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RESEARCH

Investigation of the Microbiocidal, Antioxidant, and Cytotoxic Effects of *Cinnamomum verum* (Cinnamon), *Origanum onites* (Oregano Balls), and *Origanum vulgare* (Black Oregano) Hydrosols: A Model Food Environment-Driven Usability Study

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Abstract

This comprehensive study evaluated the antimicrobial efficacy, antioxidant capacity, and cytotoxicity of cinnamon (*Cinnamomum verum*), black oregano (*Origanum vulgare*), and ball oregano (*Origanum onites*) hydrosols, with particular focus on acidified cinnamon variants (3% ascorbic acid, CHC; 3% malic acid, CHM). GC–MS analysis revealed distinct chemotypes: cinnamon hydrosol was cinnamaldehyde-dominant (76.66%), while oregano hydrosols exhibited carvacrol-rich profiles. Black oregano demonstrated superior antioxidant capacity, with a total phenolic content (TPC) of 1286 mg GAE/L and DPPH/ABTS values of 3.07 and 4.24 mmol TE/L, respectively. Antimicrobial testing revealed that cinnamon-based hydrosols produced the largest inhibition zones against *Candida albicans* (34.06 ± 2.00 mm) and *Aspergillus flavus* (26.23 ± 0.50 mm), while acidification significantly enhanced their antibacterial potency. CHM achieved the lowest MIC values for multiple pathogens, including *Klebsiella pneumoniae* (1.45 ± 0.01 µL/mL) and *Bacillus cereus* (1.99 ± 0.01 µL/mL). In Kashar cheese washing trials, CHM demonstrated superior performance with mean log reductions of 2.52 across five pathogens at 45 min, with most antimicrobial effect occurring within the first 15 min. Zeta potential analysis revealed CH maintained electrostatic stability (-22 to -23.5 mV), while CHM's near-neutral charge (-2.55 to -3.52 mV) facilitated enhanced bacterial contact. Cytotoxicity assays identified workable dilution ranges preserving ≥ 70% fibroblast viability (in L929), supporting short contact and rinse-type use. These findings position acidified cinnamon hydrosols, particularly CHM, as promising natural surface decontaminants for food systems.

Keywords Hydrosol · Natural · Model food · Antioxidant · Antimicrobial · Cytotoxicity · Cheese

Introduction

Growing consumer demand for clean-label foods and gentle, bio-based antimicrobials has sparked renewed interest in plant-derived distillates for food protection and surface hygiene (Chorianopoulos et al., 2008; Karampoula et al., 2016). While essential oils (EOs) are widely studied for their potent antimicrobial and antioxidant properties, their pungent aroma, hydrophobic nature, and potential cytotoxicity at higher doses can limit their direct use in food systems (Hyldgaard et al., 2012; Maurya et al., 2021; Reis et al., 2022). Hydrosols, also known as hydrolates or aqueous distillates, are the water-based co-products of steam or hydrodistillation, providing a complementary and often underutilized alternative (Almeida et al., 2024; Tavares et al., 2022). They contain tiny droplets of volatile

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compounds and water-soluble small molecules from the same aromatic plants, usually at much lower concentrations than EOs, resulting in a milder sensory effect, easier handling in aqueous environments, and a safer profile for topical and rinsing applications (Di Vito et al., 2021; Šilha et al., 2020). From a sustainability standpoint, elevating hydrosols from mere by-products to valuable ingredients aligns with circular processing and waste reduction in botanical manufacturing (Almeida et al., 2024). Hydrosols also have clear industrial relevance because they are generated as unavoidable aqueous co-products during steam/hydrodistillation for essential-oil production, and they can therefore be available at scale as a low-cost, water-compatible ingredient stream (Almeida et al., 2024; Politi et al., 2020; Tavares et al., 2022). Their direct compatibility with aqueous processing makes them practical candidates for contact-time–controlled interventions, such as immersion washing or spray/rinse steps used to reduce surface contamination on foods and processing surfaces, where rapid action and ease of rinsing are operational priorities (Chorianopoulos et al., 2008; D'Amato et al., 2018; Karampoula et al., 2016). However, wider implementation requires addressing key limitations, including relatively low and variable concentrations of active volatiles compared to essential oils, potential batch-to-batch compositional variation, and stability and microbiological quality considerations during storage. These constraints make chemical fingerprinting, formulation strategies, and validation in realistic food-matrix scenarios essential to support reproducible industrial use (Almeida et al., 2024; D'Amato et al., 2018; Jakubczyk et al., 2021; Tavares et al., 2022).

Hydrosols have demonstrated measurable antimicrobial effects, though generally weaker than their corresponding EOs. Nevertheless, they are often effective for surface rinses, hurdle approaches, and product washing where direct contact and residence time are controllable. Origanum hydrosols rich in carvacrol demonstrate broad-spectrum antimicrobial activity against key foodborne pathogens. Specific studies have shown oregano hydrosols to be effective against *Salmonella* Typhimurium, with industrial oregano hydrolate eliminating strains immediately after inoculation (Gavriil et al., 2021). Other research has demonstrated a significant reduction of *Listeria monocytogenes* populations by oregano hydrolates, even seven days after application on fresh produce (Xylia et al., 2022). Additionally, oregano hydrosols have shown efficacy against *Escherichia coli* O157:H7, *Staphylococcus aureus*, and various spoilage fungi (Almeida et al., 2024; Bairamis et al., 2024; Cid-Pérez et al., 2019; D'Amato et al., 2018). On the other hand, cinnamon-derived distillates containing cinnamaldehyde have been reported to exhibit strong anti-yeast and antifungal activity, as well as inhibit various foodborne pathogens (Beyaz et al., 2025; Guo et al., 2024; Jaramillo Jimenez et al., 2024).

Beyond their antimicrobial effects, hydrosols possess antioxidant properties derived from their phenolic compounds and oxygenated terpenoids. These substances can neutralize free radicals and bind to pro-oxidant metals (Almeida et al., 2024). Although this antioxidant activity is typically lower than in essential oils or alcohol-based plant extracts, it can still be beneficial in aqueous or surface applications where direct contact and repeated reapplication are possible. Standard tests, such as total phenolic content (TPC), DPPH, and ABTS, offer different ways to measure electron transfer and radical-quenching abilities and are often used to compare botanical distillates (Jakubczyk et al., 2021; Politi et al., 2020, 2022; Shimamura et al., 2014). Reports indicate that the chemotype of Origanum containing carvacrol or thymol demonstrates robust antioxidant properties, while cinnamaldehyde and phenylpropanoids in Cinnamomum are similarly linked, with co-extracted phenolics contributing to radical scavenging contingent upon concentration and matrix (Guo et al., 2024; Li et al., 2021; Lombrea et al., 2020; Mączka et al., 2023).

From an application perspective, hydrosols are appealing as direct-use aqueous rinses and surface decontamination agents for fresh produce, meats, and dairy products, as well as in oral care and cosmetic formulations, where mildness and water compatibility are beneficial (Almeida et al., 2024; Bairamis et al., 2024; Gavriil et al., 2021; Hyldgaard et al., 2012). Their generally lower volatility and reduced hydrophobic load compared to EOs can result in acceptable sensory impact at effective doses, while regulatory assessments classify key actives like carvacrol as safe for specific food uses (GRAS) with defined intake limits (Lei et al., 2019; Munro & Danielewska-Nikiel, 2006). However, systematic evaluations of cytotoxicity in mammalian cells exposed to these applications are essential for establishing safe working limits, especially for short-term contact rinses and surface treatments.

Despite growing interest in hydrosols as clean-label antimicrobials, the field still lacks integrated studies that jointly (i) link hydrosol volatile fingerprints and colloidal properties to bioactivity, (ii) define cytocompatible working dilutions relevant to short-contact use, and (iii) validate performance in a realistic dairy-surface context. In addition, simple food-grade acidification strategies are promising for improving antimicrobial performance; however, they are rarely compared across acids with different functional roles in foods (e.g., malic acid as an acidulant and potential chelating weak acid, and ascorbic acid as an acidulant with redox activity). Such comparisons are important because "acidification" can influence activity through multiple routes, including pH-dependent microbial susceptibility and formulation-dependent interfacial behavior.

In this study, we hypothesized that acidifying hydrosols with malic acid or ascorbic acid could significantly

broaden their antimicrobial spectrum by leveraging pH-driven susceptibility and formulation-dependent interfacial effects, all while ensuring cytocompatibility at practical dilutions suitable for rinse-based applications. Based on our findings about cinnamon hydrosol's broad-spectrum microbiocidal activity, we specifically tested this hypothesis by using it to enhance its effects. Therefore, we extensively chemically profiled hydrosols from *Cinnamomum verum*, *Origanum onites*, and *Origanum vulgare*, demonstrating their potential. Then, we evaluated their antioxidant capacities using TPC, DPPH, and ABTS assays, highlighting their promising antioxidative properties. Their antimicrobial effectiveness was rigorously assessed through agar diffusion and broth microdilution methods, underscoring their strong antimicrobial potential. We also examined cytotoxicity in L929 and HT29 cell lines to ensure safety and broad applicability. Furthermore, we characterized the droplet size and zeta potential of cinnamon formulations, providing critical insights. Most importantly, the decontamination efficacy of these formulations was validated via timed immersion washing on Kashar cheese surfaces inoculated with representative bacterial and fungal pathogens, confirming their practical effectiveness.

