

Infrared Processing of Chili Oil at Different Exposure Times for Enhanced Biological Performance and Phytochemical Modulation

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Infrared (IR) processing of chili oil significantly changed its phytochemical composition and biological activity. GC–MS analysis revealed that unexposed chili oil was rich in eugenol (32.7%), caryophyllene (8.2%), and polyunsaturated fatty acids (PUFAs). Short-term IR exposure (5 min) reduced eugenol content to 25.9% and generated a positional isomer at 10.9% along with diynoic acid esters and alkyne fatty-acid derivatives, indicating isomerization and partial PUFA degradation. Prolonged IR exposure (10 min) further decreased volatile phenolics and PUFA esters while enriching high-molecular-weight alcohols (1-heptatriacotanol, 7.6%) and chlorinated derivatives (9,12-octadecadienoyl chloride, 21.0%). The 5-min IR treatment showed the highest antimicrobial activity among the tested exposure times, with inhibition zones increasing for *Bacillus subtilis* (25 ± 0.2 mm), *Staphylococcus aureus* (23 ± 0.7 mm), *Escherichia coli* (18 ± 0.5 mm), *Salmonella typhi* (19 ± 0.2 mm), and *Candida albicans* (25 ± 0.3 mm), and MIC/MBC values notably reduced (15.62 µg/mL for *B. subtilis* and *C. albicans*). IR-treated chili oil also displayed strong dose- and time-dependent biofilm inhibition. Five-minutes IR-treated chili oil reduced *B. subtilis* from 3.2×10⁵ to 2.1×10³ and *S. aureus* from 2.4×10⁵ to 2.6×10³ CFU/mL. Furthermore, antioxidant activity peaked at 5 min exposure, while anti-inflammatory activity was enhanced (IC₅₀ = 2.72 ± 0.14 µg/g).

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INTRODUCTION

Essential oils have long been recognized for their strong bioactivity and their ability to inhibit a wide variety of microorganisms, making them increasingly valuable in food preservation and safety applications (Al-Rajhi and Abdel Ghany 2023). Due to their plant-derived nature and broad consumer preference, these compounds are considered promising substitutes for synthetic preservatives, especially as they can function both as antimicrobial agents and flavor enhancers in food systems. Numerous investigations have confirmed the capacity of essential oils to suppress food-borne pathogens, highlighting their potential for industrial use (Alsolami *et al.* 2023; Qanash *et al.* 2023; Al-Rajhi *et al.* 2024). Chili peppers (*Capsicum* spp.) are among the medicinal plants widely studied for their rich profile of bioactive metabolites. These phytochemicals, particularly capsaicin, exhibit a range of pharmacological activities. While the health advantages of chili consumption continue to be evaluated, evidence suggests that chili compounds may play significant roles in improving human health either independently or synergistically (Powis *et al.* 2013). Capsaicin has been shown to inhibit carcinogenesis and promote apoptosis in cancer cells originating from skin, colon, bladder, breast, prostate, and lung tissues (Tsou *et al.* 2006). Similar anticancer effects have been observed in hepatocellular carcinoma, colon cancer, leukemia, gastric cancer (Huh *et al.* 2011; Amruthraj *et al.* 2014), and lung cancer models (Anandakumar *et al.* 2012). Extracts from *Capsicum chinense* have further demonstrated the ability to suppress the proliferation of HepG2 cells across reactive oxygen species (ROS) making, activation of apoptosis, and induction of autophagy *via* a caspase-3-dependent mechanism. Beyond anticancer activity, chili-derived compounds have also been informed to have anti-allergic and anti-inflammatory effects, with capsaicin being recognized as a vital giver to these responses (Sancho *et al.* 2002).

The antimicrobial influence of chili extracts is well established, with several studies documenting activity against *Clostridium sporogenes*, *Streptococcus pyogenes*, *Sarcina lutea*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Careaga *et al.* 2003; Omolo *et al.* 2014). Chili oil and chili seed oil also demonstrate strong antibacterial properties. For example, chili seed oil has shown repressive influences toward *E. coli* and *Salmonella* sp. (Rather *et al.* 2025), while chili oil itself has been confirmed to exhibit antimicrobial activity (Omolo 2014). Antifungal action has also been observed; liquid soap formulated with extract of red chili (*Capsicum annuum* L. var. *longum*) produced a potent inhibition against growth of *Candida albicans* (Madia *et al.* 2024).

Comparative testing of five common spices—ginger, garlic, turmeric, chili, and onion—revealed that all extracts inhibited *Staphylococcus aureus* and *Klebsiella pneumoniae*, with chili showing the greatest antibacterial effect, producing a 26 mm inhibition zone against *S. aureus* (Maharjan *et al.* 2019). Microscopic analysis further supports the mechanism of action of chili extracts. Treatment of *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus* with 10% chili crude extract resulted in clear inhibition zones and notable morphological damage, including cell wall collapse and membrane rupture. In contrast, *E. coli* exposed to 40% dried chili extract exhibited irregular cell shapes without complete cell wall rupture. This variation may be explained by differences in the cell wall architecture between Gram-positive and Gram-negative bacteria (Muangkote *et al.* 2019).

Chili oil, produced by infusing dried chili peppers in vegetable oil, is a widely used seasoning known for its distinctive aroma and moderate spiciness. Beyond its culinary role, this oil contains numerous bioactive ingredients that participate to its antimicrobial, antioxidant, and anti-inflammatory potential, increasing its relevance in both food and health-related applications (Peng *et al.* 2024).

Recent advances in oil modification technologies have demonstrated significant potential for enhancing the biological activities of plant oils. Various physical and chemical techniques have been explored to improve pathogen-inhibitory, antioxidant, inflammation-suppressing, and anticancer activities. For example, ozonation has emerged as an efficient tool for enriching oils with reactive oxygen species that promote deeper biochemical changes. Ozone-modified pumpkin seed oil exhibited strong anti-*Helicobacter pylori*, tumor-suppressive, blood glucose-lowering, and lipid-reducing activities, confirming the ability of ozone treatment to potentiate therapeutic efficacy (Alsalamah *et al.* 2025a). Similar enhancements were reported for ozonized propolis, where the treatment significantly improved its biological profiles, supported by molecular docking results (Al-Rajhi *et al.* 2025a). In addition, ozonation of peanut oil and black seed oil resulted in improved phytochemical composition and enhanced biological activities, including antioxidant, antimicrobial, and antineoplastic effects (Al-Rajhi *et al.* 2024, 2025b). Beyond ozonation, UV-C radiation, for instance, was shown to effectively alter the chemical profile of *Aloe vera* oil, leading to improved pharmacological activities *in vitro* (Alsalamah *et al.* 2025b). These advancements indicate that exposure to specific forms of energy, whether oxidative or radiation that can significantly upgrade the biological performance of plant-derived oils. Infrared radiation (IR), which located within the electromagnetic spectrum and characterized by wavelengths extending from 0.78 to 1000 μm (Fernando and Amaratunga 2022), has been applied in the drying of agricultural products due to its ability to promote rapid and efficient moisture removal. However, despite the growing body of research on ozonation and UV-based treatments, little attention has been given to the potential of infrared (IR) radiation as a tool for enhancing the biological activities of edible and medicinal oils. No previous study has investigated the effect of IR radiation on chili oil or evaluated how IR-induced modifications may influence its antimicrobial, antioxidant, or anti-inflammatory properties. Accordingly, the present study sought to address this knowledge gap by examining the impact of infrared radiation on the bioactivity of chili oil, providing a novel perspective compared with earlier approaches and addressing an important unmet need in oil bio-enhancement research.

EXPERIMENTAL

Chili Oil Treatment with Infrared (IR)

Chili oil prepared in-house by heating 50 g of dried red chili flakes (*Capsicum annum* var. longum, Mountain Spice Co., Asheville, NC, USA, batch no. CH-2024-09) in 200 mL of refined peanut oil (Lion & Globe, lot no. LPG7890, Lam Soon Edible Oils, Malaysia) at 120 °C for 15 min with stirring, followed by vacuum filtration through Whatman No. 1 paper (lot no. 1001-125, Cytiva). The resulting oil was stored at 4 °C in amber glass bottles and used within 7 days. For comparison, a commercial chili oil (Lee Kum Kee Chiu Chow Oil, lot no. LKK-789-ABC) was used as received.

