

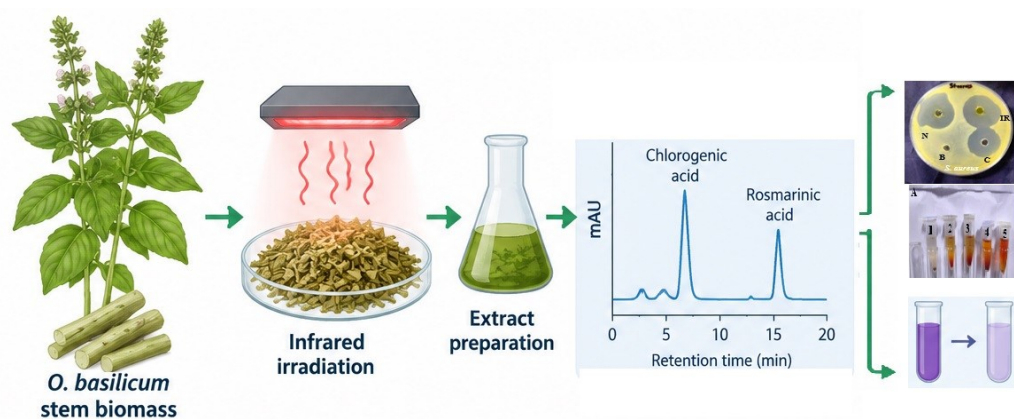
Chemical Reconfiguration of *Ocimum basilicum* Stem Biomass by Infrared Processing and Its Impact on Antimicrobial and Antioxidant Activities

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GRAPHICAL ABSTRACT



Chemical Reconfiguration of *Ocimum basilicum* Stem Biomass by Infrared Processing and Its Impact on Antimicrobial and Antioxidant Activities

Hanan Moawad ^{a,*} and Aisha M. H. Al-Rajhi ^b

This study evaluated the effect of controlled IR irradiation on the phenolic profile, and bioactivities of *Ocimum basilicum* stem biomass extract. Polyphenolic profiling was performed using HPLC. Antimicrobial activity was assessed using agar diffusion and Time–kill kinetics. Antioxidant activity was evaluated using DPPH assay. HPLC analysis revealed increased chlorogenic acid and rosmarinic acid after IR treatment. MIC values decreased fourfold for *E. faecalis* and *P. aeruginosa*, and twofold for *S. aureus* and *C. albicans*. Time–kill assays showed accelerated bactericidal activity, with complete eradication within 3 h for most strains following IR treatment. Antioxidant activity and antihemolytic protection were also enhanced changed to: IR treatment enhanced antioxidant activity, reducing the IC₅₀ from 9.52 ± 0.78 to 6.55 ± 0.03 µg/mL, while antihemolytic protection increased from 32%, 51%, and 68% to 46%, 69%, and 84% at 25%, 50%, and 75% MIC, respectively. Controlled IR irradiation selectively modulated the phenolic content of *O. basilicum* stem extract, resulting in measurable enhancement of antimicrobial and antioxidant performance. These findings suggest that IR treatment may be a useful approach for modifying the phytochemical and functional properties of plant extracts, although the contributions of IR-specific and thermal effects require further investigation.

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Keywords: Medicinal herb stems; *Ocimum basilicum*; Infrared irradiation; Polyphenolic profiling; Antimicrobial; Antihemolytic activity

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INTRODUCTION

Microbial diseases remain a persistent and evolving threat to global public health. The rapid emergence of antimicrobial resistance, the spread of opportunistic pathogens in healthcare and community settings, and the limited pace of new antibiotic discovery have intensified the search for alternative therapeutic strategies (Sandu *et al.* 2025). Bacterial and fungal infections continue to impose substantial morbidity, mortality, and economic burden worldwide. In this context, identifying safe, accessible, and biologically effective agents—particularly from natural sources—has become a research priority (Al-Rajhi *et al.* 2023; Salam *et al.* 2023; Liu *et al.* 2025). Natural products have historically served as a foundation for drug discovery and remain a rich reservoir of bioactive molecules. Plant-derived extracts contain diverse secondary metabolites, including polyphenols, flavonoids,

terpenoids, and alkaloids, many of which exhibit different biological activities (Abdou *et al.* 2025; Alghamdi *et al.* 2026; Alharthi *et al.* 2026). These compounds may act through multiple mechanisms, such as disruption of microbial membranes, inhibition of enzymatic systems, interference with quorum sensing, and modulation of oxidative stress (Alsalamah *et al.* 2026). Compared with synthetic drugs, plant extracts often provide complex phytochemical matrices that can produce synergistic biological effects (Pérez-Flores *et al.* 2025). Consequently, medicinal plants are increasingly investigated as potential alternatives or adjuncts to conventional antimicrobial therapies (Seukep *et al.* 2025).

Among aromatic medicinal plants, *Ocimum basilicum* (sweet basil) occupies a prominent position in traditional medicine and ethnopharmacology. Belonging to the family Lamiaceae, this annual herb is widely cultivated across temperate and tropical regions (Azizah *et al.* 2023). It is characterized by its fragrant leaves, which are rich in essential oils and phenolic constituents (Zhakipbekov *et al.* 2024). In folk medicine, different parts of *O. basilicum* have been used to manage respiratory disorders, gastrointestinal disturbances, inflammation, headaches, and infections. Phytochemical investigations have identified numerous bioactive compounds, including phenolic acids, flavonoids, and volatile constituents, which contribute to its reported antimicrobial and antioxidant properties (Antonescu *et al.* 2021). Despite extensive recent investigations into the phytochemistry and biological activities of *O. basilicum*, contemporary studies continue to emphasize leaf material and essential oils, whereas stem tissues are commonly grouped within aerial biomass and rarely subjected to independent phytochemical or pharmacological characterization. This indicates a relative underrepresentation of stem-derived extracts in current research (Vassilina *et al.* 2025).