Materials and Methods

Materials

Plant materials were acquired from a local supplier in Kayseri Province, Türkiye. Samples of *Cinnamomum verum* (cinnamon), *Origanum onites* (oregano balls), and *Origanum vulgare* (black oregano) were preserved in a dry environment at 25 °C, enclosed in a clear pouch and protected from light until hydrodistillation. The chemicals and culture media utilized in this study include: Ethanol (Merck-109171), Ascorbic acid (Merck), Malic acid (Merck), DPPH: 2,2-diphenyl-1-picrylhydrazyl (Sigma-300267), ABTS:2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma-11557), Folin-Ciocalteu′s phenol reagent (Supelco-1.09001), Sodium carbonate (Sigma, 223,530), Nutrient broth (Merck-105443), Nutrient agar (Merck-105450), Malt Extract Agar (Merck-146151), Plate Count Agar (Merck-146365), DRBC Agar (Merck-100466), (MRD) Maximum Recovery Diluent (Merck-146809), Cell Counting Kit-8 (CCK-8, MedChemExpress, HY-K0301), Sorbitol MacConkey (SMAC) Agar (Merck-109207), Modified Brilliant-green Phenol-red Lactose Sucrose (BPLS) Agar (Merck-110747), Baird-Parker Agar (Millipore- 1.05406), and Dichloran Rose Bengal Cholamphenicol (DRBC) Agar (Merck-100466).

Methods

Hydrodistillation

Before hydrodistillation, 100 g of material from each plant species was pulverized using a laboratory blender (Waring-Commercial, Stamford, CT, USA). Powder samples were placed in a volumetric flask (2 L) with 1 L of distilled water and extracted for one hour using a bottle heater (Electromantle EM, Cole-Parmer, IL, USA) with a Clevenger hydrodistillation apparatus. Then, after separating the collected volatile oil, the remaining aqueous part was transferred into a separate tube and stored at + 4 °C for analysis (Lucchesi et al., 2004). Cinnamon hydrosol was coded as CH, black oregano hydrosol as BTH, and ball oregano hydrosol as BH. The antimicrobial substance obtained by adding 3% ascorbic acid to cinnamon hydrosol was coded as CHC, and the antimicrobial substance obtained by adding 3% malic acid was coded as CHM. The pH values of hydrosols and their derivatives were measured with a pH meter (Mettler Toledo, Fiveeasy Plus FP20, UK).

A modified Salting-Out procedure was applied for the determination of Total organic compounds found in the hydrosols (Hyde et al., 2017; Zhang et al., 2009). For this purpose, 25 g of NaCl was initially dissolved in 100 mL of each sample. This step prevented the loss of volatile organic compounds (VOCs) and aided their transfer to hexane. Subsequently, three successive hexane extractions were conducted by swirling the mixture for 2 min each time. In each extraction, the hexane phase was separated using a separation funnel (Isolab, Germany). The collected hexane was then stored in a borosilicate amber bottle and kept at −86 °C overnight to allow VOCs to crystallize and precipitate. The cooled hexane samples were lyophilized using a laboratory-scale freeze dryer (Christ, Alpha 2–4 LSCplus, Germany). The total organic matter content was calculated by subtracting the weight of the empty bottle from that of the final, filled bottle.

Characterization of Volatile Composition with GC–MS

The volatile content of hydrosols (cinnamon, black oregano, and ball oregano) utilized in this study was assessed following the methodology of Beyaz et al. (2025) with a few modifications. Each sample consisted of 20 µL of hydrosol dissolved in 2 mL of n-hexane and was analyzed using a triple quadrupole GC–MS/MS instrument (Thermo-Fisher Scientific, USA, GC: TRACE GC Ultra/MS–MS: TSQ Quantum GC) fitted with a TG-5MS capillary column at the central laboratory of Hitit University (Çorum, Türkiye). Helium was used at a flow rate of 1 mL/min as the carrier gas. The column temperature was programmed to increase from 50 °C to 160 °C in 3 °C/min increments for 0.67 min

and then to decrease from 160 °C to 50 °C in 5 °C/min increments for 5 min. After that, identified compounds were confirmed by comparing with data from the WILEY-NIST (12th Edition, 2020) GC–MS spectral database.

Analysis of the Total Phenolic Content (TPC)

The total phenolic content of the hydrosols was measured using the Folin-Ciocalteu method, as proposed by Singleton et al. (1999), with minor modifications. A 96-well polystyrene microplate was filled with 20 µL of the hydrosol, 100 µL of ten-fold diluted Folin-Ciocalteu reagent, and 80 µL of sodium carbonate (75 g L^{-1}). Following 1 h of incubation at room temperature, the absorbance of the mixtures was read using a microplate reader (SPECTROstar Nano, BMG Labtech GmbH, Germany) at a wavelength of 765 nm, with pure water as the blank reference. The results were presented as milligrams of gallic acid equivalents per liter.

DPPH Radical Scavenging Activity Assay

The antiradical activity of hydrosols against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was examined using a modified method based on Brand-Williams et al. (1995). Initially, 5 µL of the hydrosol sample was combined with 195 µL of a DPPH solution (25 mg/L in methanol). The mixture was vigorously shaken for 10 s before being positioned at room temperature in the microplate reader for 30 min. The absorbance of the mixture was measured using a microplate reader at 515 nm, with methanol serving as the blank at the final stage of the incubation. A calibration curve was created using various concentrations of trolox. The DPPH radical scavenging activity of each hydrosol sample was expressed as the Trolox equivalent antioxidant capacity (TEAC (mmol/L)). All experiments for hydrosol samples were performed in quintuplicate.

$ABTS^+$ Radical Cation Scavenging Activity Assay

The ABTS cation was freshly generated by combining 7 mM ABTS with an equal amount of 2.46 mM potassium persulfate to produce the radical cation ($ABTS^+$). It was then stored in the dark at 25 °C for 16 h before use. Following appropriate dilution in phosphate buffer saline (PBS), an ABTS-radical cation solution was used for the assay. Then, each hydrosol sample underwent a fourfold dilution with phosphate buffer at pH 7.2. Fifty microliters of the hydrosol sample were combined with 1.95 ml of ABTS solution, incubated in the dark for five minutes, and the absorbance was measured in quintuplicate at 734 nm. Distilled water was used as the blank solution, and the ABTS solution, free of hydrosols, served as the control. Trolox served as the standard synthetic antioxidant, and a standard curve was generated as described in the DPPH section. The $ABTS^+$ reduction capacity of hydrosols has been expressed as TEAC (mmol/L), in line with the approach employed in the DPPH radical scavenging activity assay (Re et al., 1999).

Antimicrobial Activity Assay and MIC

The test microorganisms, detailed in Tables 3 and 4, were obtained from the Culture Collection of Erciyes University, Department of Food Engineering. The antimicrobial effect of hydrosols was evaluated using the agar well diffusion technique. The bacterial test pathogen strains were grown at 35 °C in sterile nutrient broth. The yeast and mold strains used in this study were cultivated at 25 °C in sterile malt extract broth. The final cell concentration of the culture suspensions was determined by comparison to 0.4–0.5 McFarland turbidity standards. The culture suspension (1% v/v) was added to 100 mL of molten nutrient agar, and 15 mL of the medium was poured into 90 mm petri dishes. Using a sterile cork borer, four wells, each 4 mm in diameter and equally spaced, were created after the agar plates had solidified at 4 °C for 1 h. Fifty microliters of hydrosol were added to the wells using a sterile micropipette. The Petri dishes were incubated for 18–24 h at 35 °C for bacterial strains, while yeast and mold Petri dishes were kept at 25 °C for 24 h. The inhibition zones were measured in millimeters. All experiments were conducted in triplicate. The hydrosols' minimum inhibitory concentration (MIC) was determined using the broth microdilution method, as described by Wiegand et al. (2008). For MIC experiments, due to its broad-spectrum activity against bacteria, yeast, and fungi, cinnamon hydrosol was selected for acidification to enhance its bactericidal effect further.

Cytotoxicity Assay

The cytotoxic effects of hydrosols have been examined on L-929 mouse fibroblast cells and HT-29 human colon cancer cell lines. The vitality of cells was assessed using the colorimetric CCK-8 test. Cells were inoculated onto 96-well cell culture plates at a density of 1×10^4 cells per well and allowed to adhere for 24 h. Following adhesion, cells were exposed to varying concentrations of hydrosols (prepared as serial two-fold dilutions of the stock sample, ranging from 256× to 1×, where 1× corresponds to the undiluted sample). Sterile dH_2O served as a negative control, ensuring dependable comparisons. After 24 h of incubation with the test samples, 10 µL of CCK-8 reagent was applied to each well, and the cells were further incubated at 37 °C for 4 h. All experiments for each cell line were carried out in quintuplicate. Cell viability was determined by measuring absorbance at 450 nm with a

microplate reader (SPECTROstar Nano, BMG Labtech GmbH, Germany). Subsequently, absorbance values were evaluated by constructing dose–response curves. IC50 values (half-maximal inhibitory concentration) and cellular viability graphs were generated using the *drc, tidyverse, broom,* and *ragg* packages within the R Studio environment (Yetiman et al., 2025). In detail, cell viability (%) as a function of hydrosol dose (expressed as hydrosol fraction, v/v; $256\times = 1/256$ to $1\times = 1$) was modelled separately for each hydrosol and cell line using a four parameter log logistic (4PL) curve in R (package *drc*), fitted with *drm (response ~ dose, fct = LL.4(…))*. The model form was $y = c + (d-c)/\{1 + exp[b(log\ x - log\ e)]\}$, where b is the slope and e is the ED_{50}(effective dose that produces a 50% effect); to match the viability scale, the lower and upper asymptotes were constrained to c = 0% and d = 100% (implemented as *LL.4(fixed = c(NA, 0, 100, NA))*). Fits used inverse variance weighting based on the reported standard deviations (weights = $1/SD^2$; with a small floor to avoid infinite weights when SD = 0). IC_{50} was defined as the dose producing 50% viability on the absolute scale and was obtained from the fitted model using ED *(model, 50, type = "absolute", interval = "delta")*; when model-based IC_{50} was unavailable, or outside the tested range, IC_{50} was approximated by log dose interpolation between the two experimental points bracketing 50% viability. Goodness of fit was assessed by confirming model convergence and through visual inspection of the observed versus fitted curves and residual patterns. The R Markdown code files are shared as supplemental files.