To prepare a thin, uniform layer, 10 mL of chili oil was placed into a sterile glass Petri dish (diameter 9 cm) at an initial oil temperature of $22\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ (ambient room temperature). An infrared lamp (150 W, 230 V, 50 Hz, Philips, USA) emitting radiation in the near- to mid-IR range (peak wavelength approximately 2.5 to $4\text{ }\mu\text{m}$) was positioned 15 cm vertically above the oil surface. The oil was exposed for either 5 minutes or 10 minutes. During exposure, the surface temperature of the oil was continuously monitored using a K-type contact thermocouple (accuracy $\pm 0.5\text{ }^{\circ}\text{C}$) attached to the underside of the Petri dish. No active temperature control (*e.g.*, feedback regulation) was applied; the lamp remained at a fixed distance and output. Under these conditions, the oil temperature increased non-linearly over time: after 2 min it reached $45\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, after 5 min it reached $68\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, and after 10 min it reached $89\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$. Control samples (0-min exposure) were kept under identical ambient conditions but without IR illumination. Immediately after exposure, the oil was allowed to cool to $25\text{ }^{\circ}\text{C}$ in the dark, then transferred into amber vials and stored at $4\text{ }^{\circ}\text{C}$ until further analysis. All irradiation experiments were performed in triplicate to account for thermal variability.

GC–MS Analysis of Chili Oil Samples

The chemical composition of the chili oil was analyzed using gas chromatography–mass spectrometry (GC–MS). Briefly, the oil sample was diluted (1:100, v/v) in analytical-grade n-hexane and filtered through a $0.22\text{ }\mu\text{m}$ syringe filter prior to analysis. GC–MS analysis was performed using an Agilent 7890 GC coupled with an Agilent 5975C MSD. Separation was achieved on a capillary column (HP-5MS, $30\text{ m} \times 0.25\text{ mm i.d.}$, $0.25\text{ }\mu\text{m}$ film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min . The injection volume was $1\text{ }\mu\text{L}$ in split mode with a split ratio of 1:20. The injector temperature was set at $250\text{ }^{\circ}\text{C}$. The oven temperature program was as follows: initial temperature of $60\text{ }^{\circ}\text{C}$ held for 2 min, increased to $200\text{ }^{\circ}\text{C}$ at a rate of $5\text{ }^{\circ}\text{C/min}$, then ramped to $280\text{ }^{\circ}\text{C}$ at $10\text{ }^{\circ}\text{C/min}$ and held for 10 min. The total run time was approximately 40 min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, with an ion source temperature of $230\text{ }^{\circ}\text{C}$ and a quadrupole temperature of $150\text{ }^{\circ}\text{C}$. Mass spectra were recorded in the scan range of m/z 40 to 600. Identification of the oil constituents was carried out by comparing their mass spectra with those in the NIST and Wiley mass spectral libraries and by comparing their calculated retention indices with literature data. The relative percentage composition of each component was calculated based on the peak area normalization method (Al-Rajhi *et al.* 2025a).

Antimicrobial Activity Assessment

The inhibitory effect of chili oil against bacterial strains *Bacillus subtilis* (*B. subtilis* ATCC 6633), *Staphylococcus aureus* (*S. aureus* ATCC 6538), *Escherichia coli* (*E. coli* ATCC 8739), *Salmonella typhi* (*S. typhi* ATCC 6539), and the fungal strain *Candida albicans* (*C. albicans* ATCC 10221) was determined using the well diffusion method. Standardized microbial suspensions were spread evenly on Mueller-Hinton agar for bacteria or Sabouraud dextrose agar for *C. albicans*. Wells of 6 mm diameter were created, and $50\text{ }\mu\text{L}$ of chili oil samples (0-, 5-, and 10-min IR exposure) were added. Standard antimicrobial agents were included for comparison: Gentamicin ($10\text{ }\mu\text{g/mL}$) for bacteria and Nystatin ($100\text{ }\mu\text{g/mL}$) for *C. albicans*. Plates were incubated at $37\text{ }^{\circ}\text{C}$ for 24 h for bacteria and 48 h for *C. albicans*, and the inhibition zones were measured in millimeters.

(Abdelghany *et al.* 2021). Minimum inhibitory concentration (MIC) values were determined using the broth microdilution method. Serial two-fold dilutions of chili oil (1–128 µg/mL) were prepared in Mueller-Hinton broth for bacteria and Sabouraud dextrose broth for *C. albicans*. Each well was inoculated with a standardized microbial suspension and incubated at 37 °C for 24 h (bacteria) or 48 h (fungus). The MIC was defined as the lowest concentration of chili oil that completely inhibited visible growth. Standard antibiotics (Gentamicin for bacteria and Nystatin for *C. albicans*) were used as positive controls. To record minimum bactericidal/fungicidal concentration (MBC/MFC) values, 10 µL from wells showing no visible growth in the MIC assay were plated onto fresh agar and incubated under the same conditions. The MBC (for bacteria) or MFC (for *C. albicans*) was defined as the lowest concentration of chili oil resulting in no colony formation, indicating complete microbial killing. All experiments were conducted in triplicate, and mean values were reported (Al-Rajhi *et al.* 2025b).

Antibiofilm Assay

Biofilm inhibition of experienced bacteria *B. subtilis*, *S. aureus*, *E. coli*, and *S. typhi* was evaluated employing a standard 96-well microplate assay. Bacterial cultures were prepared in TSYB (10⁶ CFU/mL), and 300 µL of the inoculum was inoculated to each well containing sublethal concentrations of the tested sample (75%, 50%, and 25% of the MBC). Wells with medium only served as blanks, and wells with untreated bacteria acted as growth controls (Selim *et al.* 2024). Following incubation at 37 °C for 48 h, non-adherent cells were removed by washing the plates. The formed biofilm was then stained with 0.1% crystal violet, followed by washing, and the retained dye was subsequently solubilized using 95% ethanol. Absorbance was measured at 570 nm. Biofilm inhibition (%) was calculated as:

$$\text{Inhibition} = \left[1 - \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})}{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{blank}})} \right] \times 100 \quad (1)$$

Killing Kinetics of IR-Treated Chili Oil against Selected Bacteria

The time-kill kinetics assay was achieved to assess the antimicrobial potential of infrared (IR)-treated chili oil against tested bacteria. Bacterial cultures were prepared at a standard inoculum and exposed to IR-treated chili oil at its MIC. Samples were collected at various time points (0, 30, 60, 120, 150, 180, and 360 min), and the surviving bacterial counts were determined as colony-forming units per milliliter (CFU/mL). Vehicle-only and growth controls were included to validate the assay. The results were expressed as mean ± standard deviation of CFU/mL, and time-kill curves were generated by plotting log CFU/mL *versus* exposure time (Al-Rajhi *et al.* 2024). Bactericidal activity was defined as a ≥3 log₁₀ reduction in CFU compared to the initial inoculum.

Antioxidant Activity Assessment by DPPH Assay

The antioxidant activity of chili oil samples subjected to infrared (IR) exposure for 0, 5, and 10 min was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A methanolic DPPH solution (0.1 mM) was freshly prepared. The chili oil samples were dissolved in methanol and serially diluted to obtain concentrations ranging from 1.95 to 1000 µg/mL. For the assay, equal volumes (100 µL) of DPPH solution and each sample were added to a 96-well microplate. The reaction mixtures were incubated

at room temperature in the dark for 30 min to ensure stability of the radical reaction. Methanol containing DPPH alone was used as the negative control, while ascorbic acid served as the positive reference standard. The absorbance was recorded at 517 nm using a microplate reader (Abdelghany et al. 2019). The ratio of DPPH radical scavenging activity was calculated as:

$$\text{Scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{chili oil}}}{A\text{Abs}_{\text{control}}} \times 100 \quad (2)$$

Assessment of Anti-Inflammatory Activity Using Protein Denaturation Inhibition

The anti-inflammatory effect of chili oil samples exposed to IR radiation for 0, 5, and 10 min was evaluated by measuring their ability to prevent protein denaturation. A 1% (w/v) solution of bovine serum albumin (BSA) was prepared in phosphate-buffered saline (pH 6.4). Chili oil samples were diluted in methanol to obtain concentrations ranging from 1.56 to 200 µg/mL. Diclofenac sodium was employed as a reference anti-inflammatory agent. Equal volumes (100 µL each) of BSA solution and chili oil sample and Diclofenac sodium (standard) were combined in test tubes. The mixtures were incubated at 37 °C for 20 min initially, followed by heating at 70 °C for 5 min to induce protein denaturation. After cooling to ambient temperature, the absorbances of the solutions were measured at 660 nm using a spectrophotometer. The inhibitory effect on protein denaturation was calculated as follows,

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{chili oil}}}{A\text{Abs}_{\text{control}}} \times 100 \quad (3)$$

where A_{control} is the absorbance of the BSA solution without sample, and $A_{\text{chili oil}}$ is the absorbance in the presence of chili oil.