Recent advances in phytochemical research have explored physical and non-thermal technologies to modify plant extracts and enhance their biological activity. The application of infrared (IR) energy has attracted increasing attention as a processing approach for natural products because of its potential to modify their chemical and functional characteristics. Previous investigations have shown that IR exposure can affect the composition of bioactive constituents and, consequently, alter the biological properties of the treated material (Abdou *et al.* 2025; Hashempur *et al.* 2025). More recently, Qanash *et al.* (2026) reported that brief post-extraction IR irradiation of bee venom modified its chemical profile and was accompanied by changes in antimicrobial, antioxidant, antibiofilm, and anti-inflammatory activities. Similar effects have been observed in plant-derived materials, suggesting that the outcome of IR treatment is influenced by the characteristics of the biological matrix, irradiation conditions, and associated thermal effects. Although IR radiation has lower photon energy than UV radiation, it can still induce chemical changes through molecular vibrational excitation and associated thermal effects. These processes may promote bond weakening, molecular rearrangement, hydrolysis, oxidation, or other temperature-dependent transformations, depending on the material and irradiation conditions. Previous studies have shown that IR treatment can modify the chemical composition and functional properties of natural-product matrices (López-Gámez *et al.* 2021; Bheemaiah *et al.* 2024). Thus, the changes observed in the phenolic profile of *O. basilicum* stem extract may plausibly result from IR-induced vibrational and thermal effects, although the underlying mechanisms require further investigation. Nevertheless, the use of short-term post-extraction IR treatment for *Ocimum basilicum* stem extracts remains insufficiently investigated. In particular, its potential effects on the phenolic composition and related antioxidant and antimicrobial activities of basil stem extracts have

not been adequately characterized. Therefore, the present study investigated the effects of IR treatment on the phytochemical composition and biological activities of *O. basilicum* stem extract. In parallel with chemical characterization, *in vitro* biological assays play a crucial role in evaluating the therapeutic potential of natural extracts. These approaches collectively offer mechanistic understanding and reproducible evidence prior to *in vivo* validation (Shahidi and Samarasinghe 2025). The present study investigated the impact of infrared irradiation on stem biomass of *O. basilicum* (i) to evaluate IR-induced alterations in the polyphenolic profile and assess changes in antimicrobial activity, antioxidant and antihemolytic properties in the presence of microbial challenge.

EXPERIMENTAL

Plant Material Collection and Authentication

Fresh specimens of *O. basilicum* were obtained from, Jazan region, Saudi Arabia. Whole plants were collected during the vegetative stage and transported to the laboratory in sterile polyethylene bags. Taxonomic identification of *O. basilicum* was confirmed using standard morphological and taxonomic keys.

Preparation of *O. basilicum* Stem Material

Stems of *O. basilicum* were carefully separated from leaves and flowers to avoid phytochemical overlap from other aerial parts. The stems were rinsed with distilled water to remove adhered particles and dried under shade at ambient temperature (25 ± 2 °C) in a well-ventilated room for 10 to 14 days to preserve thermosensitive constituents. The dried *O. basilicum* stems were pulverized using a laboratory electric grinder (Retsch GmbH, Haan, Germany) to obtain a homogeneous powder. The powdered material was stored in amber-colored airtight containers at 4 °C until infrared irradiation and extraction (Shoeib *et al.* 2022).

Infrared Irradiation of *O. basilicum* Stem Biomass

A portion of the dried powdered material of *O. basilicum* stem biomass was subjected to infrared (IR) irradiation to investigate potential structural modification of polyphenolic compounds. IR exposure was performed using a near-infrared lamp (Philips Infrared Lamp, 150 W, Eindhoven, Netherlands). Approximately 5 g of powder was evenly distributed as a thin layer (<2 mm thickness) in a sterile glass Petri dish to ensure homogeneous irradiation. The IR source was positioned at a fixed distance of 20 cm from the extract surface and applied for 10 minutes. Surface temperature was continuously monitored using a digital infrared thermometer (Fluke Corporation, USA) to ensure that it did not exceed 45 °C, minimizing thermal degradation of bioactive constituents. Following irradiation, the treated *O. basilicum* stem biomass was stored in containers until chromatographic and biological analyses.

Extraction of Phenolic and Flavonoid Compounds from *O. basilicum* Stems

Twenty grams of dried *O. basilicum* stem powder (IR-treated and untreated) was extracted using 80% methanol (analytical grade, Merck, Darmstadt, Germany). The powder was soaked in 200 mL solvent and agitated at 150 rpm in a temperature-controlled orbital shaker (Heidolph Instruments GmbH, Germany) at 25 °C for 48 h. After extraction,

the mixture was filtered through Whatman No. 1 filter paper. The residual plant material was re-extracted under identical conditions to maximize recovery of phenolic and flavonoid constituents. The combined filtrates were concentrated under reduced pressure using a rotary evaporator (Buchi R-300, Switzerland) at 40 °C. The resulting crude extract of *O. basilicum* stems was further dried in a vacuum oven to constant weight and stored at -20 °C pending analysis (Romano *et al.* 2022).

HPLC Characterization of Polyphenols and Flavonoids

Quantitative and qualitative profiling of flavonoid and phenolic constituents in crude and infrared-treated extracts of *O. basilicum* stems was conducted using a high-performance liquid chromatography system (Shimadzu LC-20AT, Kyoto, Japan) coupled with a photodiode array (PDA) detector. Detection was performed at 285 nm, selected based on optimal absorbance of phenolic structures. Chromatographic separation was achieved using a reversed-phase C18 column (Phenomenex Luna C18, 4.6 × 250 mm, 5 µm particle size), thermostatically maintained at 40 °C. The injection volume for all samples was 10 µL. The mobile phase consisted of solvent A (0.05% trifluoroacetic acid in ultrapure water) and solvent B (HPLC-grade acetonitrile). Elution was carried out under a programmed gradient system as follows: 0 to 5 min (80% A), 5 to 8 min (65% A), 8 to 12 min (60% A), 12 to 15 min (82% A), 15 to 18 min (80% A), and 18 to 23 min (80% A) for column re-equilibration. The flow rate was maintained at 1.0 mL/min. Peak identification was performed by comparing retention times and UV spectral characteristics with authenticated standards. The standard mixture contained 18 compounds which purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). It included: gallic acid, chlorogenic acid, catechin, methyl gallate, caffeic acid, syringic acid, rutin, ellagic acid, p-coumaric acid, vanillin, ferulic acid, naringenin, rosmarinic acid, daidzein, quercetin, cinnamic acid, kaempferol, and hesperetin. Quantification was based on external calibration curves generated from serial dilutions of reference compounds, and results were expressed as µg/g dry extract (Al-Rajhi *et al.* 2025).

Antimicrobial Activity of Crude and IR-Treated Extracts

The antimicrobial activity of crude and IR-treated *O. basilicum* stem extracts was evaluated against *Enterococcus faecalis* (ATCC 29213), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 13315), *Candida albicans* (ATCC 90028), and *Aspergillus terreus* (AUMC 15762). For the agar well diffusion assay, bacterial suspensions were spread onto Mueller–Hinton agar, whereas fungal strains were inoculated onto malt extract agar. Wells (6 mm) were prepared in the agar, and 100 µL of each extract concentration was added. Gentamicin (10 µg/mL) and fluconazole (500 µg/mL) were used as positive controls for bacterial and fungal assays, respectively. Plates were pre-incubated at 4 °C for 30 min to facilitate diffusion and then incubated at 37 °C for 24 h for bacteria and 28 °C for 3 to 5 days for fungi. Inhibition zones were measured in millimeters (Al-Rajhi *et al.* 2023).