Kashar Cheese Model Food Environment Trials

Kashar cheese environment trials were designed with modifications based on the parameters described by Spanu et al. (2014). To examine the protective efficacy of hydrosols against foodborne pathogens, Kashar cheese samples artificially inoculated with five distinct microorganisms (*Escherichia coli* O157:H7, *Salmonella enterica* sv. Typhimurium, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus flavus*) underwent washing treatments with CH, CHC, and CHM, and the levels of inhibition were determined. The initial inoculation concentration was adjusted to approximately 10^8 CFU/mL for all test microorganisms. Using a sterile knife, Kashar cheese samples were cut into pieces (approximately 1 cm^3, or 1 g). This was done to increase the surface area. Ten sliced cheese samples (1 g each) were immersed in the inoculum solutions (sample-to-inoculum ratio 1:5, w/v). To ensure homogeneous distribution of the inoculum (50 mL), the samples were shaken for 1 min at 22 ± 2 °C and then refrigerated (Anonymous, 2010). The inoculated, cut cheese pieces were washed by immersing the samples in sterile bottles containing each hydrosol (CH, CHC, CHM) for 0, 15, 30, and 45 min. Control samples were immersed in sterile tap water. The bottles were sealed after sample additions and gently agitated at 5-min intervals throughout the incubation period. Following the hydrosol treatment for each test microorganism, 10 g of the Kashar cheese sample was transferred into sterile bottles, mixed with 90 mL of sterile peptone water, and vortexed vigorously for 1 min. Next, serial decimal dilutions were prepared using MRD. Enumeration of *E. coli* O157:H7, *S. enterica* sv. Typhimurium and *S. aureus* were fulfilled with spread plating using SMAC, BPLS, and Baird-Parker agars, respectively. All bacterial plates were incubated under anaerobic conditions at 37 °C for 48 h. The enumeration of *C. albicans* and *A. flavus* was also carried out on DRBC agar with spread plating, and the plates were kept at 28 °C for 5 days.

Zeta Potential and Droplet Size Analyses

The changes in zeta potential and droplet size of the produced cinnamon hydrozole and its blends (supplemented with 3% ascorbic and malic acid) were monitored every 7 days during the initial production phase and during a subsequent 4-week storage period at room temperature, using a Malvern ZS90 (Malvern Panalytical, UK) Zetasizer and dynamic light scattering (DLS) device. Prior to analysis, the hydrosol blends underwent homogenization for 3 min using an Ultra-Turrax (Heidolph, Silent Crusher M, Germany) at a speed of 10,000 rpm, following the addition of the aforementioned organic acids (Beyaz et al., 2025).

Statistical Analyses

The data acquired during the study were analyzed by one-way ANOVA followed by Tukey's HSD multiple comparison test ($\alpha = 0.05$) using Minitab 19 (Minitab Ltd., Coventry, UK). Replication differed by assay, as stated in the relevant Methods subsections (e.g., antimicrobial assays in triplicate, and antioxidant and cytotoxicity assays in quintuplicate). Results are reported as mean ± SD.

Results and Discussion

Phytochemical Composition of Hydrosols

GC–MS analysis of the volatile fraction recovered from the hydrosols identified distinct chemotypes for cinnamon (*Cinnamomum verum*), black oregano (*Origanum vulgare*), and ball oregano (*Origanum onites*), as presented in Table 1. The cinnamon hydrosol was mainly composed of the cinnamaldehyde, which is a phenylpropanoid aldehyde (76.66% of total peak area), alongside sesquiterpenes such

Table 1 Results of volatile identification via GC–MS for *Cinnamomum verum* (cinnamon), *Origanum onites* (oregano balls), and *Origanum vulgare* (black oregano) hydrosols. Major volatile compounds are specifically pointed out

		Cinnamon	Black Oregano	Ball Oregano
RT[a]	Compound	Area (%)	Area (%)	Area (%)
6.99	Thujene	-	-	0,27
7.21	α-Pinene	0,29	-	0,23
7.7	Camphene	0,17	-	-
8.66	2-β-Pinene	0,09	-	-
9.2	β-Myrcene	-	-	0,32
9.66	Benzaldehyde	0,23	-	-
10.45	o-Cymene	-	-	0,99
10.67	1,8-Cineole	1	-	-
11.82	γ-Terpinene	-	-	2,33
13.52	Linalool	-	-	2,88
15.96	2-Metylbenzofurane	0,1	-	-
16.32	Borneol	0,3	0,07	2,06
16.71	Benzenepropanal	1,9	-	-
16.89	4-Terpineol	-	0,04	0,87
17.55	α-Terpineol	-	-	0,17
19.97	Carvone	-	-	0,2
21.19	Cinnamaldehyde	**76,66**	-	-
22.01	Thymol	-	-	1,88
22.5	Carvacrol	-	**99,67**	**84,15**
25.24	α-Copaene	9,71	-	-
25.96	Sativene	1,04	-	-
26.46	Isosativene	0,26	-	-
26.77	Caryophyllene	0,45	-	-
26.77	Trans-caryophyllene	-	-	1,28
27.09	Germacrene-D	0,02	-	-
27.41	Aromadendrene	0,08	-	0,29
27.86	α-Humulene	0,49	-	0,47
28.09	α-Muurolene	0,13	-	-
29.6	β-Bisabolene	-	-	0,82
29.96	γ-cadinene	7,08	-	-
31.6	Caryophyllene oxide	-	-	0,53
33.08	γ-Cadinene	-	-	0,27
39.13	Docosane	-	0,19	-

[a] Retention Time

as α-copaene (9.71%) and γ-cadinene (7.08%), as well as trace amounts of oxygenated monoterpenes like 1,8-cineole (1.00%) and borneol (0.30%). Trace components were benzenepropanal (1.90%), α-pinene (0.29%), camphene (0.17%), and 2-methylbenzofuran (0.10%), among others. The dominance of cinnamaldehyde correlates with the reported profiles for *C. verum* bark essential oils (Beyaz et al., 2025; Guo et al., 2024; Li et al., 2021), which are generally rich in trans-cinnamaldehyde. Additionally, the detectable presence of oxygenated monoterpenes aligns with the enhanced aqueous solubility of these compounds and their tendency to migrate into the hydrosol during hydrodistillation (Almeida et al., 2024; Francezon & Stevanovic, 2017). Cinnamaldehyde, predominantly found as the trans (E) isomer, provides flavor and fragrance to cinnamon. It is produced naturally by the shikimate metabolic pathway, appearing as a pale yellow, viscous liquid. It derives from the bark of cinnamon trees and other plants of the *Cinnamomum* genus (Jaramillo Jimenez et al., 2024; Wang et al., 2025).

Both Origanum hydrosols exhibited a high degree of carvacrol-type profiles, but with different degrees of compositional simplicity. The black oregano hydrosol was nearly monocomponent in GC–MS results, with carvacrol accounting for 99.67% of the volatile fraction; only traces of borneol (0.07%), 4-terpineol (0.04%), and docosane (0.19%) were detected. By contrast, the ball oregano hydrosol, while also carvacrol-dominant (84.15%), contained a more diverse set of accompanying volatiles: thymol (1.88%), linalool (2.88%), γ-terpinene (2.33%), borneol (2.06%), trans-caryophyllene (1.28%), α-humulene (0.47%), caryophyllene oxide (0.53%), and small amounts of α-pinene (0.23%), o-cymene (0.99%), 4-terpineol (0.87%), and others. This pattern of carvacrol as the principal phenolic monoterpene with minor thymol and oxygenated monoterpenes is well documented for *O. onites* and carvacrol-chemotype *O. vulgare* from Anatolia, Eastern Mediterranean, and Asia (Almeida et al., 2024; Bairamis et al., 2024; Gavriil et al., 2021; Verma, 2012). The elevated carvacrol percentage observed in black oregano hydrosol compared to ball oregano suggests that carvacrol, despite its largely hydrophobic nature, partitions favorably into the aqueous phase during hydrodistillation. While carvacrol possesses a phenolic hydroxyl group capable of hydrogen bonding, its overall hydrophobicity limits its water solubility to approximately 1.25 g/L at room temperature (Mączka et al., 2023). The high carvacrol content in hydrosols likely results from a combination of factors including: (1) steam distillation efficiency in extracting volatile phenolic compounds, (2) the presence of co-distilled hydrophilic components that may enhance carvacrol's apparent solubility through micelle formation or emulsification, and (3) the relatively high vapor pressure of carvacrol (236–237 °C boiling point) facilitating its co-distillation with water. The near-monocomponent profile of black oregano hydrosol may reflect both the plant's chemotype and distillation conditions that favor carvacrol transfer over other terpenoids (Khan et al., 2018; Martins et al., 2017). Carvacrol has a broad-spectrum antimicrobial activity and is effective against fungi and foodborne bacterial pathogens, and it exhibits notable antioxidant capacity. The US Food and Drug Administration (FDA) defines

carvacrol as Generally Recognized as Safe (GRAS) for specified uses in foods. European regulatory assessments likewise consider it safe for consumers, citing a maximum daily intake of 0.0003 mg per kilogram of body weight and reporting a threshold for observable effects of approximately 500 mg/kg body weight per day (Lei et al., 2019; Munro & Danielewska-Nikiel, 2006).

Across all three hydrosols, oxygenated monoterpenes (e.g., linalool, borneol, 4-terpineol, 1,8-cineole) constituted most of the minor components. As aforecited, this is anticipated due to their greater polarity and affinity for water compared to hydrocarbon monoterpenes, which aids in their integration into the aqueous distillate (Almeida et al., 2024; Politi et al., 2020). In the ball oregano hydrosol, for instance, the cumulative contribution of linalool, borneol, 4-terpineol, and 1,8-cineole exceeded 5%, whereas hydrocarbon terpenes were comparatively scarce. Conversely, cinnamon hydrosol's sesquiterpene signature (α-copaene, γ-cadinene, α-humulene) suggests co-distillation of heavier volatiles from bark tissue along with the major phenylpropanoid component (Beyaz et al., 2025; Gagliano Candela et al., 2019; C.-H. Wang et al., 2024). These distinct chemical fingerprints not only validate the identity and quality of the hydrosols but also provide a compelling rationale for their varying biological activity, which will be examined in the following sections.