Statistical Analysis

All experiments were conducted in triplicate, and data are presented as mean ± SD. Statistical analyses were performed using GraphPad Prism. One-way ANOVA followed by Tukey's post-hoc test was used for antimicrobial assays (inhibition zones, MIC, and MBC/MFC). Biofilm inhibition data were analyzed using two-way ANOVA with Šidák's multiple comparisons test to evaluate the effects of IR exposure time and MBC concentration. Killing kinetics (log₁₀ CFU/mL) were assessed by repeated-measures two-way ANOVA with Geisser-Greenhouse correction. IC₅₀ values for anti-inflammatory and antioxidant activities were determined by non-linear regression and compared using the extra sum-of-squares F test. Statistical significance was set at $p < 0.05$, with significance levels indicated as $p < 0.05$, * $p < 0.01$, ** $p < 0.001$, and ns (not significant).

RESULTS AND DISCUSSION

From the current investigation, the volatile constituents of chili oil, along with their retention times (R.T.), molecular formulas, relative abundance (area %), and molecular weights were recognized *via* GC–MS (Table 1 and Fig. 1). The chromatographic profile showed a chemically diverse oil dominated by fatty acid derivatives, esters, and terpenoid-

related compounds. The most abundant component was 9,12-octadecadienoyl chloride (*Z,Z*), accounting for 23.6% of the total area, highlighting the prevalence of linoleic acid–derived chlorinated fatty acyl compounds in the oil. This was followed by *E*-8-methyl-9-tetradecen-1-ol acetate (19.1%) and 2,3-dihydroxypropyl palmitate (8.83%), suggesting a substantial contribution of long-chain fatty acid esters that are often related with bioactivity and lipid functionality. Moderate levels of *tert*-hexadecanethiol (6.97%) and 9,12,15-octadecatrienoic acid diacetate ester (6.58%) were also detected, signifying the occurrence of sulfur-containing composites and polyunsaturated fatty acid derivatives. Minor constituents comprised caryophyllene (3.71%), reflecting a sesquiterpenoid fraction, along with numerous unsaturated fatty acids and alcohols, such as oleic acid (*Z*), 17-octadecynoic acid, and 12-methyl-*E,E*-2,13-octadecadien-1-ol, each contributing less than 5%. Supporting these findings, Sotelo-Méndez *et al.* (2023) indicated that chili oil represents a valuable edible oil option, because of its enhanced oxidative stability and extended storage potential, which were associated with reduced unsaturation levels and consistently low acid and peroxide indices.

The comparative GC–MS studies showed notable qualitative and quantitative changes in the chemical composition of chili oil after infrared (IR) radiation. Eugenol (32.7%) was the main volatile ingredient in the unexposed oil (Table 1), which was primarily composed of phenolic components and fatty acid derivatives. Other substances found were caryophyllene (8.21%), caryophyllene oxide, a number of methoxy-phenolic derivatives, a range of polyunsaturated fatty acids (PUFAs), including 9,12-octadecadienoyl chloride (16.9%), esters of linoleic and linolenic acids, and long-chain aliphatic alcohols. After subjecting the oil to IR for a duration of 5 min, notable alterations were noted (Table 2). The eugenol concentration diminished to 25.9%, suggesting initial thermal volatilization and minor degradation, while a thermally generated positional isomer (phenol, 2-methoxy-3-(2-propenyl), 10.9%) appeared, thereby confirming the isomerization of allylphenolic structures when exposed to heat. Caryophyllene experienced a slight increase to 9.39%, likely resulting from improved release from the oil matrix at moderate temperatures. Notably, several new compounds were produced, including diynoic acid esters, hormonal-like derivatives (such as pregnenolone analogs), and alkenyne or alkyne fatty-acid derivatives. These thermally produced molecules signify a combination of oxidation, dehydrogenation, and rearrangement reactions triggered by brief IR exposure.

Polyunsaturated fatty acids exhibited distinct degradation patterns. Derivatives of linoleic and linolenic acids diminished, whereas compounds containing alkyne, such as 17-octadecynoic acid (8.32%) and 13-heptadecyn-1-ol (3.15%), emerged, indicating thermal dehydrogenation and partial cleavage of PUFA chains. Concurrently, the plentiful chlorinated fatty acid derivative, 9,12-octadecadienoyl chloride, decreased from 16.9% to 5.51%, implying thermal dechlorination or volatilization. In summary, the 5-min exposure led to moderate compositional changes marked by isomerization, partial degradation of PUFA, and the emergence of intermediate thermo-oxidative products. Prolonging the IR exposure to 10-min heightened these transformations. Eugenol continued to exhibit a slight decrease (25.9% to 25.6%), while its positional isomer remained stable at 8.57%, indicating ongoing structural rearrangement. Caryophyllene fell to 7.05%, which aligns with further thermal degradation or oxidation of sesquiterpenes. A remarkable change was the notable increase in 9,12-octadecadienoyl chloride, which reached 21.0%, exceeding even the level

observed without exposure. This notable rebound may be attributed to concentration effects, where the loss of more volatile compounds enhances the relative abundance of high-molecular-weight, thermally stable components (Table 3). Identification based on mass spectral libraries and retention index comparison is tentative; confirmation with authentic standards is recommended for future studies. The chlorinated fatty acid derivatives detected in chili oil (*e.g.*, 9,12-octadecadienoyl chloride) are unusual but not unprecedented. Their identification was based on mass spectral matching with NIST library and confirmation of the characteristic chlorine isotope pattern ($M: M+2 \approx 3: 1$). While authentic standards were not available for absolute confirmation, the consistent detection across independent samples and the strong isotopic evidence support their presence. Future studies using high-resolution mass spectrometry or synthesized standards are recommended for definitive confirmation.

Extended IR heating resulted in a notable buildup of stable long-chain alcohols, particularly 1-heptatriacotanol (7.65%), suggesting that high-molecular-weight aliphatic compounds are resistant to volatilization and become more concentrated with longer heating durations. At the same time, the majority of polyunsaturated fatty acid (PUFA) derivatives decreased to minimal levels (<1%), indicating their vulnerability to thermal oxidation, chain scission, and transformation into alkyne or chlorinated derivatives. Finally, the short-term exposure (5-min) mainly induced early-stage chemical modifications such as isomerization, mild lipid oxidation, and generation of alkyne intermediates while retaining much of the original volatile profile. Longer exposure (10-min) resulted in profound compositional restructuring, characterized by degradation of volatile phenolics and PUFA esters, enrichment of heat-stable high-molecular-weight constituents, and the formation or concentration of chlorinated and oxygenated lipid derivatives. The results suggest that IR exposure promoted volatilization, rearrangement, and thermal degradation, potentially affecting the chemical integrity and bioactivity of chili oil. Some changes appeared in the infrared spectra of the oil after IR exposure, consistent with earlier observations that IR treatment causes limited shifts in lipid functional groups (Suri *et al.* 2019). Although structural alterations are minimal, IR processing is known to influence bioactive components. Far infrared (FIR) treated sesame meal, for example, showed nearly double the phenolic content and stronger antioxidant activity than untreated seeds (Lee *et al.* 2005).

