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Research Article

Quorum quenching and antimicrobial potential of natural compounds against foodborne biofilm-forming bacteria

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Abstract

Background: Biofilm formation represents a major challenge for food safety, contributing to persistent contamination, compromised process hygiene, and increased antimicrobial resistance. Because quorum sensing (QS) regulates biofilm development, interference with QS signaling (quorum quenching [QQ]) has emerged as a promising strategy for biofilm control.

Aims: This study aimed to evaluate the biofilm-forming capacity and QS (N-acyl homoserine lactones [AHL] and autoinducer-2 [AI-2]) activity of foodborne bacterial isolates and to assess the antimicrobial and QQ potential of selected natural products under *in vitro* conditions.

Methods: A total of 109 bacterial isolates from dairy processing lines were assessed for biofilm formation using a 96-well microtiter assay. QS activity was detected using indicator microorganisms for AHL and AI-2 signaling. Selected natural products (1 mg/mL) were screened for antimicrobial and QQ activity, with QS inhibition evaluated independently of growth suppression.

Results: A total of 89% of the isolates were classified as strong biofilm producers, with *Bacillus* and *Pseudomonas* species predominating. Plant-derived extracts and natural products, including *Calendula officinalis*, propolis, nisin, and *Hypericum perforatum*, exhibited measurable antimicrobial and antibiofilm activity. Propolis and *C. officinalis* reduced QS-associated biofilm responses independently of growth inhibition, indicating potential QQ activity.

Conclusion: These findings highlight the potential of natural extracts as eco-friendly alternatives to chemical disinfectants in food processing environments.

Özet

Dayanak: Biyofilm oluşumu, gıda güvenliği açısından kalıcı kontaminasyona, proses hijyeninin bozulmasına ve artan antimikrobiyal dirence yol açan önemli bir sorundur. Biyofilm gelişimini düzenleyen quorum sensing (QS) sisteminin engellenmesi (quorum quenching [QQ]), biyofilm kontrolü için umut vadeden bir yaklaşımdır.

Amaçlar: Bu çalışmada, süt ürünleri proses hatlarından izole edilen bakteri izolatlarının biyofilm oluşturma kapasiteleri ile QS'e bağlı (N-asil homoserin laktonu [AHL] ve otoindükleyici-2 [AI-2]) sinyal aktivitelerinin değerlendirilmesi ve seçilmiş doğal ürünlerin *in vitro* koşullarda antimikrobiyal ve QQ potansiyellerinin belirlenmesi amaçlanmıştır.

Yöntemler: Toplam 109 izolatın biyofilm oluşumu 96 kuyucuklu mikroplak yöntemi ile belirlenmiş, QS aktivitesi indikatör mikroorganizmalar kullanılarak saptanmıştır. Doğal ürünler (1 mg/mL) antimikrobiyal ve QQ aktiviteleri açısından tarama düzeyinde incelenmiş, QS inhibisyonu büyüme baskılanmasından bağımsız olarak değerlendirilmiştir.

Bulgular: İzolatların %89'u güçlü biyofilm üreticisi olup, *Bacillus* ve *Pseudomonas* türleri baskın bulunmuştur. *Calendula officinalis*, propolis, nisin ve *Hypericum perforatum* belirgin antimikrobiyal ve antibiyofilm etki göstermiştir. Propolis ve *C. officinalis*, QS ile ilişkili biyofilm yanıtlarını büyüme inhibisyonundan bağımsız olarak azaltmıştır.

Sonuç: Doğal ekstraktlar, gıda işleme ortamlarında kimyasal dezenfektanlara çevre dostu alternatifler sunma potansiyeline sahiptir.

Keywords: Antibiofilm, antimicrobial, dairy processing, food safety, natural compounds, quorum quenching

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Introduction

Biofilm is a critical contributor to cross-contamination, antimicrobial resistance, biological pollution, energy inefficiency, and economic loss in various environments. Microorganisms use quorum sensing (QS) as a communication mechanism to assess their environment, regulate gene expression, and coordinate phenotypes such as antibiotic production, virulence, motility, sporulation, and biofilm formation. Disruption of QS systems often results in looser, less structured biofilms (Si & Quan, 2017; Suntharalingam & Cvitkovitch, 2005).