Antioxidant Activity

The oxidation step is considered one of the primary causes of food deterioration; therefore, antioxidants are highly valued in the food industry. In recent decades, plants with natural antioxidant properties have attracted significant interest as natural alternatives to synthetic preservatives (D'Amato et al., 2018). Hydrosols are gaining popularity as natural antioxidants due to their bioactive compounds, such as monoterpenoids, aromatic hydrocarbons, polyphenols, ethers, and aldehydes, which are byproducts of essential oil production (Xin et al., 2023). Despite concerns that synthetic antioxidants such as BHA and BHT may be harmful to human health (Aebisher et al., 2021), hydrosols are considered safe compounds, and their usage does not pose a threat to human health (Diniz Do Nascimento et al., 2020). As illustrated in Table 2, the total phenolic content (mg GAE/mL), the DPPH (mg TE/mL), and the ABTS (mg TE/mL) antioxidant capacities of the hydrosols obtained from ball oregano, black oregano, and cinnamon are presented. Markedly higher total phenolic content (1286 mg GAE/L) and the highest total antioxidant content based on DPPH (4.24 mmol TE/L) and ABTS (3,07 mmol TE/L) scavenging activity were detected in black oregano hydrosol. This was followed by ball oregano and cinnamon hydrosols, respectively. The radical scavenging activities (DPPH and ABTS) and total phenolic content observed in the hydrosols are predominantly attributed to their major bioactive components, namely carvacrol in oregano hydrosols (Ramos et al., 2014) and cinnamaldehyde in cinnamon hydrosols (Almeida et al., 2024). The higher antioxidant properties of black oregano hydrosol compared to ball oregano hydrosol may be explained by its carvacrol concentration of over 99%. As well as the major compounds, the ball oregano hydrosol included γ-terpinene, linalool, borneol, and thymol as bioactive compounds, whereas the cinnamon hydrosol contained α-copaene and γ-cadinene (Gonzalez-Burgos & Gomez-Serranillos, 2012). In a study examining cinnamon essential oil, Beyaz et al. (2025) revealed that its antioxidant properties may be attributable not only to cinnamaldehyde but also to the compounds α-copaene and γ-cadinene present within it. Furthermore, it has been demonstrated that the compounds α-copaene and γ-cadinene in cinnamon hydrosol contribute antioxidant capacity through synergistic effects, as reported by Li et al. (2021) in cinnamon essential oil. They emphasize that these components in cinnamon essential oil exhibit a positive correlation with the major component, thereby enhancing the antioxidant capacity. From an alternative perspective, phenolic compounds and flavonoids function as radical scavengers through hydrogen atom donation. The observed DPPH radical scavenging activity in Cinnamomum

Table 2 Results of Total Phenolic Content (TPC), $ABTS^{+}$, and DPPH radical scavenging activity assays. Superscripts compare means within each column. The mean ± SD (α = 0.05) is found per cell

Hydrosol	Total organic compounds (mg/mL)	Major volatile compound and amount (mg/mL)	TPC* (mg GAE/L)	ABTS** (mmol TE/L)	DPPH** (mmol TE/L)
Cinnamon (*Cinnamomum verum*)	$3,444^{b}$	Cinnamaldehyde (2,638)	$299,17 \pm 2,03^{c}$	$0,76 \pm 0,00^{c}$	$2,54 \pm 0,00^{c}$
Black Oregano (*Origanum vulgare L.*)	$2,231^{a}$	Carvacrol (2,224)	$1286 \pm 3,82^{a}$	$3,07 \pm 0,00^{a}$	$4,24 \pm 0,00^{a}$
Ball Oregano (*Origanum onites L.*)	$2,585^{c}$	Carvacrol (2,175)	$1170 \pm 3,84^{b}$	$2,43 \pm 0,00^{b}$	$3,13 \pm 0,00^{b}$

* GAE stands for the gallic acid equivalent

** TE stands fort he trolox equivalent

Table 3 Results of the antimicrobial activity assay for the hydrosols. Uppercase superscripts compare means within each row (across CH, BH, BTH, CHC, CHM). Lowercase superscripts compare means within each column (across test pathogens). The mean ± SD ($\alpha = 0.05$) is found per cell

Test Pathogens	CH (pH:4.42)*	BH (pH:7.97)*	BTH (pH:7.63)*	CHC (pH:3.03)*	CHM (pH:3.09)*
Klebsiella pneumoniae ATCC 13883	19.24 ± 1.7^{Aa}	17.24 ± 0.40^{Ba}	12.57 ± 0.10^{Ca}	18.05 ± 0.30^{Aa}	23.67 ± 0.60^{Da}
Escherichia coli O157:H7 ATCC 43897	18.62 ± 0.9^{Aa}	19.27 ± 2.20^{Aa}	16.68 ± 1.20^{Ab}	17.75 ± 0.50^{Aa}	21.24 ± 0.30^{Bb}
Escherichia coli ATCC 11230	23.65 ± 1.5^{Ab}	33.19 ± 2.40^{Bb}	19.54 ± 1.90^{Cc}	18.00 ± 0.40^{Ca}	22.90 ± 0.70^{Aa}
Proteus mirabilis ATCC 29906	16.97 ± 0.3^{Aa}	18.45 ± 0.90^{Ba}	17.97 ± 1.10^{Ab}	17.78 ± 0.10^{Aa}	16.6 ± 0.20^{Ac}
Proteus vulgaris ATCC 13319	17.14 ± 1.1^{Aa}	17.23 ± 1.50^{Aa}	27.97 ± 0.50^{Bd}	16.93 ± 0.60^{Aa}	20.10 ± 0.40^{Cb}
Salmonella enterica sv. Typhimurium ATCC 14028	17.19 ± 0.7^{Aa}	17.76 ± 0.80^{Aa}	15.53 ± 1.40^{Ab}	19.12 ± 0.30^{Bb}	20.58 ± 1.50^{Bb}
Yersinia enterocolitica ATCC 9610	18.53 ± 0.5^{Aa}	18.14 ± 0.20^{Aa}	17.20 ± 0.30^{Bb}	17.76 ± 0.60^{Aa}	22.40 ± 0.40^{Ca}
Bacillus cereus ATCC 33019	20.11 ± 0.40^{Ac}	24.18 ± 1.10^{Bc}	10.59 ± 0.10^{Ca}	17.66 ± 0.50^{Da}	22.06 ± 0.50^{Ea}
Staphylococcus aureus ATCC 25923	20.06 ± 1.20^{Ac}	28.14 ± 1.80^{Bd}	20.16 ± 1.60^{Ac}	20.00 ± 0.50^{Ab}	21.87 ± 1.10^{Aa}
Listeria monocytogenes ATCC 7644	18.24 ± 0.80^{Aa}	22.67 ± 0.50^{Bc}	19.65 ± 0.30^{Cc}	24.25 ± 0.30^{Dc}	27.62 ± 0.50^{Ed}
Candida albicans ATCC 10231	34.06 ± 2.00^{Ad}	25.06 ± 2.20^{Bc}	23.98 ± 1.70^{Be}	33.57 ± 1.10^{Ad}	34.17 ± 1.40^{Ae}
Aspergillus flavus ATCC 9643	26.23 ± 0.50^{Ab}	17.44 ± 1.10^{Ba}	13.08 ± 0.30^{Ca}	22.20 ± 1.20^{De}	26.21 ± 0.50^{Ad}

* *CH* cinnamon hydrosol, *BH* ball oregano hydrosol, *BTH* black oregano hydrosol, *CHC* 3% ascorbic acid supplemented Cinnamon hydrosol, *CHM* 3% malic acid supplemented Cinnamon hydrosol

species may also indicate their capacity to act as hydrogen donors (Table 3, pH 4.42) (Ho et al., 2019). Conversely, Al-Mansori et al. (2020) found that carvacrol has a more potent antioxidant effect than thymol, and its combined effect with thymol was reported to be much weaker than a single one. In light of this, the presence of constituents such as γ-terpinene, linalool, borneol, and thymol in ball oregano hydrosol might diminish its antioxidant activity via antagonistic interactions. Similar to our findings, researchers studying two different thyme and oregano hydrosols belonging to the *Lamiaceae* family have reported that the hydrosols contain rich antioxidant compounds (Ahmed et al., 2024; Cid-Pérez et al., 2019; El Bouny et al., 2021). The study by Al-Juhaimi et al. (2025) reported that the total phenolic content (625,79 mg/L) and DPPH antioxidant capacity (1,98 mmol/L) of thyme hydrosol are lower than those found in the present study. They highlighted that while longer extraction times increased the phenolic content, the antioxidant properties of thyme hydrosol actually declined after just one hour of distillation, underscoring the importance of optimizing extraction conditions for maximum bioactive benefit. Another study by Smiljanić et al. (2023) investigated the antioxidant properties of hydrosols from nine *Lamiaceae* family plant species. The total phenolic content of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) hydrosols was found to be 204,2 and 141,4 mg/L, respectively. The total phenolic content of the hydrosols was found to be lower than that of the oregano species identified in our study. No comparison of ABTS and DPPH antioxidant activity was performed. Researchers have stressed that total phenolic, DPPH, and ABTS antioxidant properties demonstrate a high degree of positive correlation; this result is similarly synchronized with the changes in our antioxidant values.

The primary mechanism may involve cross-reactions among radical species arising from interactions between highly reactive peroxyl radicals and antioxidant compounds (Rossetto et al., 2002). According to Öztürk (2012), Terpenes reduce ABTS and DPPH radicals by removing allylic hydrogen atoms, a process enabled by the conjugated double bonds in their molecular structures. Furthermore, Olszowy and Dawidowicz (2016) proposed that terpenoid antioxidants convert to resonance-stabilized forms, presumably via intramolecular charge delocalization driven by π-bond conjugation. Integrating our results with the cytotoxicity data presented in Fig. 1 reveals that hydrosols demonstrating significant bioactivity may serve as natural preservative agents in dairy systems at appropriate dilution levels, thereby offering a safer alternative to synthetic antioxidants that can be potentially harmful to human health.