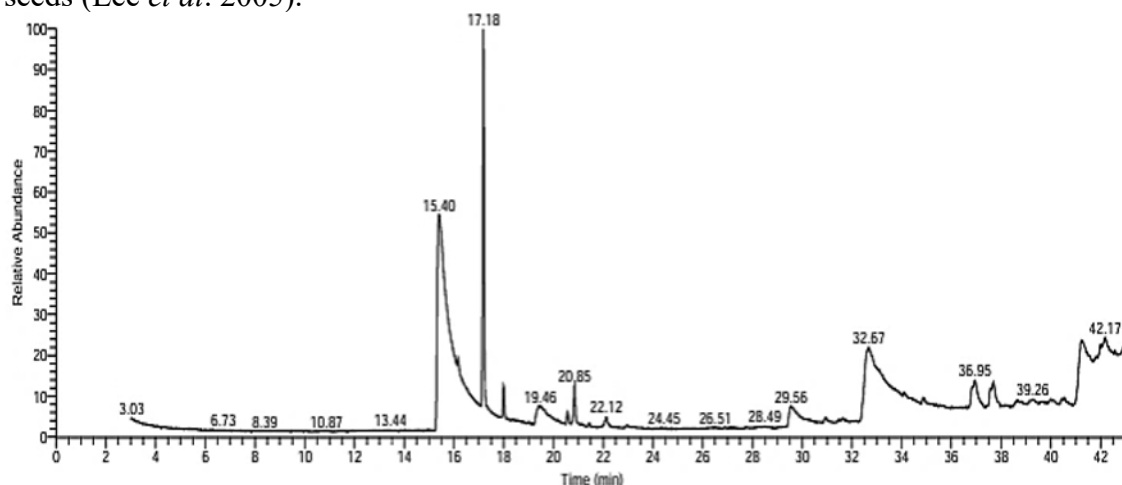


Fig. 1. Chemical composition of chili oil before IR treatment as determined by GC–MS

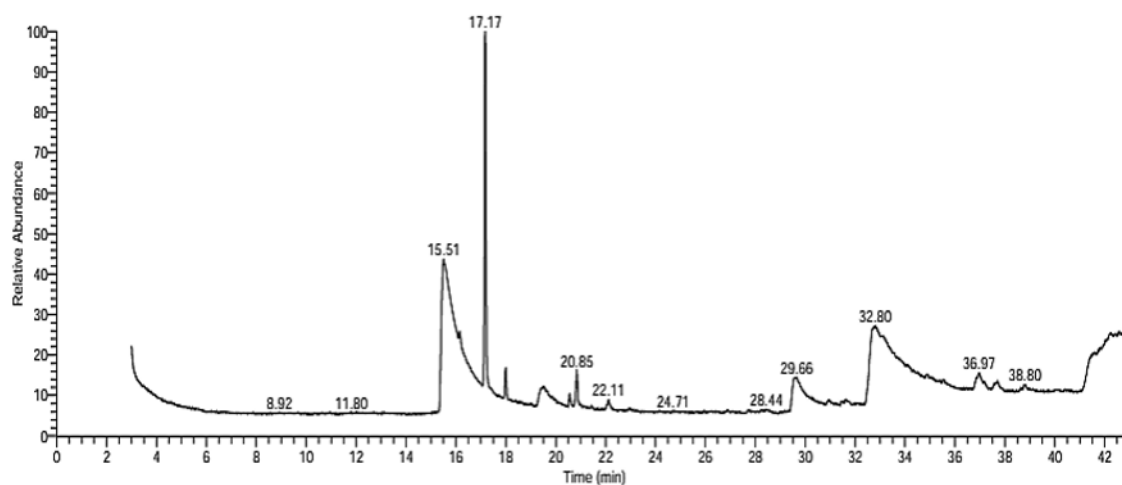


Fig. 2. Chemical composition of chili oil after IR-treatment for 5- min as determined by GC–MS

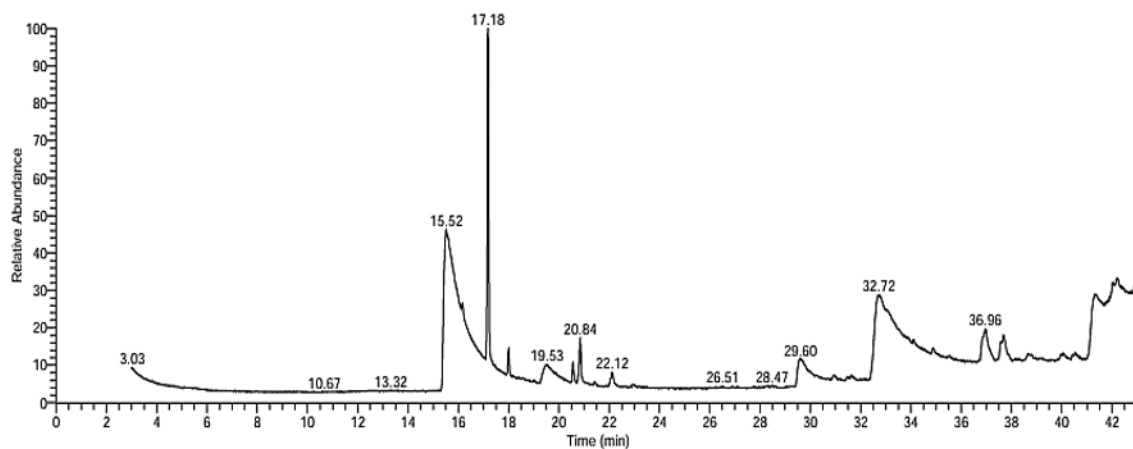


Fig. 3. Chemical composition of chili oil after IR-treatment for 10-min as determined by GC–MS

Table 1. Analysis of Unexposed Chili Oil to Infrared Radiation *via* GC-MS, Including Experimental and Literature Retention Indices (RI)

RT (min)	Area (%)	Compound	Formula	MW (g/mol)	RI _{exp.}	RI _{lit.}
15.39	32.7	Eugenol	C ₁₀ H ₁₂ O ₂	164	1350	1352
17.18	8.2	Caryophyllene	C ₁₅ H ₂₄	204	1425	1427
19.47	1.8	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	C ₁₂ H ₁₄ O ₃	206	1520	1523
19.64	0.9	(E)-Phenol, 2-methoxy-5-(1-propenyl)-	C ₁₀ H ₁₂ O ₂	164	1532	1530
20.56	0.5	4-Allylphenol	C ₉ H ₁₀ O	134	1585	1588
20.85	1.3	Caryophyllene oxide	C ₁₅ H ₂₄ O	220	1602	1600
22.12	0.6	2,5-Octadecadiynoic acid, methyl ester	C ₁₉ H ₃₀ O ₂	290	1720	1725
29.55	3.1	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282	2155	2158
30.29	0.3	Hexadecanoic acid, 2,3-dihydroxypropyl ester	C ₁₉ H ₃₈ O ₄	330	2200	2198
30.94	0.3	2-Aminoethanethiol hydrogen sulfate (ester)	C ₂ H ₇ NO ₃ S ₂	157	2235	—
32.67	16.9	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298	2340	2345
33.70	0.8	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	2380	2382
37.57	0.6	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	306	2560	2563
37.69	1.4	7,11-Hexadecadienal	C ₁₆ H ₂₈ O	236	2572	2570
38.66	0.4	Diacetyl glyceryl linolenate	C ₂₅ H ₄₀ O ₆	436	2625	—
38.82	0.2	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536	2638	2640
39.99	0.3	13-Heptadecyn-1-ol	C ₁₇ H ₃₂ O	252	2760	—
40.43	0.2	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	2805	—
40.55	0.4	1,25-Dihydroxyvitamin D3, TMS derivative	C ₃₀ H ₅₂ O ₃ Si	488	2818	—
41.22	8.3	Glyceryl linoleate	C ₂₁ H ₃₈ O ₄	354	2880	2885
41.81	1.2	Butyl 6,9,12-hexadecatrienoate	C ₂₀ H ₃₄ O ₂	306	2925	—
41.99	1.6	Epoxy dimethyltridecadienoate-d ₃	C ₁₈ H ₂₇ D ₃ O ₃	297	2940	—
42.17	3.2	Bis-TMS glyceryl linolenate	C ₂₇ H ₅₂ O ₄ Si ₂	496	2960	—

Notes: RI_{exp} experimental retention index calculated using a homologous series of n-alkanes (C8–C40) on an HP-5MS column.