Biofilms form on both living and non-living surfaces and are commonly found in hospitals, food processing facilities, and wastewater treatment plants. A range of bacteria—*Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Aeromonas*, *Bacillus*, *Klebsiella*, *Streptococcus*, *Listeria*, *Enterobacter*, *Ochrobacterium*, *Geobacillus*, and *Shigella*—have been implicated in biofilm formation, and many retain active QS systems that enhance their resilience (Lade et al., 2014; İpek, 2017). Analytical profiling of QS signals in biofilm-producing isolates has revealed the presence of both long-chain N-acyl homoserine lactones (AHLs) and autoinducer-2 (AI-2) signaling molecules, indicating that these molecules are actively produced by strong biofilm formers and are therefore relevant to understanding chemical communication within complex microbial communities (İpek, 2023). Such persistent biofilm communities pose a serious threat to food safety and public health. Conventional sanitation in the food industry relies on harsh chemicals—caustic soda, nitric and hydrochloric acids, and chlorine. While effective, these agents carry significant drawbacks: they can cause respiratory and dermatological issues, generate toxic by-products such as trihalomethanes and haloacetic acids, and contaminate soil, water, and air (Hegstad et al., 2010; Huang et al., 2012). Thus, there is growing interest in safer, eco-friendly alternatives for ensuring hygiene in food processing.

Natural compounds, particularly plant-derived extracts, have shown promise both as antimicrobials and QS agents. For instance, essential oils from cassia, tea tree, and red thyme were found to be more effective against *Pseudomonas* and *S. aureus* biofilms than antibiotics like colistin and gentamicin (Kavanaugh & Ribbeck, 2012). Likewise, *Camellia sinensis* extract was shown to inhibit QS signals of *P. aeruginosa* by mimicking autoinducer molecules and binding to their receptors (Mihalik et al., 2008).

Furthermore, pathogenic bacteria embedded in biofilms frequently develop resistance to disinfectants used in food process environments. Resistant strains have been isolated from dairy plants, potable water, wastewater systems, and environmental sediments, highlighting the need for dual-function natural agents that combine antimicrobial and antibiofilm capabilities (Castillo-Juárez et al., 2015; Jeong et al., 2018).

Recent studies continue to support the efficacy of phytochemicals—including flavonoids and phenolic acids—as QS inhibitors and biofilm disruptors (Fydrych et al., 2025; Shariati et al., 2024). Despite growing evidence on the antimicrobial and anti-quorum-sensing properties of plant-derived compounds, biofilm persistence in dairy processing environments remains a major hygienic challenge. Conventional sanitation strategies mainly target planktonic cells and often neglect QS mechanisms that stabilize biofilms. Moreover, comparative evaluation of AHL- and AI-2–mediated signaling inhibition in foodborne isolates is still limited. In this study, we characterized the biofilm-forming and QS profiles of 109 dairy-associated isolates and evaluated the antimicrobial and quorum quenching (QQ) activities of selected natural products under standardized *in vitro* conditions. This integrated approach provides evidence for natural compounds as potential dual-function agents targeting both bacterial growth and QS-regulated biofilm persistence.

Materials and Methods

Bacterial Strains and Culture Conditions

A total of 109 bacterial isolates previously obtained from dairy process lines (İpek & Zorba, 2018) were used in this study. Reference strains *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 6538P, and *Micrococcus luteus* ATCC 4698 were included as controls. Isolates were maintained on tryptic soy agar (TSA, Merck, Germany) at –18 °C and cultured in tryptic soy broth (TSB) at 30 °C prior to assays.

For QS assays, the biosensor strains *Chromobacterium violaceum* CV026, *Agrobacterium tumefaciens* A136, and *Vibrio harveyi* BB170 were used. The biosensor cultures were grown in Luria–Bertani broth supplemented with the appropriate antibiotics and incubated at 30 °C for 24 h under standard conditions. For AI-2 assays, *V. harveyi* BB170 was cultivated in Marine Broth (2216; BD Difco, USA) for 24 h, and a 1:5000 dilution was prepared using autoinducer bioassay medium prior to inoculation (İpek, 2017; Lade et al., 2014; Park et al., 2014).

Natural Compounds

The tested agents comprised plant-derived ethanolic extracts (*Calendula officinalis* L., *Hypericum perforatum*, *C. sinensis*, *Panax ginseng*, *Ganoderma lucidum*, *Prunella vulgaris*, *Sambucus nigra*, *Cichorium intybus*, and *Thymus vulgaris*), bee-derived propolis extract, and commercially available natural-origin compounds (nisin, resveratrol, vanillin, curcumin, and apple vinegar). All extracts were prepared at a concentration of 1 mg/mL, with plant materials extracted in 70% ethanol, agitated at 180 rpm for 6 h, and sterilized using 0.2 µm membrane filtration, and then stored at 4 °C until use (Lin et al., 2017).