MIC values were determined by broth microdilution in sterile 96-well microplates. Two-fold serial dilutions of the extracts were prepared in Mueller–Hinton broth at final concentrations of 1 to 1000 µg/mL. Microbial suspensions were adjusted to 0.5 McFarland (1×10^6 CFU/mL) and appropriately diluted before inoculation. Plates were incubated at 37 °C for 24 h for bacteria and 28 °C for 48 to 72 h for yeast. The MIC was defined as the

lowest concentration showing no visible growth. For MBC determination, aliquots from wells without visible growth were subcultured onto fresh agar and incubated under the corresponding conditions. The MBC (for bacteria) and MFC (for fungi) were defined as the lowest concentration showing no colony formation (Alawlaqi *et al.* 2023).

Time–Kill Kinetic Assay

The bactericidal dynamics of the extracts were assessed through time–kill analysis. Bacterial suspensions adjusted to 0.5 McFarland were inoculated into broth media containing extract concentrations equivalent to the determined MIC. At predetermined intervals (0, 30, 60, 90, 120, 150, and 180 min), 0.5 mL aliquots were withdrawn, serially diluted, and plated onto nutrient agar. Plates were incubated overnight at 37 °C, and colony-forming units (CFU/mL) were enumerated. The reduction in viable count at each time point was calculated relative to the initial inoculum. Control samples lacking extract exposure were processed in parallel. Log reductions were plotted to determine time-dependent antimicrobial activity (Appiah *et al.* 2017).

Antihemolytic Activity Examination

To assess protective effects against hemolysis, bacterial cultures were treated with sub-inhibitory concentrations (25%, 50%, and 75% of MIC) of *O. basilicum* stem extracts. After incubation, cultures were centrifuged at $16,000 \times g$ for 20 min, and supernatants were collected. Fresh human erythrocytes (2% v/v suspension in isotonic saline) were mixed with supernatants and incubated at 37 °C for 2 h. Following centrifugation, hemoglobin release was quantified spectrophotometrically at 545 nm. Complete hemolysis induced by 0.1% sodium dodecyl sulfate (SDS) served as positive control, while untreated erythrocytes in saline served as negative control (Olchowik-Grabarek *et al.* 2023). Hemolysis percentage was calculated using Eq. 1,

$$\% \text{Hemolysis} = \frac{(A_{\text{sample}} - A_{\text{negative}})}{(A_{\text{positive}} - A_{\text{negative}})} \times 100 \quad (1)$$

where A_{sample} , A_{positive} , and A_{negative} are the absorbance of the sample, positive control, and negative control, respectively.

DPPH Radical Scavenging Evaluation

The antioxidant capacity of crude and IR-treated *O. basilicum* extracts was evaluated using the DPPH free radical scavenging method. Extracts were prepared in methanol at concentrations up to 1000 µg/mL. An equal volume (100 µL) of 0.1 mM DPPH solution was mixed with 100 µL of each test sample in a 96-well plate. After incubation in the dark at 30 °C for 30 min, absorbance was measured at 517 nm using a microplate reader (BioTek Instruments, USA). Ascorbic acid was used as a reference antioxidant. Radical scavenging activity (%) was calculated relative to a control containing DPPH without extract (Alsalamah *et al.* 2023). Scavenging activity has now been added to the Methods section, as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A_0 - A_1)}{(A_0)} \times 100 \quad (2)$$

where A_0 is the absorbance of the DPPH control and A_1 is the absorbance of the sample containing the extract.

Statistical Analysis

All experiments were performed independently in triplicate, and data are presented as mean $\pm$ SD. Differences among groups were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, USA). Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

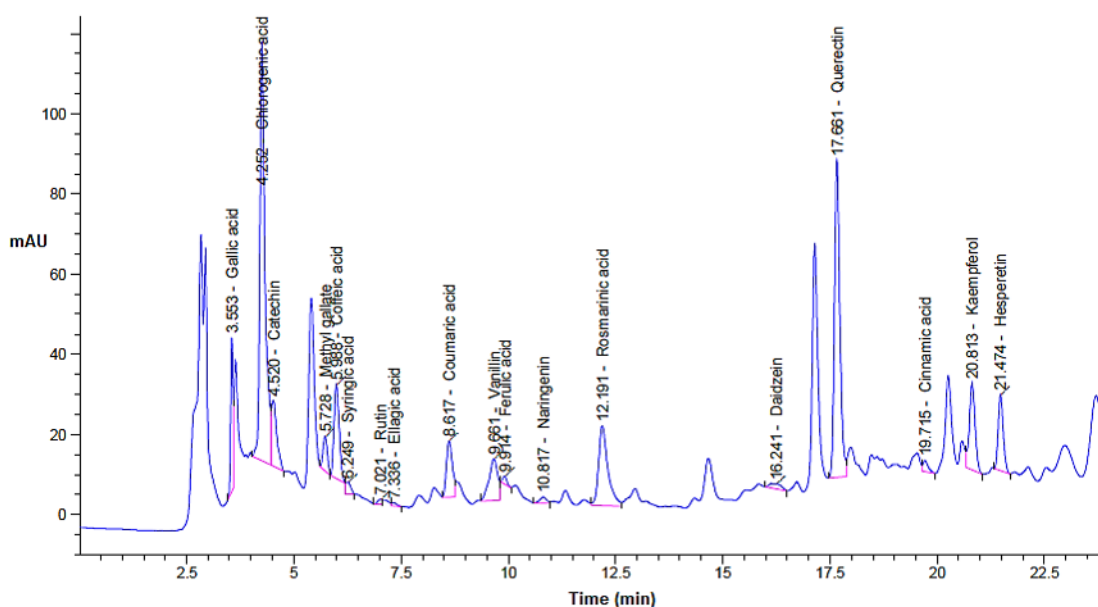
HPLC Profiling of Crude and Irradiated *O. basilicum* Stem Extracts