RI_{lit} = literature retention index from NIST Chemistry WebBook or published data on DB-5/HP-5 columns; “—” indicates no literature value available

Table 2. Analysis of Exposed Chili Oil to IR-Radiation for 5-min via GC-MS, Including Experimental and Literature Retention Indices (RI)

RT (min)	Area (%)	Compound	Formula	MW (g/mol)	RI _{exp}	RI _{lit}
15.50	25.9	Eugenol	C ₁₀ H ₁₂ O ₂	164	1352	1352
16.16	10.9	Phenol, 2-methoxy-3-(2-propenyl)	C ₁₀ H ₁₂ O ₂	164	1390	1392
17.17	9.4	Caryophyllene	C ₁₅ H ₂₄	204	1424	1427
17.99	0.7	10-Heptadecen-8-ynoic acid, methyl ester, (E)	C ₁₈ H ₃₀ O ₂	278	1485	—
19.86	0.5	Methyl 2,4-tridecadiynoate	C ₁₄ H ₂₀ O ₂	220	1560	—
20.22	0.1	Methyl 5,7-hexadecadiynoate	C ₁₇ H ₂₆ O ₂	262	1582	—
20.56	0.5	7-Hydroxytetralylmethyl acetate	C ₁₃ H ₂₀ O ₃	224	1595	—
20.84	1.2	Caryophyllene oxide	C ₁₅ H ₂₄ O	220	1610	—
20.99	0.1	2,5-Octadecadiynoic acid, methyl ester	C ₁₉ H ₃₀ O ₂	290	1625	—
21.43	0.1	Preg-4-en-3-one, 17 α -hydroxy-17 α -cyano-	C ₂₀ H ₂₇ NO ₂	313	1660	—
22.12	0.5	β -Cyclocitral derivative	C ₂₃ H ₃₂ O	324	1720	—
28.45	0.1	Tetraneurin - A - Diol	C ₁₅ H ₂₀ O ₅	280	2100	—
29.56	1.2	Hexadecanoic acid, 2,3-dihydroxypropyl ester	C ₁₉ H ₃₈ O ₄	330	2158	2198
29.60	0.6	Oleic Acid	C ₁₈ H ₃₄ O ₂	282	2160	2162
29.66	3.8	Estra-1,3,5(10)-trien-17 α -ol	C ₁₈ H ₂₄ O	256	2165	—
30.24	0.3	Dasycarpidan-1-methanol, acetate (ester)	C ₂₀ H ₂₆ N ₂ O ₂	326	2205	—
30.34	0.3	Alanine, 3-(benzyloxy)-, L-	C ₁₀ H ₁₃ NO ₃	195	2220	—
30.41	0.2	Pentadecanoic Acid	C ₁₅ H ₃₀ O ₂	242	2230	2232
30.48	0.2	25-Dihydroxyvitamin D3, TMS derivative	C ₃₀ H ₅₂ O ₃ Si	488	2240	—
30.62	0.2	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536	2255	2640
30.93	0.5	2-Aminoethanethiol hydrogen sulfate (ester)	C ₂ H ₇ NO ₃ S ₂	157	2280	—
31.49	0.2	Hexadecadienoic acid, methyl ester	C ₁₇ H ₃₀ O ₂	266	2320	2325
31.62	0.2	9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254	2335	2338
32.81	5.5	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298	2410	2345
33.11	8.3	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	2435	—
33.71	3.2	13-Heptadecyn-1-ol	C ₁₇ H ₃₂ O	252	2480	—
41.40	1.4	Butyl 6,9,12-hexadecatrienoate	C ₂₀ H ₃₄ O ₂	306	2255	2640
41.75	0.5	Glycidyl oleate	C ₂₁ H ₃₈ O ₃	338	1352	1352
42.02	0.6	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	1390	1392
42.08	0.5	Glyceryl linoleate	C ₂₁ H ₃₈ O ₄	354	1424	1427
42.89	0.2	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308	1485	—
43.00	0.1	Epoxy tricyclotriacontane	C ₃₀ H ₅₂ O ₂	444	1560	—

Notes: RI_{exp} experimental retention index calculated using a homologous series of n-alkanes (C₈–C₄₀) on an HP-5MS column; RI_{lit} = literature retention index from NIST Chemistry WebBook or published data on DB-5/HP-5 columns; “—” indicates no literature value available. Some compounds may show RI differences due to thermal modifications after IR exposure.

Table 3. Analysis of Exposed Chili Oil to IR-Radiation for 10-min *via* GC-MS, Including Experimental and Literature Retention Indices (RI)

RT (min)	Area (%)	Compound	Formula	MW (g/mol)	RI _{exp}	R _{lit}
15.52	25.6	Eugenol	C ₁₀ H ₁₂ O ₂	164	1353	1352
16.16	8.6	Phenol, 2-methoxy-3-(2-propenyl)	C ₁₀ H ₁₂ O ₂	164	1391	1392
17.18	7.1	Caryophyllene	C ₁₅ H ₂₄	204	1426	1427
19.88	0.3	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	C ₁₂ H ₁₄ O ₃	206	1562	1523
20.56	0.7	4-Allylphenol	C ₉ H ₁₀ O	134	1586	1588
20.84	1.5	Caryophyllene oxide	C ₁₅ H ₂₄ O	220	1603	1600
21.43	0.1	6,9,12-Octadecatrienoic acid, methyl ester	C ₁₉ H ₃₂ O ₂	292	1662	1660
29.58	4.1	Hexadecanoic acid, 2,3-dihydroxypropyl ester	C ₁₉ H ₃₈ O ₄	330	2160	2198
30.21	0.7	Aminoethanethiol hydrogen sulfate (ester)	C ₂ H ₇ NO ₃ S ₂	157	2202	—
30.57	0.1	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282	2245	2162
30.65	0.1	Tetraneruin - A diol	C ₁₅ H ₂₀ O ₅	280	2258	—
30.71	0.1	9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254	2265	2338
30.94	0.4	1,25-Dihydroxyvitamin D ₃ , TMS derivative	C ₃₀ H ₅₂ O ₃ Si	488	2285	—
31.47	0.1	Hexadecadienoic acid, methyl ester	C ₁₇ H ₃₀ O ₂	266	2322	2325
32.73	21.0	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298	2405	2345
33.89	0.9	9,12-Octadecadienoic acid (Z,Z)	C ₁₈ H ₃₂ O ₂	280	2490	2382
34.89	0.3	Hexyl bicyclopropyl octanoate	C ₂₁ H ₃₈ O ₂	322	2550	—
36.97	3.1	Monolinolein	C ₂₁ H ₃₈ O ₄	354	2655	2885
37.58	0.7	8,11,14-Eicosatrienoic Acid, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	306	2690	2563
37.68	1.6	13-Heptadecyn-1-ol	C ₁₇ H ₃₂ O	252	2705	—
38.67	0.4	2,3-Diacetyloxypropyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	C ₂₅ H ₄₀ O ₆	436	2770	—
40.08	0.4	Cholest-5-en-3-ol (3á)-	C ₂₇ H ₄₆ O	386	2860	2865
41.30	7.7	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536	2935	2640
42.02	2.5	D ₃ -epoxy fatty acid methyl ester	C ₁₈ H ₂₇ D ₃ O ₃	297	2985	—
42.37	0.5	2-Monolinenin,2TMS derivative	C ₂₇ H ₅₂ O ₄ Si ₂	496	3010	—
42.96	0.2	7,10,13-Eicosatrienoic acid, methyl ester	C ₂₁ H ₃₆ O ₂	320	3050	3055

Notes: RI_{exp} experimental retention index calculated using a homologous series of n-alkanes (C₈–C₄₀) on an HP-5MS column.

RI_{lit} = literature retention index from NIST Chemistry WebBook or published data on DB-5/HP-5 columns; “—” indicates no literature value available

RI values for some compounds may shift after prolonged IR exposure due to thermal degradation or isomerization.

Similar enhancements in carotenoids and free fatty acids have been reported in black cumin and grape seed oils following IR heating (Fu *et al.* 2018; Suri *et al.* 2019). Such findings indicate that IR treatment can enrich antioxidant constituents while maintaining the main fatty acid profile, consistent with the compositional changes observed in IR-treated *Lepidium sativum* oil in this study. Improvements in phenolic and flavonoid levels following infrared exposure have also been documented for *Angelica gigas* Nakai, along with increased antioxidant capacity (Azad *et al.* 2018). Topical investigation by Calleja-Gomez *et al.* (2024) verified that IR treatment can boost the yield of antioxidant constituents and pigments, reporting a notable 10.06% improvement in the recovery of chlorophylls, carotenoids, and phenolic antioxidants.

Likewise, Kim *et al.* (2006) exhibited that integrating IR heating into green tea production significantly strengthened its phytochemical profile. Replacing the traditional roasting step with IR exposure at 90 °C for 10-min led to noticeable rises in total phenolics (from 476 to 811 mg g⁻¹) and total flavanols (from 176 to 209 mg g⁻¹). Levels of key catechins—including epigallocatechin and epigallocatechin gallate—also increased substantially, contributing to improved flavor and overall tea quality. According to Anumudu *et al.* (2024), IR heating offers technological advantages in food processing by promoting efficient heat transfer while protecting nutritional and sensory attributes. Extraction yields, fatty acids composition, and functional properties of vegetable oil were improved *via* IR processing (Pandiselvam *et al.* 2025).