Biofilm Formation Assay

Biofilm production was evaluated using the 96-well microtiter plate method (Christensen et al., 1985). Overnight cultures adjusted to 10^8 colony-forming unit (CFU)/mL were inoculated into TSB, incubated at 30 °C for 24 h, washed, and stained with 1% crystal violet. The bound dye was solubilized in 96% ethanol, and absorbance was measured at 600 nm (optical density [OD_{600}]).

Isolates were classified as weak, moderate, or strong biofilm producers according to established cut off values (İpek, 2017).

QS Signal Detection

AHL production was determined using *C. violaceum* CV026 (short-chain AHLs) and *A. tumefaciens* A136 (long-chain AHLs with X-gal indicator). Autoinducer-2 (AI-2) activity in Gram-positive isolates was detected by luminescence induction in *V. harveyi* BB170 as described by Sun et al. (2014). Biosensor strains were cultivated under standard conditions prior to assays. AHL production was determined based on pigment or color development in the CV026 and A136 indicator systems. For AI-2 detection, *V. harveyi* BB170 luminescence was measured following incubation with test samples, and signal intensity was quantified using a microplate reader by recording optical density at 600 nm (OD_{600}) together with luminescence values, as previously described (Lade et al., 2014; Park et al., 2014).

Antimicrobial Activity of Natural Compounds

The antimicrobial activity of natural compounds was assessed by the disc diffusion method following European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2014) guidelines. Briefly, bacterial suspensions (10^8 CFU/mL) were inoculated onto Mueller-Hinton agar plates, and sterile discs loaded with 15 µL of extract were applied. Plates were incubated at 30 °C for 24 h, and inhibition zones were measured. No antibiotic was used as a positive control in this study; 70% ethanol was included as the control to evaluate any solvent-related effects on bacterial growth. Sterile water was used as the negative control (<6 mm).

QQ Assays

QQ activity of natural compounds was evaluated using disc diffusion assays for AHL-mediated signaling and microtiter plate–based luminescence assays for AI-2–dependent signaling, as previously described (Truchado et al., 2015; Zhu et al., 2016). For AHL inhibition, sterile discs impregnated with test compounds were placed onto indicator strain–inoculated agar plates and incubated under standard conditions. For AI-2 inhibition, luminescence produced by *V. harveyi* BB170 in the presence of test samples was measured using a microplate reader. Zones showing loss of pigment formation or a significant reduction in luminescence relative to untreated controls were considered indicative of QQ activity. Sterile distilled water was used as the negative control, while 70% ethanol (used as the extraction solvent) was included as the solvent control where applicable.

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentrations (MBC), and Inhibition Ratios

MICs were determined by the broth microdilution method in 96-well microplates using serial two-fold dilutions of the extracts, in accordance with EUCAST guidelines (EUCAST, 2014). Wells were inoculated with standardized bacterial suspensions, and MIC was defined as the lowest concentration showing no visible growth, confirmed by OD_{600} measurements and 2,3,5-triphenyltetrazolium chloride colorimetric assessment. MBCs were determined by subculturing aliquots from non-turbid wells onto agar plates and identifying the lowest concentration yielding no colony growth. Solvent control wells containing 70% ethanol (at the same final concentration used for extract preparation) were included as controls. Sterile water was used as the negative control.

Inhibition ratios (%) were calculated based on optical density values using the following formula:

Inhibition (%) = [(OD_{control} − OD_{treated}) / OD_{control}] × 100

Statistical Analysis

Statistical analysis was performed using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA). All experiments were conducted in triplicate (n = 3) unless otherwise stated. Results are expressed as mean ± standard deviation. Differences between groups were evaluated using one-way analysis of variance, followed by Tukey's post hoc test for multiple comparisons. A p-value of <0.05 was considered statistically significant.

Results

Biofilm Formation by Isolates

Among the 109 bacterial isolates obtained from dairy process lines, 89% (n = 94) were classified as strong biofilm producers, whereas only 9 and 2 isolates showed moderate and weak adherence, respectively. Genera such as *Bacillus* and *Pseudomonas* were dominant among strong producers. *L. innocua*, *B. subtilis*, *B. megaterium*, and *P. fluorescens* displayed moderate adherence, whereas *K. oxytoca* and *B. pumilus* were weak producers. None of the isolates were biofilm-negative. The biofilm-forming capacities of bacterial isolates obtained from dairy industry surfaces, as determined by the 96-well microtiter plate assay, are presented in Table S1. These findings confirm the high prevalence of biofilm-forming bacteria in dairy environments (Figure 1).