HPLC analysis enabled the identification and quantification of 18 phenolic and flavonoid compounds in the crude stem extract of *O. basilicum* extract (Table 1 and Fig. 1). The chromatographic profile revealed that chlorogenic acid (6110 $\mu\text{g/g}$) and quercetin (4430 $\mu\text{g/g}$) were the predominant constituents, followed by catechin (1640 $\mu\text{g/g}$), rosmarinic acid (1330 $\mu\text{g/g}$), and kaempferol (1030 $\mu\text{g/g}$). Moderate levels of gallic acid (507 $\mu\text{g/g}$), caffeic acid (588 $\mu\text{g/g}$), and hesperetin (360 $\mu\text{g/g}$) were also detected. Minor constituents included ellagic acid (35.0 $\mu\text{g/g}$), ferulic acid (38.4 $\mu\text{g/g}$), rutin (65.4 $\mu\text{g/g}$), and cinnamic acid (24.5 $\mu\text{g/g}$). The total quantified phenolic/flavonoid compounds indicated a rich amount of polyphenolic molecules in the crude stem extract. Meanwhile, infrared (IR) irradiation induced measurable alterations in the phenolic profile. For example, ferulic acid increased markedly from 38.4 to 72.7 $\mu\text{g/g}$; rosmarinic acid increased from 1330 to 1480 $\mu\text{g/g}$. Gallic acid rose from 507 to 567 $\mu\text{g/g}$, and catechin increased from 1640 to 1710 $\mu\text{g/g}$. Conversely, quercetin decreased from 4430 to 3960 $\mu\text{g/g}$. Notable reductions were observed in kaempferol (1030 to 650 $\mu\text{g/g}$), rutin (65.4 to 25.7 $\mu\text{g/g}$), and syringic acid (78.6 to 15.8 $\mu\text{g/g}$) (Table 1 and Fig. 2).

The comparative HPLC analysis demonstrated that infrared irradiation selectively modulated the phenolic composition of *O. basilicum* stem extract rather than causing generalized degradation. The significant increase in chlorogenic acid and rosmarinic acid after IR exposure suggests enhanced liberation or structural reorganization of bound phenolics. Similar increases in hydroxycinnamic acids following controlled physical or thermal treatments have been reported in Lamiaceae species, where moderate energy input facilitated the release of esterified phenolics without compromising structural integrity (López-Gómez *et al.* 2021; Rostamabadi *et al.* 2023). Chlorogenic acid and rosmarinic acid are widely recognized as dominant phenolic acids in *O. basilicum* and related aromatic herbs, frequently identified through HPLC analysis (Kritsi *et al.* 2024). Their elevation after IR treatment may be attributed to conformational relaxation of plant matrix components or partial cleavage of phenolic–cell wall linkages, mechanisms previously described in studies examining infrared and mild thermal processing of plant materials (Bheemaiah *et al.* 2024). The reduction in glycosylated flavonoids, including rutin, kaempferol, and quercetin, may reflect structural rearrangement, deglycosylation, or transformation into derivatives not fully detected under the applied chromatographic conditions. Similar decreases in flavonoid glycosides following energy-assisted processing have been attributed to hydrolytic or oxidative modifications (Pérez-Martínez *et al.* 2026).

Table 1. Quantitative Phenolic and Flavonoid Composition of Crude and IR Irradiation *O. basilicum* Stem Extract

Compounds	<i>O. basilicum</i> Stem Extract			
	Crude		IR irradiated	
	Area (mAU/s)	Conc. ($\mu\text{g/g}$)	Area (mAU/s)	Conc. ($\mu\text{g/g}$)
Gallic acid	140.56	507	166.54	567
Chlorogenic acid	876.47	6110	1077.95	7090
Catechin	141.59	1640	157.19	1710
Methyl gallate	59.98	1690	58.00	154
Caffeic acid	197.35	590	218.83	620
Syringic acid	24.67	78.6	5.26	15.8
Rutin	9.05	65.4	3.76	25.6
Ellagic acid	6.20	35.1	5.37	28.7
Coumaric acid	135.76	250	110.18	190
Vanillin	146.97	245	159.07	250
Ferulic acid	13.33	38.3	26.79	72.7
Naringenin	17.22	82.8	16.33	74.1
Rosmarinic acid	300.64	1330	354.06	1480
Daidzein	22.82	64.9	5.24	14.1
Quercetin	688.04	44330	650.60	3960
Cinnamic acid	22.78	24.5	21.71	22.1
Kaempferol	201.41	1030	135.06	650
Hesperetin	152.87	360	129.30	287

**Fig. 1.** HPLC chromatogram of crude *O. basilicum* stem extract

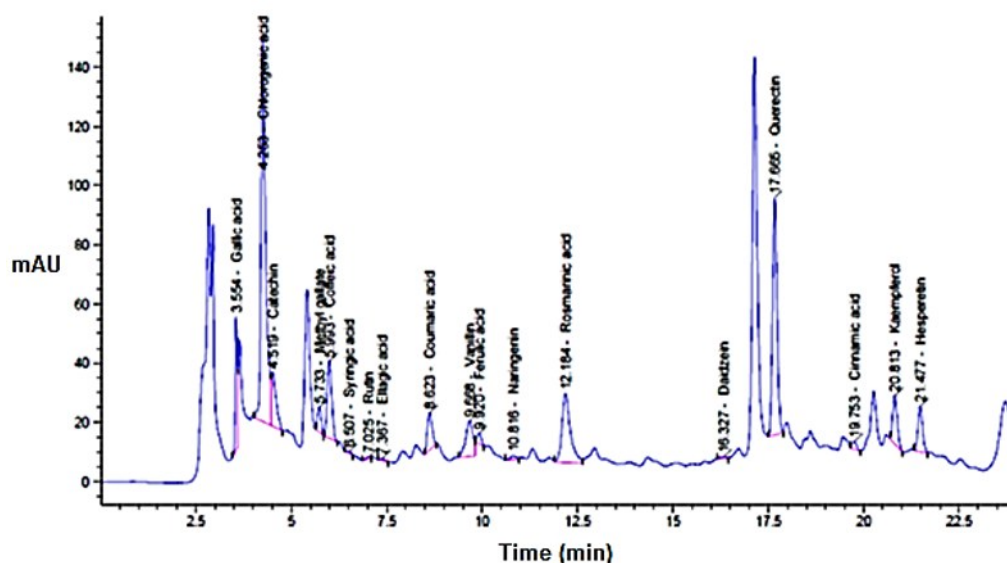


Fig. 2. HPLC chromatogram of IR-treated *O. basilicum* stem extract

The increase in ferulic acid may likewise result from cleavage of complex phenolic esters, as reported during the processing of phenolic-rich matrices (Rostamabadi *et al.* 2023). These changes may therefore represent redistribution rather than absolute compound loss, indicating a qualitative rebalancing of the phenolic profile following IR treatment. The increased levels of chlorogenic and rosmarinic acids may contribute to the enhanced antioxidant and antimicrobial activities observed, consistent with previous reports linking phenolic enrichment with improved bioactivity following optimized plant processing (Antonescu *et al.* 2021; Mańkowska and Dems-Rudnicka 2025).