The quantitative data indicate that chili oil exposed to IR for 5-min achieved the highest inhibitory activity among all treatments. At this exposure time, the oil produced the largest inhibition zones: *B. subtilis* increased from 22 ± 0.4 mm to 25 ± 0.2 mm, *S. aureus* from 19 ± 0.5 mm to 23 ± 0.7 mm, *E. coli* from 14 ± 0.1 mm to 18 ± 0.5 mm, *S. typhi* from 13 ± 0.2 mm to 19 ± 0.2 mm, and *C. albicans* from 23 ± 0.6 mm to 25 ± 0.3 mm. This enhancement was further supported by reductions in MIC values, which dropped from 31.25 to 15.62 µg/mL for *B. subtilis* and *C. albicans*, and from 62.5 to 31.25 µg/mL for *S. aureus*, *E. coli*, and *S. typhi*. Similarly, the MBC/MFC values decreased significantly, such as the decline from 125 to 31.25 µg/mL for *S. typhi* and from 62.5 to 31.25 µg/mL for *B. subtilis*. Notably, the inhibition zones of exposed and exposed chili oil exposed to IR for different times were comparable to those produced by the standard antimicrobial agent, highlighting the strong bioactivity achieved after exposure to IR for 5 min. These quantitative findings confirm that 5-min IR exposure resulted in the maximum antimicrobial inhibition and represented the optimal treatment time for enhancing the bioactivity of chili oil. This suggests that short-term IR treatment may induce chemical or structural modifications in the oil that strengthen its interaction with microbial cells.

Eugenol, identified as the predominant constituent in the oil, has been reported to exhibit a broad spectrum of biological activities. Previous research has highlighted its strong antioxidant, anti-inflammatory, analgesic, and antimutagenic potential, along with notable anti-platelet and anti-allergic effects (Ulanowska and Olas 2021). In addition, eugenol exhibits pronounced antimicrobial activity against several clinically relevant bacteria and fungi, including *S. aureus*, *Pseudomonas aeruginosa*, and *E. coli*. This activity is largely attributed to the presence of its free phenolic hydroxyl group. Enhanced antimicrobial properties have also been reported for chemically modified eugenol derivatives; for example, Da Silva *et al.* (2018) found that esterified or functionally substituted forms of eugenol displayed lower MIC values (500 µg/mL) than the parent

molecule (1000 µg/mL). These documented biological effects of eugenol help to explain the strong antimicrobial and biofunctional responses observed in the IR-treated oil examined in the present study. Eugenol also exhibits considerable antifungal activity, with documented effects against *Candida albicans*, *Penicillium italicum*, *Aspergillus niger*, *Fusarium oxysporum*, *Trichophyton* spp., and other clinically relevant fungi. Its antifungal mechanism involves perturbation of membrane function, suppression of virulence factors, and prevention of biofilm creation (Marchese *et al.* 2017).

Infrared-treated chili oil demonstrated a clear dose- and exposure-time-dependent biofilm inhibition against all tested bacteria. At 25% MBC, biofilm inhibition ranged from $41.95 \pm 1.53\%$ to $78.46 \pm 2.52\%$ for *B. subtilis*, $38.23 \pm 2.52\%$ to $80.76 \pm 2.52\%$ for *S. aureus*, $55.61 \pm 2.52\%$ to $74.94 \pm 2.00\%$ for *E. coli*, and $42.11 \pm 2.00\%$ to $74.52 \pm 2.08\%$ for *S. typhi*, with the highest inhibition consistently observed after 5-min of IR exposure. At 50% MBC, inhibition further increased, reaching $70.04 \pm 2.08\%$ to $86.59 \pm 2.52\%$ for *B. subtilis*, $60.73 \pm 2.52\%$ to $86.18 \pm 2.08\%$ for *S. aureus*, $83.06 \pm 2.00\%$ to $87.56 \pm 1.53\%$ for *E. coli*, and $71.56 \pm 2.08\%$ to $92.03 \pm 2.00\%$ for *S. typhi*. The maximum biofilm inhibition was observed at 75% MBC, where values ranged from $82.09 \pm 2.08\%$ to $93.95 \pm 2.00\%$ for *B. subtilis*, $83.40 \pm 2.52\%$ to $95.23 \pm 2.00\%$ for *S. aureus*, $87.88 \pm 2.00\%$ to $95.12 \pm 2.00\%$ for *E. coli*, and $82.33 \pm 2.00\%$ to $94.19 \pm 2.00\%$ for *S. typhi*. Across all concentrations, 5-min of IR exposure consistently yielded the highest inhibition, indicating that a brief IR treatment significantly enhances the antibiofilm potential of chili oil. The chili oil treated with IR exhibited a distinct biofilm inhibition that was dependent on both the dosage and the duration of exposure against all tested bacteria. At 25% MBC, the biofilm inhibition varied from $41.95 \pm 1.53\%$ to $78.46 \pm 2.52\%$ for *B. subtilis*, from $38.23 \pm 2.52\%$ to $80.76 \pm 2.52\%$ for *S. aureus*, from $55.61 \pm 2.52\%$ to $74.94 \pm 2.00\%$ for *E. coli*, and from $42.11 \pm 2.00\%$ to $74.52 \pm 2.08\%$ for *S. typhi*, with the most inhibition consistently noted after 5-min of IR exposure. At 50% MBC, the inhibition further escalated, achieving values between $70.04 \pm 2.08\%$ and $86.59 \pm 2.52\%$ for *B. subtilis*, from $60.73 \pm 2.52\%$ to $86.18 \pm 2.08\%$ for *S. aureus*, from $83.06 \pm 2.00\%$ to $87.56 \pm 1.53\%$ for *E. coli*, and from $71.56 \pm 2.08\%$ to $92.03 \pm 2.00\%$ for *S. typhi*. The peak biofilm inhibition was recorded at 75% MBC, where the values ranged from $82.09 \pm 2.08\%$ to $93.95 \pm 2.00\%$ for *B. subtilis*, from $83.40 \pm 2.52\%$ to $95.23 \pm 2.00\%$ for *S. aureus*, from $87.88 \pm 2.00\%$ to $95.12 \pm 2.00\%$ for *E. coli*, and from $82.33 \pm 2.00\%$ to $94.19 \pm 2.00\%$ for *S. typhi*. Throughout all concentrations, a 5-min IR exposure consistently resulted in the highest inhibition, suggesting that a short IR treatment notably boosts the antibiofilm efficacy of chili oil. Von Borowski *et al.* (2019) reported the inhibition of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* biofilms with 60% and 80%, respectively by extract from seeds of *Capsicum* red peppers. In another study biofilm of various *Candida* including *C. tropicalis* and *C. glabrata* was inhibited with level 80% at 187.5 µg/mL MBC of chilli extract (Menezes *et al.* 2022). In previous investigation (Sora *et al.* 2025), fatty acids contents of *Capsicum* extracts demonstrated inhibition of *Candida* spp.

The killing kinetics of IR-treated chili oil demonstrated a time- and exposure-dependent reduction in bacterial viability. At 30 min, 5-min IR exposure reduced *B. subtilis* from $3.2 \times 10^5 \pm 0.4$ CFU/mL to $2.1 \times 10^3 \pm 1.0$ CFU/mL and *S. aureus* from $2.4 \times 10^5 \pm 0.7$ to $2.6 \times 10^3 \pm 1.0$ CFU/mL, while 10-min exposure showed slightly higher reductions. Gram-negative bacteria, *E. coli* and *S. typhi*, also exhibited decreases; for example, *E. coli* dropped from $2.1 \times 10^5 \pm 0.5$ to $2.5 \times 10^2 \pm 3$ CFU/mL after 5-min IR treatment at 60 min.

Substantial reduction ($\geq 3 \log_{10}$) in microbial viability was observed at 360 min, with no detectable colonies for some treatment combinations for all IR-exposed samples, while untreated samples retained higher CFU counts. These outcomes indicate that 5 to 10 min IR exposure noticeably improved the bactericidal effect of chili oil, with Gram-positive bacteria being slightly more susceptible. Time killing analysis of chili crude extract was assessed by Thavornsawadi *et al.* (2021); that study visualized completed destruction of bacterial mixture at doses above 10.0% w/v.

