinning, and drawing. Among these, electrospinning is the most straightforward and versatile method, allowing precise control over fiber morphology and composition [45].

Currently, no studies have reported the anti-dermatophyte effects of PEO nanofibers loaded with ELOP. In this study, the antifungal activity of PEO/ELOP nanofibers was evaluated against *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*, the main causative agents of dermatophytosis. The results showed significant anti-dermatophyte effects, suggesting that these nanofibers could serve as natural antifungal agents. The fatty acids incorporated in the nanofibers likely exert their effect by disrupting the fungal cell membrane, leading to cell death.

Given these promising findings, future studies should investigate the broader antifungal spectrum of PEO/extracted lipid nanofibers against additional fungal pathogens. Their in vivo efficacy and safety in treating fungal infections should also be evaluated. Furthermore, formulation optimization could improve controlled release, stability, and therapeutic performance, paving the way for innovative, natural, and biocompatible antifungal wound dressings or topical treatments with minimal side effects.

In 2018, Golestaneh et al. successfully synthesized eutectic nanofibers of fatty acids using the co-electrospinning technique, demonstrating that fatty acids can form stable nanofibrous structures. The present study confirms and extends these findings by fabricating PEO nanofibers loaded with ELOP via electrospinning, validating this method for producing natural lipid-based nanofiber systems [46].

In 2019, Khajeh Amiri and Zibasarsht fabricated electrospun fibers containing a ternary eutectic fatty acid mixture for textile applications. Their FT-IR analysis indicated no chemical reactions between the constituents, and SEM imaging confirmed smooth fibers without nodes [47]. Similarly, in the present study, PEO/ELOP nanofibers were characterized by FT-IR and SEM, showing stable fiber formation without nodes. Given the challenges of electrospinning lipid-containing fibers, various parameters were systematically optimized to achieve uniform and well-formed fibers.

Fatty acid-based nanofibers have not been previously explored for treating dermatophytosis. In contrast, Misra et al. (2017) developed electrospun nanofibers embedded with a thiocarbamate derivative of tolnaftate for dermatophyte treatment. Their in vitro results showed approximately 95% inhibition of *Trichophyton rubrum*, while effects against *Microsporum canis* were limited. In vivo studies on *T. rubrum*-infected Swiss albino mice demonstrated complete fungal inhibition after seven consecutive days of topical application [48]. In the present study, PEO nanofibers loaded with ELOP showed a minimum inhibitory concentration (MIC) of 21 µg/mL against *Microsporum canis*, demonstrating significant antifungal activity. These results indicate that PEO/extracted lipid nanofibers are a promising natural antifungal strategy with distinct efficacy against *M. canis*, complementing previous studies on synthetic antifungal-loaded nanofibers.

Electrospun nanofibers have recently emerged as effective platforms for treating microbial infections due to their advantages in targeted delivery and active wound dressing applications. In this study, nanofibers composed of PEO integrated with naturally extracted lipids were evaluated against the in vitro growth of dermatophytes. This approach leverages the biocompatibility, eco-friendliness, and natural origin of the lipid components, offering a sustainable and effective alternative to conventional synthetic antifungal agents. By combining the unique physicochemical properties of electrospun nanofibers with bioactive fatty acids derived from olive oil pulp, this study contributes to the development of innovative, side-effect-minimized antifungal therapies.

For comparison, studies such as "Introducing electrospun polylactic acid incorporating etched halloysite nanotubes for controlled release of Amoxicillin" illustrate highly engineered systems for sustained and targeted delivery of synthetic antibiotics. In that case, PLA-based nanofibers integrated with etched halloysite nanotubes modulate the release kinetics of amoxicillin, optimizing drug availability at the infection site.

In contrast, the present study focuses on naturally derived lipid extracts embedded in PEO nanofibers as intrinsic antifungal agents. Without synthetic drugs, these nanofibers showed significant inhibition of dermatophyte fungi, highlighting their potential as low-cost, biocompatible, and environmentally friendly materials for antifungal wound care. This strategy emphasizes natural simplicity, using bioactive compounds inherent in the lipid extracts to exert therapeutic effects.

Both approaches advance functional electrospun nanofibers but with different objectives: the PLA/EHNT system offers precise control over drug release, whereas the PEO/lipid nanofibers focus on natural bioactivity, safety, and simplified formulation. Future research could integrate these strategies to develop multifunctional nanofibrous materials that combine natural antifungal activity with engineered release control, optimizing clinical outcomes.

5. Conclusion

In recent years, resistance of pathogenic fungi, particularly dermatophytes, to available antifungal agents has risen at an alarming rate. This trend,

combined with the adverse effects of synthetic drugs, highlights the urgent need for safer and more effective alternatives. Natural compounds are promising candidates due to their bioactivity and reduced side effects.

In this study, lipids produced by the oleaginous yeast *Yarrowia lipolytica* were investigated as natural antifungal agents. Olive oil pressing pulp, a low-cost agricultural by-product, served as the carbon source, yielding 4.07 mg/ml of microbial lipids. GC-MS analysis identified key fatty acids such as oleic, linoleic, palmitic, and stearic acids, compounds known for their antimicrobial properties.

The extracted lipids demonstrated antifungal activity against *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*. The minimum inhibitory concentrations (MICs) were 21, 21, and 44 µg/ml, respectively, compared to 4 µg/ml for the standard drug clotrimazole. Importantly, incorporation of these lipids into electrospun PEO nanofibers did not diminish their antifungal efficacy. The nanofiber formulation also offers practical benefits, including improved handling, localized drug delivery, and potential application as wound dressings.

These findings underscore the feasibility of combining microbial biotechnology with nanotechnology to generate value-added products from agro-industrial wastes. Lipid-containing nanofibers represent a biocompatible and innovative platform for managing dermatophyte infections. Future in vivo studies will be essential to validate their therapeutic efficacy and safety in clinical contexts.

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Authors Contribution
Mohaddeseh Larypoor developed the original idea and the protocol, abstracted and analyzed data, study the concept and design, wrote the manuscript and critical revision of the manuscript for important intellectual content and is guarantor. Helia Ramezani contributed to the development of the protocol, abstracted data, and prepared the manuscript.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests
There is no conflict of interest in this article.

Reference

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