Assessment of Antimicrobial Activity for Extracts

The crude extract exhibited significant antimicrobial activity against all tested microorganisms except *A. terreus*.

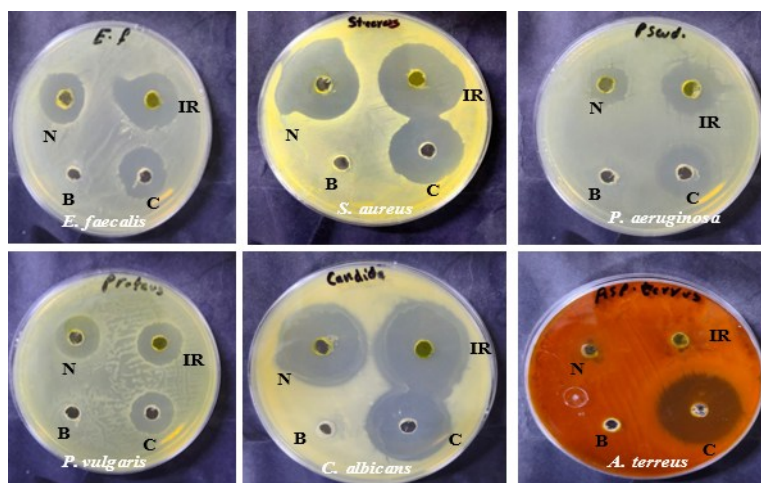


Fig. 3. Comparative inhibition zones (mm) of untreated/crude (N) and infrared treated (IR) *O. basilicum* stem extracts, with solvent control (B) and positive control (C: gentamicin for bacteria and fluconazole for fungi), against the tested microbial strains using the agar well diffusion assay.

Inhibition zones ranged from 14 ± 0.1 mm (*P. aeruginosa*) to 29 ± 1.8 mm (*S. aureus*). Following IR treatment, inhibition zones slightly increased for most strains. *E. faecalis* increased from 21 ± 0.4 to 26 ± 1.5 mm, *S. aureus* from 29 ± 1.8 to 33 ± 1.2 mm, and *P. aeruginosa* from 14 ± 0.1 to 22 ± 0.3 mm. *C. albicans* increased from 29 ± 0.9 to 32 ± 0.1 mm. Only *P. vulgaris* showed minimal change (17 ± 0.7 to 18 ± 0.2 mm) (Fig. 3 and Table 2). The lack of detectable activity against *A. terreus* may be attributed to its lower susceptibility to the bioactive constituents of the extract, potentially due to differences in cell-wall composition, membrane permeability, or tolerance to phenolic compounds.

Table 2. Inhibition Zones (mm) of Crude and IR-treated Extracts with MIC and MBC (for bacteria) or MFC (for fungi) Levels Values of Crude and IR-treated Extracts

Microbes	Inhibition Zones (mm)			MIC Levels		MBC or MFC Levels	
	Crude extract	Crude extract + IR	Reference drug	Crude extract	Crude extract + IR	Crude extract	Crude extract + IR
<i>E. faecalis</i>	21 ± 0.4	26 ± 1.5	20 ± 0.8	$62.5 \pm 0.1^{***}$	$15.62 \pm 0.2^{***}$	$125 \pm 0.2^{***}$	$31.25 \pm 0.2^{***}$
<i>S. aureus</i>	29 ± 1.8	33 ± 1.2	26 ± 1.0	$15.62 \pm 0.3^{**}$	$7.8 \pm 0.1^{**}$	$31.25 \pm 0.3^{**}$	$15.62 \pm 0.1^{**}$
<i>P. aeruginosa</i>	14 ± 0.1	22 ± 0.3	20 ± 0.7	$62.5 \pm 0.2^{***}$	$15.62 \pm 0.2^{***}$	$125 \pm 0.2^{***}$	$31.25 \pm 0.3^{***}$
<i>P. vulgaris</i>	17 ± 0.7	18 ± 0.2	19 ± 0.6	$31.25 \pm 0.4^{\#}$	$31.25 \pm 0.3^{\#}$	$62.5 \pm 0.4^{*}$	$31.25 \pm 0.4^{*}$
<i>C. albicans</i>	29 ± 0.9	32 ± 0.1	31 ± 0.5	$31.25 \pm 0.2^{**}$	$15.62 \pm 0.2^{**}$	$125 \pm 0.4^{***}$	$31.25 \pm 0.4^{***}$
<i>A. terreus</i>	0.0	0.0	30 ± 0.3	--	--	--	--

Note: Data are written as means $\pm$ SD; where * $P \leq 0.05$, ** $P \leq 0.01$; *** $P \leq 0.001$; # non-significant

Table 3. Time–kill Kinetics (CFU/mL) of Crude and IR-treated *O. basilicum* Stem Extract against Selected Bacterial Strains at MIC Concentrations

Time	Crude <i>O. basilicum</i> Stem Extract			
	CFU/mL For <i>E. faecalis</i>	CFU/mL For <i>S. aureus</i>	CFU/mL For <i>P. aeruginosa</i>	CFU/mL For <i>P. vulgaris</i>
0	$82 \times 10^5 \pm 1.0$	$53 \times 10^5 \pm 0.9$	$66 \times 10^5 \pm 1.5$	$35 \times 10^5 \pm 1.1$
30 min	$147 \times 10^4 \pm 0.9$	$235 \times 10^4 \pm 0.5$	$65 \times 10^4 \pm 1.0$	$64 \times 10^4 \pm 1.2$
1h min	$189 \times 10^3 \pm 1.0$	$194 \times 10^3 \pm 0.4$	$132 \times 10^3 \pm 1.1$	$158 \times 10^3 \pm 1.0$
2h	$215 \times 10^2 \pm 0.2$	$172 \times 10^2 \pm 1.5$	$141 \times 10^2 \pm 0.4$	$195 \times 10^2 \pm 2.0$
2.30 h	$115 \times 10 \pm 1.1$	$91 \times 10^2 \pm 0.9$	$106 \times 10 \pm 0.6$	$77 \times 10 \pm 0.6$
3 h	129 ± 0.3	52	93 ± 1.0	115
6h	0	0	0	10
Time	IR-treated <i>O. basilicum</i> Stem Extract			
	CFU/mL For <i>E. faecalis</i>	CFU/mL For <i>S. aureus</i>	CFU/mL For <i>P. aeruginosa</i>	CFU/mL For <i>P. vulgaris</i>
0	$82 \times 10^5 \pm 1.2$	$53 \times 10^5 \pm 0.9$	$66 \times 10^5 \pm 1.5$	$35 \times 10^5 \pm 1.1$
30 min	$24 \times 10^4 \pm 1.0$	$121 \times 10^3 \pm 0.5$	$18 \times 10^4 \pm 1.0$	$18 \times 10^4 \pm 0.7$
1h min	$161 \times 10^2 \pm 0.8$	$34 \times 10^2 \pm 0.7$	$255 \times 10^2 \pm 1$	$16 \times 10^3 \pm 1$
2h	317 ± 1.0	134 ± 0.5	182 ± 1	$112 \times 10 \pm 1$
2.30 h	45	39	98	255
3 h	0	0	0	15
6h	0	0	0	0