Table 4. Antimicrobial Activity of Chili Oil Exposed to IR at Different Times (0-, 5-, and 10-min)

Tested Microbes	Zone of Inhibition (mm)			MIC ($\mu\text{g/mL}$)			MBC/MFC ($\mu\text{g/mL}$)		
	0 min	5 min	10 min	0 min	5 min	10 min	0 min	5 min	10 min
<i>B. subtilis</i>	22 ± 0.4^b	25 ± 0.2^a	23 ± 0.1^b	31.25 ^b	15.62 ^a	31.25 ^b	62.5 ^b	31.25 ^a	62.5 ^b
<i>S. aureus</i>	19 ± 0.5^b	23 ± 0.7^a	19 ± 0.3^b	62.5 ^b	31.25 ^a	62.5 ^b	125 ^b	62.5 ^a	125 ^b
<i>E. coli</i>	14 ± 0.1^c	18 ± 0.5^a	16 ± 0.1^b	62.5 ^b	31.25 ^a	62.5 ^b	125 ^b	62.5 ^a	125 ^b
<i>S. typhi</i>	13 ± 0.2^c	19 ± 0.2^a	17 ± 0.2^b	62.5 ^b	31.25 ^a	31.25 ^a	125 ^c	31.25 ^a	62.5 ^b
<i>C. albicans</i>	23 ± 0.6^b	25 ± 0.3^a	22 ± 0.5^b	31.25 ^b	15.62 ^a	31.25 ^b	62.5 ^b	31.25 ^a	62.5 ^b

Superscript letters that differ within the same row indicate a significant difference ($p < 0.05$).

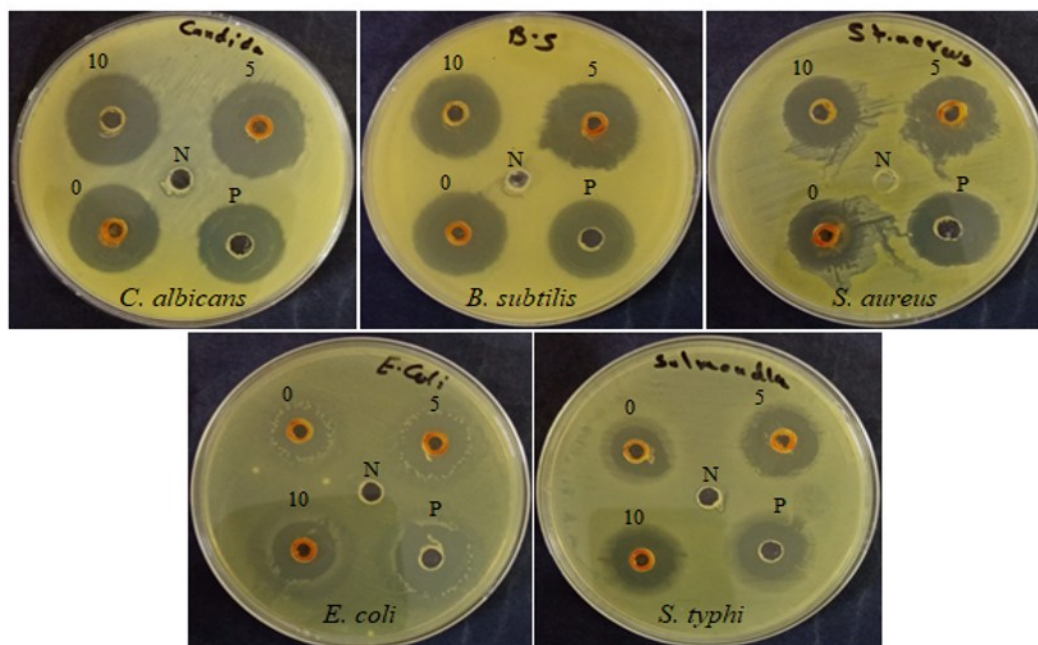


Fig. 4. Well diffusion assays were achieved to evaluate the antimicrobial outcome of chili oil following IR treatment at three exposure times (0, 5, and 10 min). The assays were conducted against various microbial strains. N denotes the negative control (solvent), and P denotes the positive control (standard antimicrobial agent).

Table 5. Biofilm Inhibition Potential of IR-Treated Chili Oil at Different Exposure Times (0-, 5-, 10- min) and Concentrations (0%, 25%, 50%, and 75% MBC)

Conc. MBC %	<i>B. subtilis</i>			<i>S. aureus</i>		
	0 min	5 min	0 min	5 min	0 min	5 min
0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
25	41.95 ± 1.53	78.46 ± 2.52	65.80 ± 2.52	38.23 ± 2.52	80.76 ± 2.52	53.62 ± 2.52
50	70.04 ± 2.08	86.59 ± 2.52	80.61 ± 2.52	60.73 ± 2.52	86.18 ± 2.08	85.19 ± 2.08
75	82.09 ± 2.08	93.95 ± 2.00	90.35 ± 2.00	83.40 ± 2.52	95.23 ± 2.00	89.26 ± 2.00
Conc. MBC %	<i>E. coli</i>			<i>S. typhi</i>		
	0 min	5 min	10 min	0 min	5 min	10 min
0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
25	55.61 ± 2.52	74.94 ± 2.00	68.07 ± 2.00	42.11 ± 2.00	74.52 ± 2.08	62.43 ± 2.00
50	83.06 ± 2.00	87.56 ± 1.53	84.64 ± 2.52	71.56 ± 2.08	92.03 ± 2.00	77.42 ± 2.00
75	87.88 ± 2.00	95.12 ± 2.00	89.74 ± 2.00	82.33 ± 2.00	94.19 ± 2.00	92.17 ± 2.00

Table 6. Killing Kinetics of IR-Treated Chili Oil against Selected Bacteria

Killing Kinetic (min)	CFU /mL					
	<i>B. subtilis</i>			<i>S. aureus</i>		
	0 min	5 min	10 min	0 min	5 min	10 min
0	32×10 ⁵ ±0.4	32×10 ⁵ ±0.4	32×10 ⁵ ±0.4	24×10 ⁵ ±0.7	24×10 ⁵ ±0.7	24×10 ⁵ ±0.7
30	11×10 ⁴ ±0.6	21×10 ³ ±1.0	34×10 ³ ±2.0	85×10 ⁴ ±0.8	26×10 ³ ±1	29×10 ⁴ ±1
60	56×10 ³ ±0.2	27×10 ² ±0.8	41×10 ² ± 0.4	121×10 ³ ±0.4	35×10 ² ±1.0	91×10 ³ ±0.8
120	49×10 ² ±0.9	12×10±1.0	29×10 ±0.	144×10 ² ±1.0	36×10±0.6	14×10 ² ±1.4
150	78×10 ±1.2	0	228±2.0	90×10 ² ±1.0	156	35×10 ±1.0
180	222±0.3	0	12	97×10	18	227
360	15	0	0	25	0	0
Killing Kinetic (min)	<i>E. coli</i>			<i>S. typhi</i>		
	0 min	5 min	10 min	0 min	5 min	10 min
0	21×10 ⁵ ±0.5	21×10 ⁵ ±0.5	21×10 ⁵ ±0.5	19×10 ⁵ ±1.0	19×10 ⁵ ±1.0	19×10 ⁵ ±1.0
30	83×10 ⁴ ±2.4	39×10 ⁴ ±1.0	55×10 ⁴ ±0.1	94×10 ⁴ ±1.2	34×10 ⁴ ±2	74×10 ⁴ ±0.5
60	35×10 ³ ±1.5	25×10 ² ±3	234×10 ³ ±0.9	153×10 ³ ±1.0	19×10 ³ ±1	32×10 ³ ±1.2
120	26×10 ² ±0.5	19×10±1.0	32×10 ² ±0.4	93×10 ² ±2.0	94×10±1	21×10 ² ±2.0
150	182×10±0.8	173	12×10±1.8	53×10 ±0.6	145	24×10 ±1.0
180	214±1	9	26	115	32	42
360	10	0	0	0	0	0