Antimicrobial Activity and MIC

Initially, it was observed that agar well diffusion screening (Table 3) demonstrated that the hydrosols had measurable inhibitory effects against a wide range of bacteria, yeast, and mold. This is consistent with reports that hydrosols (aqueous distillates) often display antimicrobial activity, albeit partially weaker than the corresponding essential oils, and are therefore especially relevant for surface/contact applications (Almeida et al., 2024; D'Amato et al., 2018; Di Vito et al., 2021; Tavares et al., 2022). Importantly, inhibition-zone diameters should be interpreted as semi-quantitative, as they depend not only on the intrinsic antimicrobial potency but also on the diffusion, volatility, and partitioning of active compounds through agar (Balouiri et al., 2016;

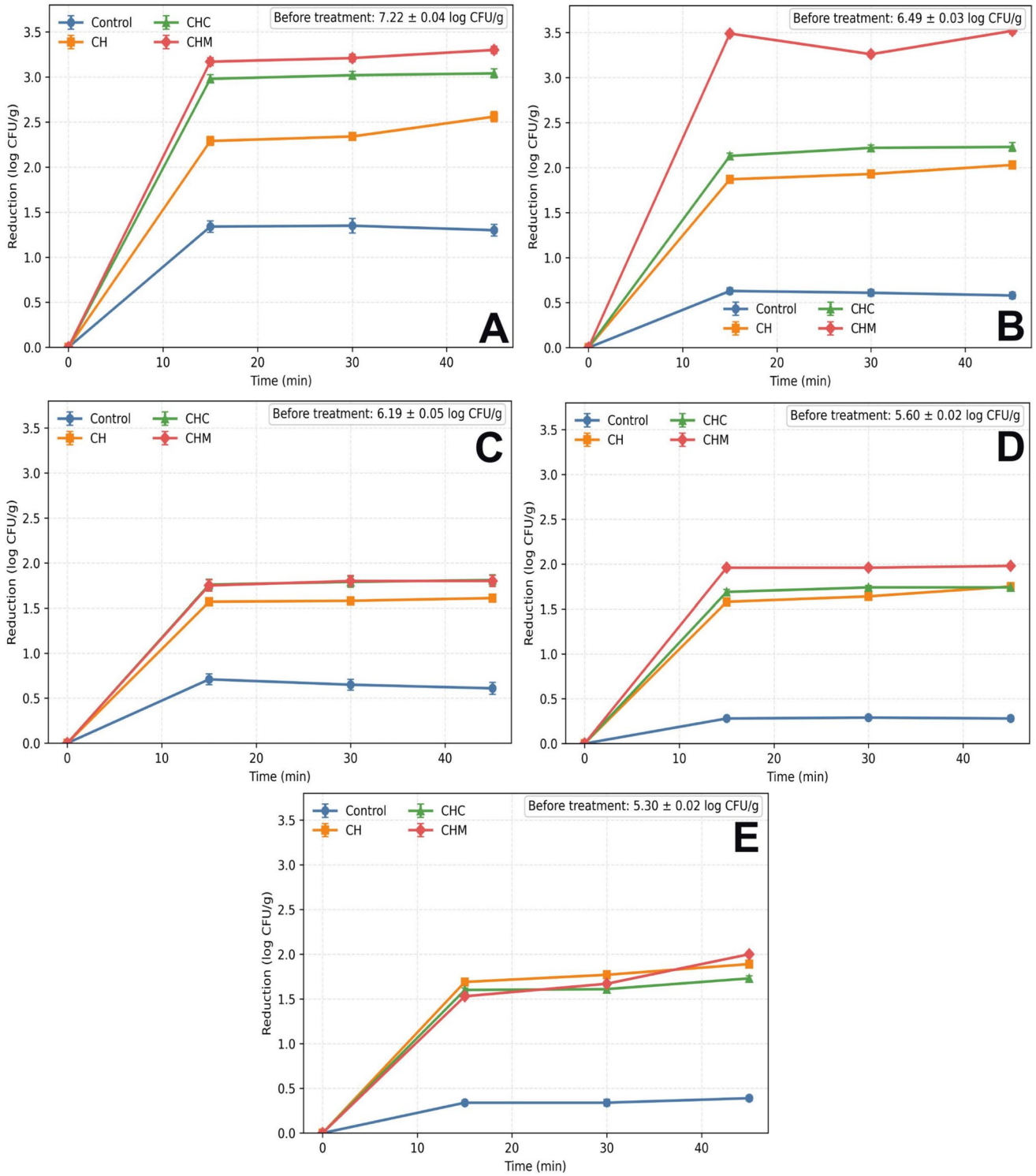

Fig. 1 The graphics depicting microbial load reduction on Kashar cheese following immersion washing with cinnamon hydrosol (CH) and its acidified derivatives, CHC (CH + 3% ascorbic acid) and CHM (CH + 3% malic acid), compared with a water control. Curves show reduction (log CFU/g) relative to the before-treatment baseline (t = 0) at 0, 15, 30, and 45 min; error bars denote mean ± SD (in some figures, error bars cannot be seen because of low SD). Figures: (**A**) *Escherichia coli* O157:H7 ATCC 43897; (**B**) *Salmonella enterica* serovar Typhimurium ATCC 14028; (**C**) *Staphylococcus aureus* ATCC 25923; (**D**) *Candida albicans* ATCC 10231; (**E**) *Aspergillus flavus* ATCC 9643

Bubonja-Šonje et al., 2020; Hyldgaard et al., 2012; Wiegand et al., 2008).

Overall, cinnamon hydrosol (CH) and its acidified formulations (CHC, CHM) produced the most prominent antifungal/anti-yeast inhibition zones, particularly against *Candida albicans* and *Aspergillus flavus* (Table 3). Given that GC–MS indicated a cinnamaldehyde-dominant cinnamon chemotype (Table 1), this pattern aligns with literature describing cinnamaldehyde-associated antifungal and anti-yeast activity through membrane perturbation and interference with key cellular processes (Jaramillo Jimenez et al., 2024; Sun et al., 2020). By contrast, the Origanum hydrosols (BH and BTH; carvacrol-rich chemotypes) displayed pathogen-dependent antibacterial activity (Table 3), which is consistent with the broad but strain- and membrane-dependent susceptibility patterns reported for carvacrol-containing oregano preparations (Bairamis et al., 2024; Cid-Pérez et al., 2019; D'Amato et al., 2018; Gavriil et al., 2021; Xylia et al., 2022).

Broth microdilution MIC testing was focused on cinnamon-based formulations (Table 4), because these showed the broadest activity in screening. In this assay, acidification notably strengthened antibacterial performance: CHM generally produced the lowest MICs across multiple bacteria, followed by CHC, while unmodified CH required higher concentrations. This "acidification gain" is mechanistically plausible for several reasons. First, Gram-negative bacteria possess an outer membrane that restricts penetration of hydrophobic antimicrobials, and permeability can increase when stabilizing divalent-cation bridges are perturbed (Nikaido, 2003; Silhavy et al., 2010). Organic-acid conditions can exacerbate membrane stress and contribute to outer-membrane destabilization, which has been demonstrated for weak acids such as lactic acid and discussed for related acid stress contexts (Alakomi et al., 2000; Clifton et al., 2015; Lund et al., 2020). Second, weak organic acids can enter cells in an undissociated form and then dissociate in the cytosol, forcing ATP-intensive pH homeostasis and amplifying growth inhibition (Hirshfield et al., 2003; Krulwich et al., 2011; Slonczewski et al., 2009). These mechanisms provide a coherent explanation for why CHM (malic-acid formulation) enhanced antibacterial efficacy across several Gram-negative and Gram-positive strains relative to CH.

At the level of the dominant cinnamon volatile, cinnamaldehyde is an electrophilic α,β-unsaturated aldehyde. It can interact with cellular targets via Schiff-base formation and Michael-type additions to nucleophilic residues, which disrupts enzyme systems and has been linked to inhibition of bacterial cell division (e.g., FtsZ-related effects) and broader physiological dysfunction (Chai et al., 2022; Domadia et al., 2007; Jaramillo Jimenez et al., 2024; Wei et al., 2011). Such multi-target activity is often invoked to explain broad-spectrum effects and the relatively strong performance of cinnamon-derived preparations in both Gram-positive and Gram-negative bacteria, especially when additional stressors (low pH/organic acids) are present (Hyldgaard et al., 2012; Lund et al., 2020).

For Origanum hydrosols, the dominant phenolic monoterpene carvacrol is widely described as a membrane-active antimicrobial. It partitions into lipid bilayers, increases membrane permeability, and can collapse the proton motive force, leading to leakage of ions and impaired energy metabolism (Gill & Holley, 2006a, b; Hyldgaard et al., 2012; Ultee et al., 1999, 2002). This mechanism is compatible with the substantial inhibition zones observed for BH/BTH against selected bacteria in agar diffusion (Table 3). Differences between BH and BTH can also be rationalized by the role

Table 4 Minimum inhibitory concentration (MIC) values of cinnamon hydrosol formulations evaluated in the present study (μL hydrosol/mL broth; final v/v). Uppercase superscripts indicate comparisons within each row (across CH, CHC, CHM). Lowercase superscripts indicate comparisons within each column (across pathogens). Values are mean ± SD (α = 0.05)

Test Pathogens	CH (μL/mL)*	CHC (μL/mL)*	CHM (μL/mL)*
Klebsiella pneumoniae ATCC 13883	6.34 ± 1.81^{Aa}	7.26 ± 0.01^{Aa}	1.45 ± 0.01^{Ba}
Escherichia coli O157:H7 ATCC 43897	18.15 ± 0.01^{Ab}	6.34 ± 1.81^{Ba}	7.26 ± 0.01^{Bb}
Escherichia coli ATCC 11230	18.15 ± 0.00^{Ab}	6.34 ± 1.81^{Ba}	7.26 ± 0.00^{Bb}
Proteus mirabilis ATCC 29906	12.70 ± 3.27^{Ac}	6.34 ± 1.81^{Ba}	6.34 ± 1.81^{Bb}
Proteus vulgaris ATCC 13319	12.70 ± 3.27^{Ac}	6.34 ± 1.81^{Ba}	1.99 ± 0.94^{Ca}
Yersinia enterocolitica ATCC 9610	15.42 ± 0.94^{Ab}	6.34 ± 1.81^{Ba}	7.26 ± 0.01^{Bb}
Staphylococcus aureus ATCC 25923	12.70 ± 1.09^{Ac}	6.34 ± 0.00^{Ba}	2.54 ± 1.81^{Ca}
Bacillus cereus ATCC 33019	15.42 ± 3.56^{Ab}	7.26 ± 1.09^{Ba}	1.99 ± 0.01^{Ca}
Salmonella enterica sv. Typhimurium ATCC 14028	18.15 ± 0.01^{Ab}	7.26 ± 0.02^{Ba}	1.99 ± 0.01^{Ca}
Listeria monocytogenes ATCC 7644	9.97 ± 0.94^{Aa}	7.26 ± 1.81^{Aa}	3.09 ± 3.27^{Ba}
Candida albicans ATCC 10231	18.15 ± 0.02^{Ab}	36.30 ± 0.01^{Bb}	31.75 ± 3.99^{Bc}

**CH* cinnamon hydrosol, *CHC* 3% ascorbic acid supplemented cinnamon hydrosol, *CHM* 3% malic acid supplemented cinnamon hydrosol

of minor co-volatiles and formulation-dependent diffusion/transport phenomena, which can influence apparent activity in agar even when a single compound dominates the profile (Hyldgaard et al., 2012; Mutlu-Ingok et al., 2020).