The IR-treated extract demonstrated comparable or superior activity to reference drugs in certain cases, particularly against *S. aureus* and *E. faecalis*. The enhanced antimicrobial activity after IR exposure correlates with the increased concentrations of chlorogenic and rosmarinic acids identified by HPLC. These compounds are known to compromise bacterial cell wall integrity and interfere with enzymatic systems (de Oliveira *et al.* 2017; Kalinowska *et al.* 2024).

The pronounced improvement against *P. aeruginosa* is notable, as this organism is typically resistant due to its outer membrane barrier. The observed enhancement may be attributed to synergistic interactions among IR-modified phenolics (Li *et al.* 2026). Comparable improvements in antimicrobial potency following mild physical treatments have been documented in plant extracts subjected to controlled irradiation or thermal modulation (Bilen *et al.* 2025; Aldayel 2026).

MIC and MBC Levels of Crude and IR-treated Extracts

The MIC and MBC values of both extracts can be seen in (Table 2). For *E. faecalis*, the MIC decreased significantly from 62.5 ± 0.1 µg/mL in the crude extract to 15.62 ± 0.2 µg/mL following IR treatment, representing a fourfold reduction ($p \leq 0.001$). Similarly, *P. aeruginosa* exhibited a reduction from 62.5 ± 0.2 to 15.62 ± 0.2 µg/mL (fourfold decrease; $p \leq 0.001$). In the case of *S. aureus*, the MIC declined from 15.6 ± 0.3 to 7.8 ± 0.1 µg/mL (twofold reduction; $p \leq 0.01$). *C. albicans* showed a significant decrease from 31.2 ± 0.2 to 15.62 ± 0.2 µg/mL (twofold reduction; $p \leq 0.01$). Conversely, *P. vulgaris* exhibited no statistically significant difference between treatments, with MIC values remaining at 31.2 ± 0.4 µg/mL (crude) and 31.2 ± 0.3 µg/mL (IR-treated) ($p > 0.05$). A similar trend was observed for MBC values. For *E. faecalis*, MBC decreased from 125 ± 0.2 µg/mL (crude) to 31.2 ± 0.2 µg/mL (IR-treated), corresponding to a fourfold reduction ($p \leq 0.001$). *P. aeruginosa* also showed a fourfold decrease from 125 ± 0.2 to 31.2 ± 0.3 µg/mL ($p \leq 0.001$). For *S. aureus*, MBC declined from 31.25 ± 0.3 to 15.62 ± 0.1 µg/mL (twofold reduction; $p \leq 0.01$). *C. albicans* demonstrated a marked decrease from 125 ± 0.4 to 31.2 ± 0.4 µg/mL of MFC (fourfold reduction; $p \leq 0.001$). Interestingly, *P. vulgaris* showed a reduction in MBC from 62.5 ± 0.4 to 31.2 ± 0.4 µg/mL (twofold decrease; $p \leq 0.05$), despite the unchanged MIC, suggesting enhanced bactericidal efficiency without a corresponding change in growth inhibition threshold. The MBC/MIC ratios were ≤ 2 for most organisms after IR treatment, indicating bactericidal rather than bacteriostatic activity. Infrared irradiation significantly enhanced the antimicrobial activity of *O. basilicum* stem extract, as indicated by the reduced MIC and MBC values. This improvement coincided with increased chlorogenic, and rosmarinic acid levels detected by HPLC, which may contribute to antimicrobial effects through membrane disruption and oxidative stress (Berecka-Rycerz *et al.* 2025; Mohagheghian *et al.* 2025).

Time–Kill Kinetic Analysis

Time–kill analysis demonstrated concentration-dependent and time-dependent reductions in viable bacterial counts for both crude and IR-treated extracts. The initial inoculum ranged from 3.5 to 8.2×10^6 CFU/mL across tested strains. For the crude extract, gradual bacterial decline was observed. *E. faecalis* decreased from 8.2×10^6 CFU/mL at 0 h to complete eradication at 6 h. A ≥ 3 log₁₀ reduction was achieved between 2 and 3 h. *S. aureus* declined from 5.3×10^6 CFU/mL to complete elimination at 6 h. *P. aeruginosa*

showed progressive reduction from 6.6×10^6 CFU/mL to zero detectable cells at 6 h. However, *P. vulgaris* exhibited slower susceptibility, with residual growth (10 CFU/mL) still detected at 6 h (Table 3). In contrast, the IR-treated extract demonstrated markedly accelerated killing kinetics. For *E. faecalis*, complete eradication occurred within 3 h, compared with 6 h for the crude extract. *S. aureus* and *P. aeruginosa* were also fully eliminated by 3 h. A ≥ 3 to 4 $\log_{10}$ reduction was observed within the first 2 h for these strains. Notably, *P. vulgaris* was eradicated by 6 h with IR treatment, whereas incomplete killing was observed with the crude extract (Table 3). Statistical analysis confirmed significant interaction ($p < 0.05$) between treatment type and time, indicating that IR exposure significantly enhanced the rate of bacterial killing. The time–kill curves indicate that IR irradiation enhanced both the inhibitory potency and bactericidal rate of *O. basilicum* stem extract, as reflected by the reduced MIC/MBC values and faster CFU/mL decline. This effect may be related to the increased chlorogenic and rosmarinic acid levels detected by HPLC, as these phenolics can contribute to membrane disruption and oxidative stress (Hernández-Miranda *et al.* 2025; Rudrapal *et al.* 2025).