Anti-inflammatory

The anti-inflammatory activity of IR-treated chili oil at different exposure times (0, 5, and 10 min) was evaluated using protein denaturation inhibition, with diclofenac sodium serving as the positive control (Table 7). In all treatments, inhibition increased dose-dependently. Higher chili oil concentrations produced stronger anti-inflammatory effects. At the lowest dose (1.56 µg/g), the 5-min and 10-min IR-treated oils already showed enhanced activity (41.9 ± 2.0% and 33.6 ± 1.5%, respectively) compared with the

untreated sample ($21.3 \pm 1.0\%$). This trend continued as the dose increased. For instance, at $25 \mu\text{g/g}$, inhibition reached $75.9 \pm 3.6\%$ (5 min) and $67.5 \pm 3.0\%$ (10 min), clearly outperforming the untreated oil ($56.7 \pm 2.4\%$). The highest activity was observed at $200 \mu\text{g/g}$, where IR-treated oils reached $92.8 \pm 4.2\%$ (5 min) and $87.4 \pm 3.8\%$ (10 min) inhibition, approaching the inhibition level of diclofenac sodium ($94.4 \pm 4.5\%$). This indicates that IR treatment improves the bioactive potency of chili oil, approaching the activity of the reference drug diclofenac sodium at the highest tested concentration at high doses. The IC_{50} values further authorize the enhancement in activity with IR exposure. The 5-min IR-treated oil exhibited the strongest effect ($\text{IC}_{50} = 2.72 \pm 0.14 \mu\text{g/g}$), even lower than the 10-min treated sample ($\text{IC}_{50} = 6.03 \pm 0.29 \mu\text{g/g}$) and noticeably better than the untreated oil ($\text{IC}_{50} = 14.99 \pm 0.75 \mu\text{g/g}$). This suggests that moderate IR exposure (5-min) optimizes the anti-inflammatory components in chili oil, possibly by improving the release or structural modification of active phytochemicals. According to Cortes-Ferre *et al.* (2022), chili pepper seeds extract exhibited anti-inflammatory effects and therefore holds promise for topical therapeutic utilizations. In addition to its antimicrobial properties, bioinformatics and molecular analyses indicate that eugenol (main constituent in chili oil) may exert notable anti-inflammatory effects by suppressing COX-2 and 5-LOX enzymatic activity (de Andrade and Mendes 2020). This aligns with cellular studies, which suggest that eugenol reduces superoxide production by inhibiting the Raf/MEK/ERK1/2/p47-phosphorylation signaling cascade and attenuating the expression of key pro-inflammatory cytokines, including IL-1 β , IL-6, and TNF- α (Hwang *et al.* 2020). Taken together, these insights highlight that the elevated eugenol content in the chili oil likely plays a major role in enhancing both its antimicrobial and anti-inflammatory efficacy.

Table 7. Anti-inflammatory Potential of IR-Treated Chili Oil at Different Exposure Times (0-, 5-, 10- min) and Doses

Dose ($\mu\text{g/g}$)	Inhibition of Protein Denaturation (%)			
	IR-Treated Chili Oil			Diclofenac Sodium
	0 min	5-min	10-min	
0.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1.56	21.3 ± 1.0^c	41.9 ± 2.0^a	33.6 ± 1.5^b	45.5 ± 2.1^a
3.125	30.5 ± 1.3^c	50.9 ± 2.2^a	42.3 ± 1.8^b	52.9 ± 2.4^a
6.25	39.6 ± 1.7^c	59.5 ± 2.9^a	50.1 ± 2.2^b	59.9 ± 2.8^a
12.5	48.1 ± 2.0^c	67.7 ± 3.3^a	58.8 ± 2.6^b	67.7 ± 3.1^a
25	56.7 ± 2.4^c	75.9 ± 3.6^a	67.5 ± 3.0^b	75.9 ± 3.4^a
50	72.4 ± 2.9^c	88.2 ± 3.9^a	81.8 ± 3.5^b	89.7 ± 4.1^a
100	65.8 ± 2.6^c	82.8 ± 3.7^a	76.3 ± 3.3^b	82.9 ± 3.8^a
200	81.9 ± 3.2^c	92.8 ± 4.2^a	87.4 ± 3.8^b	94.4 ± 4.5^a
$\text{IC}_{50} (\mu\text{g/g})$	14.99 ± 0.75	2.72 ± 0.14	6.03 ± 0.29	2.31 ± 0.12

Values in the same row with different superscript letters are significantly different ($p < 0.05$).

Antioxidant

Table 8 shows the DPPH radical scavenging potential of IR-treated chili oil at different exposure times (0, 5, 10 min) and doses, compared with ascorbic acid. The antioxidant activity increased dose-dependently for all samples. Among IR-treated chili oil samples, 5-min exposure showed the strongest activity, achieving $96.4 \pm 1.91\%$ at $1000 \mu\text{g/mL}$ with an IC_{50} of $5.65 \pm 0.20 \mu\text{g/mL}$. This was followed by 10 min (achieving $92.6 \pm 1.914\%$ at $1000 \mu\text{g/mL}$ with an IC_{50} of $17.26 \pm 0.50 \mu\text{g/mL}$) and 0 min exposures, indicating that IR treatment for 5-min enhances radical scavenging. Ascorbic acid exhibited the highest activity, reaching $97.7 \pm 1.45\%$ at $1000 \mu\text{g/mL}$ with an IC_{50} of $2.30 \pm 0.10 \mu\text{g/mL}$. The IC_{50} values further confirm that longer exposure required higher concentrations to reach 50% inhibition, while shorter exposure significantly improved antioxidant potency. These results demonstrate that IR treatment effectively increased the antioxidant activity of chili oil, making it a promising natural radical scavenger. The antioxidant potential of red pepper seed was reported (Bal *et al.* 2022). Previous investigation by Medina-Juarez *et al.* (2012) indicated that antioxidant activity of chili pepper depends on its chemical content which may agreement with the present investigation.

Table 8. DPPH Radical Scavenging Potential of IR-Treated Chili Oil at Different Exposure Times (0-, 5-, 10- min) and Doses

Dose ($\mu\text{g/mL}$)	DPPH Scavenging (%)			
	0 min	5 min	10 min	Ascorbic Acid
0.0	0.00 ± 0.00	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
1.95	26.6 ± 7.58^c	37.9 ± 7.58^b	23.5 ± 7.58^d	44.8 ± 0.95^a
3.9	34.8 ± 6.52^c	44.5 ± 6.52^b	32.1 ± 6.52^d	52.7 ± 1.05^a
7.81	42.7 ± 6.34^c	52.6 ± 6.34^b	40.8 ± 6.34^d	60.6 ± 1.10^a
15.62	51.6 ± 5.50^c	59.7 ± 5.50^b	49.2 ± 5.50^d	68.3 ± 1.15^a
31.25	60.1 ± 5.56^c	68.3 ± 5.56^b	57.7 ± 5.56^d	76.3 ± 1.20^a
62.5	69.1 ± 4.93^c	76.4 ± 4.93^b	67.0 ± 4.93^d	81.5 ± 1.25^a
125	76.3 ± 5.19^c	84.5 ± 5.19^b	74.9 ± 5.19^d	87.9 ± 1.30^a
250	84.4 ± 4.14^c	90.7 ± 4.14^b	82.9 ± 4.14^d	93.3 ± 1.35^a
500	90.2 ± 2.51^c	93.6 ± 2.51^b	88.7 ± 2.51^d	95.9 ± 1.40^a
1000	94.1 ± 1.91^c	96.4 ± 1.91^b	92.6 ± 1.91^d	97.7 ± 1.45^a
$\text{IC}_{50} \mu\text{g/mL}$	14.10 ± 0.40	5.65 ± 0.20	17.26 ± 0.50	2.30 ± 0.10

Values in the same row with different superscript letters are significantly different ($p < 0.05$).

CONCLUSIONS

1. Infrared (IR) treatment of chili oil was effective for modulations of oil chemical composition and enhancing its biological activities.
2. A 5-min IR exposure may have contributed to partial isomerization of phenolics and moderate degradation of polyunsaturated fatty acids, potentially generating new bioactive compounds.
3. This treatment maximized antimicrobial efficacy against both bacterial and fungal strains, pointedly reduced MIC/MBC values, and strongly inhibited biofilm formation in a dose- and time-dependent manner.
4. Additionally, antioxidant and anti-inflammatory activities were highest after 5-min of IR exposure

5. Short-term IR processing may optimize chili oil's phytochemical profile, suggesting enhanced potential.

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