A notable assay-dependent discrepancy was observed for C. albicans: cinnamon-based formulations generated very large inhibition zones on agar, yet in broth MIC testing, unmodified CH performed better than acidified CHC/CHM (Table 4). Such divergence is not unusual because agar diffusion and broth microdilution probe different constraints (diffusion/partitioning versus sustained exposure in a liquid phase) (Balouiri et al., 2016; Bubonja-Šonje et al., 2020; Hyldgaard et al., 2012; Wiegand et al., 2008). Additionally, *C. albicans* is capable of robust extracellular pH modulation and can utilize or transport certain carboxylic acids, which can contribute to acid tolerance in liquid environments (Alves et al., 2020; Danhof et al., 2016). Therefore, while acidification may enhance antibacterial performance through membrane and acid-stress mechanisms, the same strategy does not necessarily improve anti-*Candida* potency in broth, where yeast physiology and acid adaptation can dominate the outcome.

Hydrosol-induced Cytotoxicity in HT29 and L929 Cells

CCK-8 evaluated the cytotoxic potential of the three hydrosols in HT29 human colorectal adenocarcinoma cells and L929 mouse fibroblasts following 24 h exposure across serial dilutions (1 × to 256 ×). All hydrosols demonstrated a dose-dependent decrease in cell viability, with varying sensitivities observed between the two cell lines. Four-parameter logistic models provided stable dose–response fits from which IC50 values were estimated (Fig. 1). Consistent with the standard 70% viability cutoff for cytotoxicity screening (Beyaz et al., 2025), broad non-cytotoxic ranges were seen at higher dilutions for both cell types. In contrast, viability gradually decreased as samples approached the more concentrated end of the range. (Fig. 1A–B).

At first glance, across cell lines, HT29 cells were generally more susceptible than L929 fibroblasts at equivalent hydrosol dilutions, reflected by left-shifted dose–response curves and lower IC50 estimates for HT29 relative to L929 in the same treatment (Fig. 1B). For ball oregano hydrosol, a significant reduction in cell viability was observed at dilutions below 32 ×, with HT29 cells being notably more sensitive than L929 cells. The estimated IC50 values were 26.67 × for HT29 and 79.48 × for L929 (Fig. 1B). Similarly, black oregano hydrosol displayed a concentration-dependent cytotoxic effect, with HT29 cells again showing high sensitivity; however, L929 cells exhibited slightly more sensitivity compared to HT29 cells. The IC50 values were 13.59 × for HT29 and 9.45 × for L929, indicating moderate selectivity. Cinnamon hydrosol demonstrated potent cytotoxicity toward both cell lines at lower dilutions; however, L929 fibroblasts showed greater resistance compared to HT29 cells (IC50: 22.15 ×). Although the IC50 (1.46 ×) value for cinnamon hydrosol is very low, more than 70% viability was observed in L929 cells after 2 × dilution. This pattern indicates a nominal degree of oncoselectivity over the 24-h exposure used here, i.e., a concentration range in which fibroblast viability remains above the 70% threshold, while HT29 viability is more substantially reduced. Among hydrosols, differences in apparent potency reflected their dominant chemotypes: cinnamon hydrosol (CH), rich in the electrophilic α, β-unsaturated aldehyde cinnamaldehyde, generally caused greater viability losses at similar dilutions, while the Origanum hydrosols (BH, BTH), dominated by the phenolic monoterpene carvacrol, showed broadly similar but often slightly less pronounced effects at comparable points on the dilution spectrum (Fig. 1).

The observed cytotoxicity patterns align with the known mechanisms of action of the dominant compounds in each hydrosol. Cinnamaldehyde, the primary component of cinnamon hydrosol, functions as an electrophilic α,β-unsaturated aldehyde capable of forming Schiff bases with primary amines and undergoing Michael-type addition to protein thiols. These covalent modifications disrupt key enzyme systems and mitochondrial function, ultimately reducing cellular metabolic activity as measured by CCK-8 assays (Jaramillo Jimenez et al., 2024; Lin et al., 2013; Ranjitkar et al., 2021). In contrast, carvacrol, the major phenolic monoterpene in both Origanum hydrosols, partitions into lipid bilayers, increasing membrane permeability and proton flux. This protonophoric action collapses electrochemical gradients and compromises energy metabolism (Fan et al., 2015; Pakdemirli et al., 2020; Sampaio et al., 2021), manifesting as reduced CCK-8 readouts at higher concentrations.

Collectively, these data identify practical working ranges where antimicrobial benefits can be achieved while maintaining acceptable viability of fibroblasts. This is particularly relevant for short-contact, rinse-type applications, which are typical of dairy-surface decontamination protocols. However, it should be noted that the CCK-8 assay employed here measures continuous 24-h exposure, which exceeds typical contact times in dairy processing scenarios. Future research should therefore verify safety during shorter exposure periods (15–45 min) using primary human epithelial or skin models. Within these established limits, effective utilization of these hydrosols as antimicrobial agents appears achievable while preserving acceptable cytocompatibility.

Table 5 Results of the cleaning trial for the microbiological load of model food (Kashar cheese) concerning each selected test pathogen (Log(cfu/g)). Uppercase superscripts compare times within each sample. Lowercase superscripts compare samples within each time. The values were shown as mean ± SD (α = 0.05)

Test Pathogens	Samples	Before treatment	15. min Log(cfu/g)	30. min Log(cfu/g)	45. min Log(cfu/g)
Escherichia coli O157:H7 ATCC 43897	Control	7.22 ± 0.04^{Aa}	5.88 ± 0.05^{Ba}	5.87 ± 0.07^{Ba}	5.92 ± 0.05^{Ba}
	CH	7.22 ± 0.04^{Aa}	4.93 ± 0.03^{Bb}	4.88 ± 0.01^{Bb}	4.66 ± 0.04^{Cb}
	CHC	7.22 ± 0.04^{Aa}	4.24 ± 0.02^{Bc}	4.20 ± 0.01^{Bc}	4.18 ± 0.03^{Cc}
	CHM	7.22 ± 0.04^{Aa}	4.05 ± 0.02^{Bd}	4.01 ± 0.01^{Bd}	3.92 ± 0.01^{Cd}
Salmonella enterica sv. Typhimurium ATCC 14028	Control	6.49 ± 0.03^{Aa}	5.86 ± 0.02^{Ba}	5.88 ± 0.02^{Ba}	5.91 ± 0.02^{Ca}
	CH	6.49 ± 0.03^{Aa}	4.62 ± 0.01^{Bb}	4.56 ± 0.02^{Cb}	4.46 ± 0.01^{Db}
	CHC	6.49 ± 0.03^{Aa}	4.36 ± 0.01^{Bc}	4.27 ± 0.01^{Cc}	4.26 ± 0.04^{Cc}
	CHM	6.49 ± 0.03^{Aa}	3.00 ± 0.01^{Bd}	3.23 ± 0.01^{Cd}	2.97 ± 0.02^{Bd}
Staphylococcus aureus ATCC 25923	Control	6.19 ± 0.06^{Aa}	5.48 ± 0.01^{Ba}	5.54 ± 0.01^{Ba}	5.58 ± 0.03^{Ca}
	CH	6.19 ± 0.01^{Aa}	4.62 ± 0.01^{Bb}	4.61 ± 0.02^{Bb}	4.58 ± 0.02^{Cb}
	CHC	6.19 ± 0.06^{Aa}	4.43 ± 0.01^{Bc}	4.40 ± 0.01^{Bc}	4.38 ± 0.01^{Bc}
	CHM	6.19 ± 0.06^{Aa}	4.44 ± 0.02^{Bc}	4.39 ± 0.01^{Bc}	4.39 ± 0.01^{Bc}
Candida albicans ATCC 10231	Control	5.60 ± 0.02^{Aa}	5.32 ± 0.01^{Ba}	5.31 ± 0.01^{Ba}	5.32 ± 0.02^{Ba}
	CH	5.60 ± 0.02^{Aa}	4.02 ± 0.02^{Bb}	3.96 ± 0.01^{Cb}	3.85 ± 0.02^{Db}
	CHC	5.60 ± 0.02^{Aa}	3.91 ± 0.02^{Bc}	3.86 ± 0.01^{Cc}	3.86 ± 0.01^{Cb}
	CHM	5.60 ± 0.02^{Aa}	3.64 ± 0.02^{Bd}	3.64 ± 0.01^{Bd}	3.62 ± 0.01^{Bc}
Aspergillus flavus ATCC 9643	Control	5.30 ± 0.02^{Aa}	4.96 ± 0.02^{Ba}	4.96 ± 0.03^{Ba}	4.91 ± 0.02^{Ca}
	CH	5.30 ± 0.02^{Aa}	3.61 ± 0.02^{Bb}	3.53 ± 0.01^{Cb}	3.41 ± 0.01^{Db}
	CHC	5.30 ± 0.02^{Aa}	3.70 ± 0.01^{Bc}	3.69 ± 0.02^{Bc}	3.57 ± 0.02^{Cc}
	CHM	5.30 ± 0.02^{Aa}	3.77 ± 0.01^{Bd}	3.63 ± 0.01^{Cd}	3.30 ± 0.01^{Dd}

Model Food Trial Results

The decontamination efficacy of cinnamon-based hydrosols was evaluated in a model food system using Kashar cheese as a representative dairy surface. This matrix was selected due to its susceptibility to surface contamination and relevance to dairy processing applications where aqueous rinses are operationally feasible. Immersion washing trials demonstrated that all cinnamon hydrosol formulations significantly reduced microbial loads across all five test pathogens compared to the water control (Table 5; Fig. 2).