The Impact of IR Irradiation on Antihemolytic Activity

The crude extract reduced hemolysis by approximately 32%, 51%, and 68% at 25%, 50%, and 75% of MIC, respectively (Figs. 4, 5). In comparison, the IR-treated extract achieved significantly higher protection of 46%, 69%, and 84% at the same respective concentrations. The enhancement was most pronounced at 75% MIC (Fig. 4A).

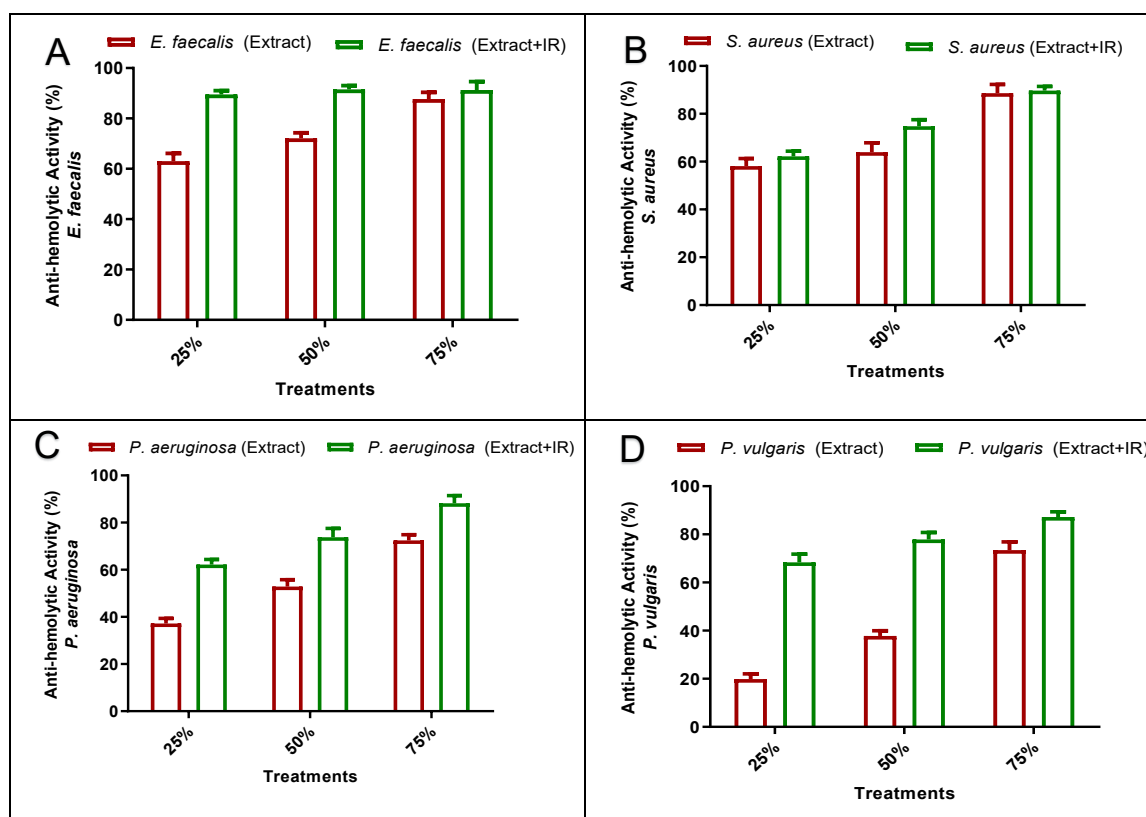


Fig. 4. Bar graphs for antihemolytic activity in the presence of bacterial challenge (A) *E. faecalis*; (B) *S. aureus*; (C) *P. aeruginosa*; (D) *P. vulgaris*. Data are represented as means $\pm$ SD.

For *S. aureus*, the crude extract provided 35%, 56%, and 70% hemolysis inhibition at 25%, 50%, and 75% MIC, respectively. The IR-treated extract improved protection to 48%, 72%, and 87% at corresponding concentrations. The difference between treatments was notable at all tested levels ($p < 0.05$), with the highest protection observed at 75% MBC (Fig. 4B). Hemolysis induced by *P. aeruginosa* supernatant was reduced by 28%, 45%, and 62% using the crude extract at increasing MBC fractions. The IR-treated extract demonstrated enhanced inhibition of 41%, 63%, and 81%, respectively. The improvement was particularly evident at 50% and 75% MIC (Fig. 4C). The crude extract reduced hemolysis by 30%, 49%, and 65% at 25%, 50%, and 75% MBC. The IR-treated extract achieved 43%, 60%, and 78% inhibition at the same concentrations (Fig. 4D).

Although the improvement was somewhat lower than that observed for Gram-positive strains, the IR-treated extract consistently showed stronger antihemolytic activity across the tested strains and concentrations, with the greatest protection at 75% MBC. The enhanced activity, particularly against *E. faecalis* and *S. aureus*, may be related to the increased chlorogenic and rosmarinic acid levels detected by HPLC, as phenolic compounds can help preserve membrane integrity and limit oxidative damage (Brglez Mojzer *et al.* 2016; Takó *et al.* 2020). The concentration-dependent protection is consistent with previous reports on the membrane-protective effects of phenolic compounds (Cyboran-Mikołajczyk *et al.* 2022; Veleva *et al.* 2025). Notably, the extract provided protection without detectable hemolytic effects under the tested conditions.

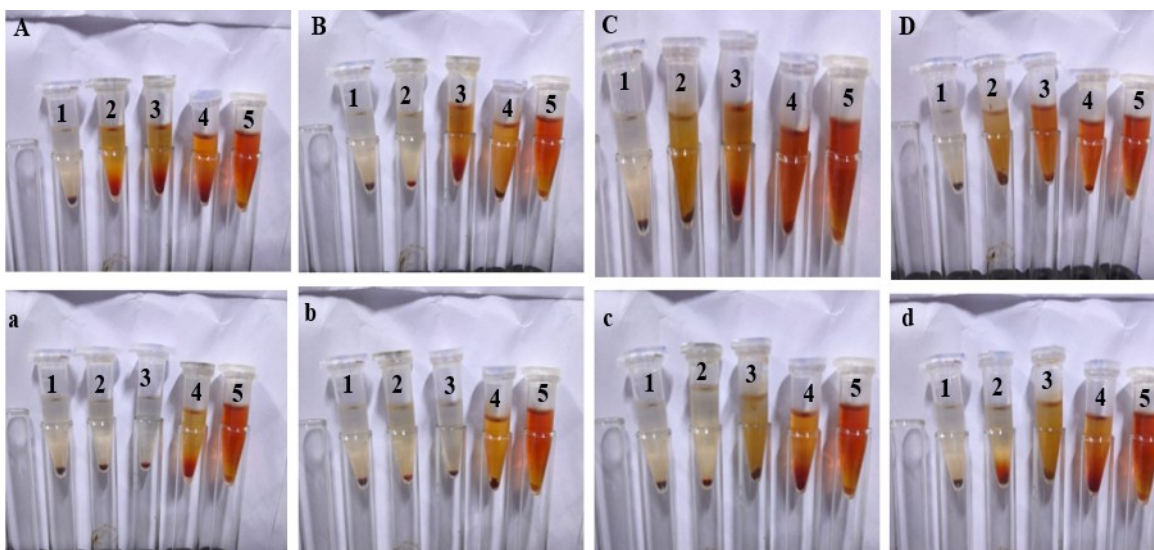


Fig. 5. Testing antihemolytic activity in the presence of various tested bacterial species upon using crude extract (upper row) and irradiated extract (lower row); Eppendorf tube 1: is the tested bacterium only (untreated) - Tubes 2, 3, and 4 treated with 25%, 50%, and 75% MIC, respectively. While tube 5: is the negative control (SDS). (A, a) *E. faecalis*, (B, b) *S. aureus*, (C, c) *P. aeruginosa*, and (D, d) *P. vulgaris*.

The Impact of IR Irradiation on Antioxidant Capacity

Both crude and IR-treated extracts demonstrated dose-dependent DPPH radical scavenging activity. The IR-treated extract exhibited significantly higher antioxidant capacity across all tested concentrations. The IC_{50} value of the crude extract was $9.52 \pm 0.78 \mu\text{g/mL}$, whereas the IR-treated extract showed a slightly lower IC_{50} value of $6.55 \pm$

0.03 $\mu\text{g/mL}$, indicating approximately 30% improvement in radical scavenging efficiency. Ascorbic acid (reference standard) exhibited an IC_{50} of $3.19 \pm 0.05 \mu\text{g/mL}$ (Fig. 6). At 100 $\mu\text{g/mL}$, the crude extract achieved 54% scavenging activity, while the IR-treated extract reached 71%. At the highest tested concentration (1000 $\mu\text{g/mL}$), both extracts exceeded 85% inhibition, although the IR-treated extract consistently showed superior activity. The significant reduction in IC_{50} after IR irradiation reflects improved antioxidant efficiency, likely attributable to increased chlorogenic and rosmarinic acid levels, as detected by HPLC. These compounds are well-recognized hydrogen-donating antioxidants capable of neutralizing DPPH radicals. The enhancement observed was moderate and statistically validated, indicating improved antioxidant performance relative to the reference standard (Aldoghachi *et al.* 2021; Aleksieva *et al.* 2025). The results further confirm that IR treatment selectively optimized the redox-active phenolic content.

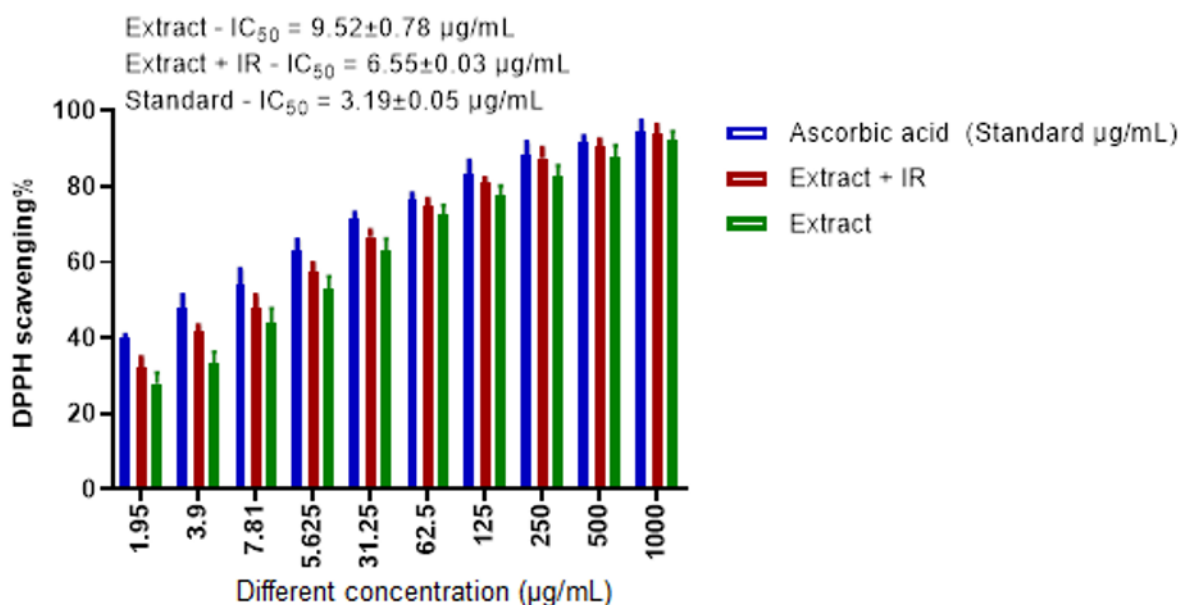


Fig. 6. DPPH radical scavenging activity (%) of crude and IR-treated *O. basilicum* stem extracts and corresponding IC_{50} values (outcomes are drawn as means $\pm$ SD)

Limitation of the Present Study

Future studies could investigate the effect of direct heating of the dried extract at 45 $^{\circ}\text{C}$ for 10 minutes to distinguish the effects of thermal exposure from those specifically associated with infrared (IR) irradiation.

CONCLUSIONS

1. Controlled infrared (IR) treatment was associated with changes in the phytochemical composition of *O. basilicum* stem biomass extract and enhanced antimicrobial, antioxidant, and antihemolytic activities under the tested conditions. These effects may reflect combined infrared-induced and thermal effects.

2. HPLC analysis confirmed selective increases in chlorogenic and rosmarinic acids, which correlated with significant reductions in MIC and MBC values and accelerated bactericidal kinetics. Importantly, antimicrobial improvements were strain-dependent, with more pronounced effects against *E. faecalis* and *P. aeruginosa*.
3. Time–kill analysis showed that IR treatment increased the speed and strength of bacterial killing. The enhanced antihemolytic and antioxidant activities observed after infrared irradiation further support the role of phenolic enrichment—particularly chlorogenic and rosmarinic acids—in improving the functional bioactivity of *O. basilicum* stem extract.

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