A clear hierarchy of antimicrobial performance emerged across treatments. The malic acid-supplemented formulation (CHM) consistently achieved the greatest log reductions, followed by the ascorbic acid-supplemented variant (CHC), with the unmodified cinnamon hydrosol (CH) showing intermediate efficacy. Water controls exhibited only marginal reductions, confirming that observed effects were attributable to the hydrosol treatments rather than mechanical washing alone.

For Gram-negative pathogens, CHM produced particularly pronounced reductions. Against *Escherichia coli* O157:H7, CHM achieved a 3.30 log CFU/g reduction after 45 min, with the majority of this effect (3.17 log reduction) occurring within the first 15 min of exposure. Similarly, against *Salmonella enterica* serovar Typhimurium, CHM reduced counts by 3.52 log CFU/g at 45 min, with 3.49 log reduction achieved within the initial 15-min period. This rapid action suggests that the antimicrobial mechanisms operate quickly upon contact with contaminated surfaces.

Against Gram-positive *Staphylococcus aureus*, both acidified formulations (CHC and CHM) performed comparably, achieving approximately 1.8 log reductions, while CH yielded a 1.61 log reduction. Notably, for this pathogen, most of the reduction occurred within the first 15 min across all hydrosol treatments, with minimal additional reduction thereafter.

The antifungal activity followed a similar pattern, with CHM demonstrating superior efficacy against both *Candida albicans* (1.98 log reduction) and *Aspergillus flavus* (2.00 log reduction). Interestingly, while *C. albicans* reductions plateaued after 15 min, *A. flavus* showed continued reduction between 15 and 45 min, suggesting differential kinetics of antifungal action that may reflect distinct cellular targets or penetration barriers.

When averaged across all five microorganisms, the mean log reductions at 45 min were 0.63 (control), 1.97 (CH), 2.11 (CHC), and 2.52 (CHM). This quantitative hierarchy underscores the enhancement conferred by acidification, with malic acid providing the most significant overall benefit. The kinetic profiles revealed that 80–95% of the final CHM effect against *E. coli* and *Salmonella* occurred within

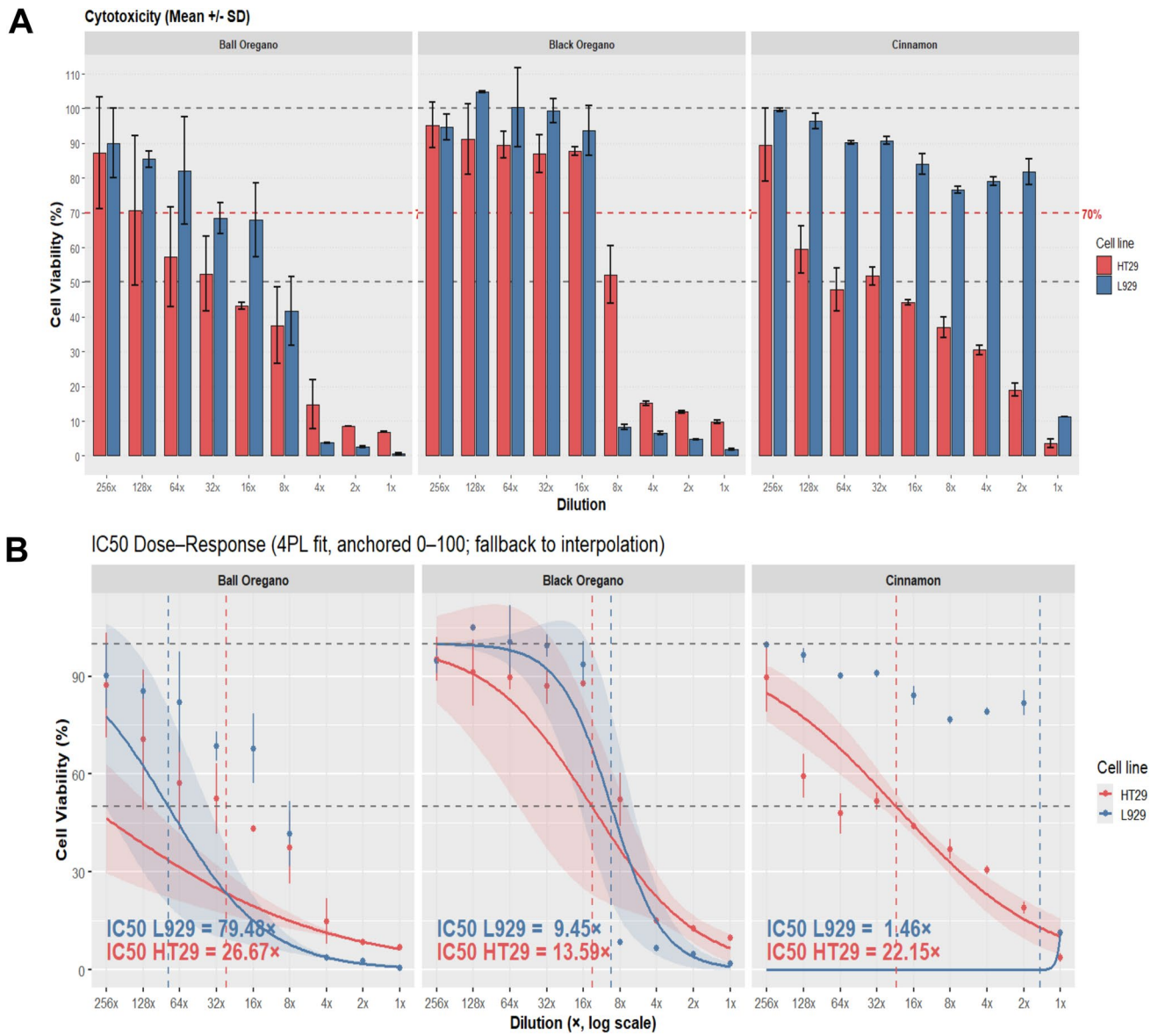

Fig. 2 Hydrosol-induced cytotoxicity in HT29 and L929 cells. (**A**) Bar plots show CCK-8 cell viability (mean ± SD) for HT29 human colorectal adenocarcinoma cells and L929 mouse fibroblasts exposed to serial dilutions of three hydrosols (Ball oregano, Black oregano, Cinnamon). The x-axis denotes fold dilution of the stock sample (256× to 1×; 1× = undiluted). Colors indicate cell line (HT29, red; L929, blue). Dashed horizontal lines mark 100% and 50% viability, and a red dashed line denotes the 70% viability threshold commonly used as a cytotoxicity cutoff; the "70%" label is shown at the right margin. (**B**) Dose–response relationships fitted with four-parameter logistic models (4PL fit) on a $\log_{10}$ dilution axis. Points show observed means with SD; shaded ribbons depict the model 95% confidence bands. Vertical dashed lines indicate the estimated IC50 positions for each cell line, and the corresponding IC50 values (expressed as × dilution) are annotated at the lower left of each facet. Across both panels, data are presented as mean ± SD from independent measurements. *Abbreviations:* IC50, half-maximal inhibitory concentration; ×, fold dilution relative to the undiluted hydrosol (1 ×)

the first 15 min, while *A. flavus* exhibited a more gradual reduction over the 45-min exposure period.

These findings demonstrate that cinnamon hydrosol washing represents a practical decontamination strategy for dairy surfaces, with acidification (particularly with malic acid) substantially enhancing efficacy against both bacterial and fungal contaminants. The rapid action observed (with the majority of the effect occurring within 15 min) aligns well with industrial processing constraints, where short contact times are often operationally necessary. The differential response patterns among pathogens suggest that formulation optimization could be tailored to specific microbial challenges in food processing environments.

The superior performance of CHM may be attributed to its near-neutral zeta potential (Table 6), which reduces electrostatic repulsion with negatively charged bacterial surfaces, facilitating closer droplet-cell contact. Additionally, the acidification likely enhances membrane permeability through pH-dependent mechanisms, as previously discussed in Section "Antimicrobial Activity and MIC". These interfacial and pH-mediated effects collectively contribute to the observed enhancement in antimicrobial activity within the food matrix context.

Zeta Potential and Droplet Size Distribution

Zeta potential and droplet size govern electrostatic interactions between dispersed particles and are key indicators of physical and chemical stability in emulsion-type systems (Liang et al., 2022). Table 6 summarizes these parameters for cinnamon hydrosol (CH) and its acidified derivatives with 3% ascorbic acid (CHC) and 3% malic acid (CHM). In aqueous dispersions, the magnitude of the zeta potential reflects the strength of electrostatic repulsion: absolute values ≥ 30 mV typically confer electrostatic stabilization against flocculation, whereas values ≤ 10 mV are considered near-neutral; the sign indicates whether droplets are (+) cationic or (-) anionic (Patravale et al., 2012; Sharma et al., 2014; Xiao et al., 2025). At week 1, zeta potentials were − 22.2 mV (CH), − 6.14 mV (CHC), and − 2.55 mV (CHM), shifting by week 4 to − 23.5, − 4.57, and − 3.52 mV, respectively. Thus, CH retained a moderately negative charge and even slightly increased in magnitude, whereas CHC and CHM remained near-neutral with only minor shifts. These trajectories align with established relationships between pH, ionic strength, and interfacial charge in emulsified systems (Liang et al., 2022; Rabinovich-Guilatt et al., 2004; Zhou et al., 2018). Acidification by organic acids partially neutralizes negative groups and increases ionic strength, compressing the electrical double layer and lowering the magnitude of the negative zeta potential (Rabinovich-Guilatt et al., 2004; Zhou et al., 2018). While CH therefore shows stronger electrostatic stabilization, CHC and CHM exhibit weaker electrostatic barriers. However, zeta potential alone does not fully determine colloidal stability; steric effects, interfacial composition, continuous-phase viscosity, and polydispersity can also contribute to maintaining kinetic stability, even at relatively low zeta-potential magnitudes (Sharma et al., 2014; Xiao et al., 2025).

Droplet-size evolution was formulation dependent (Fig. 3). During storage, CH and CHC became finer (CH: 628.5 → 468.0 nm; CHC: 605.4 → 422.7 nm), whereas CHM coarsened from a smaller initial size (297.7 → 432.1 nm). None of the dispersions reached the sub-200 nm domain often associated with maximized resistance to gravitational separation and extended shelf life (McClements, 2021). By week 4, all systems converged to a similar size range (≈420–470 nm), indicating broadly comparable gravitational behavior at the end of storage and suggesting that large differences in final droplet size do not drive differences in antimicrobial performance.

Table 6 Zeta potential and particle size measurement results for *Cinnamomum verum* (cinnamon) hydrosol and its derivatives

Hydrosol	Sampling Time	Zeta Potential (mV)	Z-average size (nm)
Cinnamon hydrosol (CH)	1 st week	−22.2	628.5
	4th week	−23.5	468
Cinnamon hydrosol supplemented with 3% Ascorbic acid (CHC)	1 st week	−6.14	605.4
	4th week	−4.57	422.7
Cinnamon hydrosol supplemented with 3% Malic acid (CHM)	1 st week	−2.55	297.7
	4th week	−3.52	432.1

These interfacial properties are closely aligned with the antimicrobial findings discussed above. Bacterial cell surfaces generally carry a net negative charge, so droplets with less negative (near-neutral) zeta potentials experience reduced electrostatic repulsion and can approach and deposit more readily on cells (Ferreyra Maillard et al., 2021). In this context, CHM's nearly neutral zeta potential, combined with its initially smaller droplets, likely facilitated closer contact between hydrosol droplets and bacterial membranes, coherently explaining the larger inhibition zones and lower MIC values observed for CHM against multiple gram-negative and gram-positive bacteria (Tables 3, 4). Acidification further enhances permeabilization of gram-negative outer membranes by perturbing divalent-cation bridges and increasing acid stress, amplifying the benefit of near-neutral droplets (Alakomi et al., 2000; Clifton et al., 2015; Nikaido, 2003).

Conclusions

The present study provides an integrated assessment of three hydrosols (cinnamon (CH), ball oregano (BH), and black oregano (BTH)) linking their volatile chemistry to antioxidant capacity, antimicrobial performance, cytotoxicity, and behavior in a model food system. GC–MS profiling revealed that cinnamon and origanum hydrosols were cinnamaldehyde- and carvacrol-dominant, respectively, corresponding to their bioactivity, with antioxidant capacity ranked as BTH > BH > CH. Antimicrobial testing displayed formulation- and pathogen-specific responses. Acidifying cinnamon hydrosol markedly enhanced antibacterial potency, with the malic-acid blend (CHM) producing

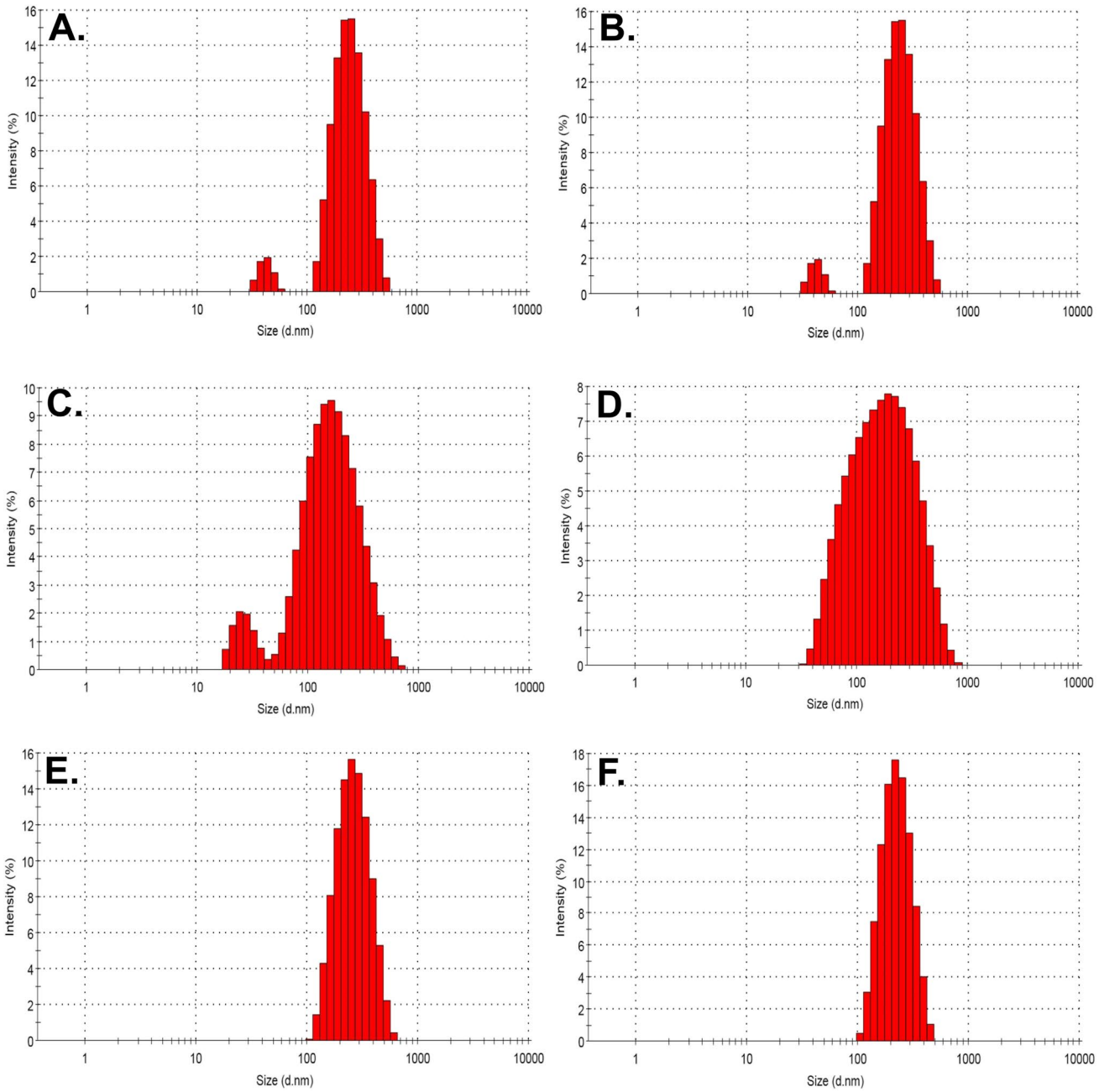

Fig. 3 Graphical illustrations showing the particle size distribution of Cinnamon hydrosols used in this study. (**A**-**B**) Cinnamon hydrosols during storage from the first to the fourth week. (**C**-**D**) Cinnamon hydrosols with 3% ascorbic acid during storage from the first to the fourth week. (**E**–**F**) Cinnamon hydrosols with 3% malic acid from the first to the fourth week

the largest inhibition zones for many gram-negative and gram-positive strains and the lowest MICs for several key pathogens. Origanum hydrosols showed notable activity with pathogen selectivity; BH, which combines carvacrol with auxiliary oxygenated monoterpenes, sometimes surpassed the nearly monocomponent BTH in agar assays. For fungi, cinnamon-based hydrosols generated the largest agar inhibition zones. In contrast, in broth MIC tests against *Candida albicans*, plain CH outperformed its acidified variants, indicating that antibacterial gains from acidification do not necessarily translate to yeasts in liquid systems.

Physical characterization of the cinnamon systems (CH, CHC, CHM) helps explain these trends. CH maintained the most negative zeta potential during storage, whereas CHC and CHM remained near-neutral; droplet sizes converged to ~ 420–470 nm, with CHM initially presenting the smallest droplets. Less negative zeta potentials, combined with smaller early droplets, coincided with more substantial

antibacterial effects, particularly for CHM, suggesting that acidification trades some electrostatic stabilization for improved droplet-cell encounters and pH-driven membrane susceptibility. Model-food trials on Kashar cheese confirmed the practical relevance of these findings: short immersion washes substantially reduced microbial loads, with CHM consistently outperforming CH and CHC, and most antibacterial effects occurring within the first 15 min. Cytotoxicity assays identified workable dilution ranges that maintained ≥ 70% fibroblast viability while retaining antimicrobial activity, supporting short-contact, rinse-type applications. Overall, malic acid-supplemented cinnamon hydrosol emerges as a promising clean-label surface decontaminant for dairy and other food products. Future work should extend validation to additional matrices and strains, optimize acidulation and processing conditions, assess sensory impact and shelf life, and refine interfacial properties to maximize efficacy while preserving cytocompatibility.

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Author Contributions Walid Bouhlel: Methodology, Investigation. Ahmet E. Yetiman: Writing—original draft, validation, project administration, methodology, investigation, formal analysis, data curation, and conceptualization. Mahmut Doğan: Writing—review & editing, validation, supervision, resources, project administration, funding acquisition, conceptualization. Mehmet Horzum: Writing—original draft, methodology, investigation. Ertan Kanbur: Methodology, Investigation.

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Data Availability Data will be made available on request.

Declarations

Competing interest The authors declare no competing interests.

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