QS Signals

Screening with biosensor strains revealed that none of the Gram-negative isolates produced short-chain AHLs detectable by *C. violaceum* CV026. However, most produced medium- or long-chain AHLs, as indicated by pigment formation in *A. tumefaciens* A136 assays; notably, 12 Gram-negative biofilm-forming isolates (22.2%) were identified as medium- and long-chain AHL producers. For Gram-positive isolates, all tested strains induced luminescence in *V. harveyi* BB170, indicating the presence of AI-2 signaling molecules (Figure 2). The presence of AI-2 signaling in Gram-positive bacterial isolates, as detected using *V. harveyi* BB170 as a biosensor strain, is presented in Table S2.

Antimicrobial Activity of Natural Compounds

The antimicrobial activity of natural compounds was tested against selected strong biofilm-forming isolates and reference strains. *C. officinalis*, propolis, nisin, and *H. perforatum* exhibited inhibition zones ranging between 7 and 10 mm, demonstrating moderate antimicrobial activity. In contrast, no inhibition zones

were observed for *C. sinensis*, *P. ginseng*, vanillin, curcumin, *G. lucidum*, *P. vulgaris*, or *S. nigra* extracts (Table 1).

QQ Activity

Several natural compounds exhibited QQ potential. Camellia sinensis extract produced the largest inhibition zone against AHL-mediated signaling, while propolis and apple vinegar also demonstrated inhibitory effects. Interestingly, nisin showed strong inhibition against long-chain AHLs, with a mean zone of 26.5 mm. AI-2 inhibitory activity varied, with propolis and apple vinegar showing inhibition ratios comparable to 70% ethanol, whereas *S. nigra* extract was the least effective (Table 2; Figure 3).

MIC, MBC and Growth Inhibition Profiles of Natural Compounds

MIC and MBC assays confirmed the antimicrobial efficacy of selected natural compounds. *C. officinalis* showed the highest inhibition against *E. coli* ATCC 25922 (72.4%), while propolis demonstrated strong inhibition against *S. lactis* (97.2%). In contrast, *H. perforatum* was generally less effective. Overall, propolis and *C. officinalis* possess both antimicrobial and QQ properties, supporting their potential use in food processing environments (Table 3).

Discussion

The high prevalence of strong biofilm formation among dairy-associated isolates confirms that structured microbial communities remain a persistent risk in food processing environments (Carrascosa et al., 2021; İpek & Zorba, 2018). The predominance of *Bacillus* and *Pseudomonas* species aligns with previous reports describing these genera as recurrent and resilient contaminants in dairy systems (İpek & Zorba, 2018). However, beyond structural persistence, the detection of QS signals emphasizes the regulatory role of microbial communication in maintaining biofilm stability (Suntharalingam & Cvitkovitch, 2005).

The observed differences between AHL- and AI-2–mediated signaling indicate distinct regulatory mechanisms. AHL systems, typically associated with Gram-negative bacteria, are closely linked to biofilm maturation and virulence-related traits (Castillo-

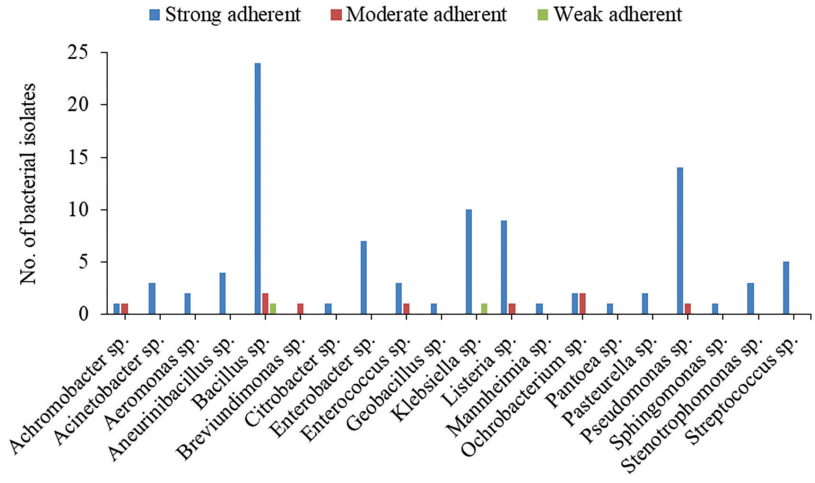

Figure 1. The number of bacterial isolates from different genera capable of forming biofilms.

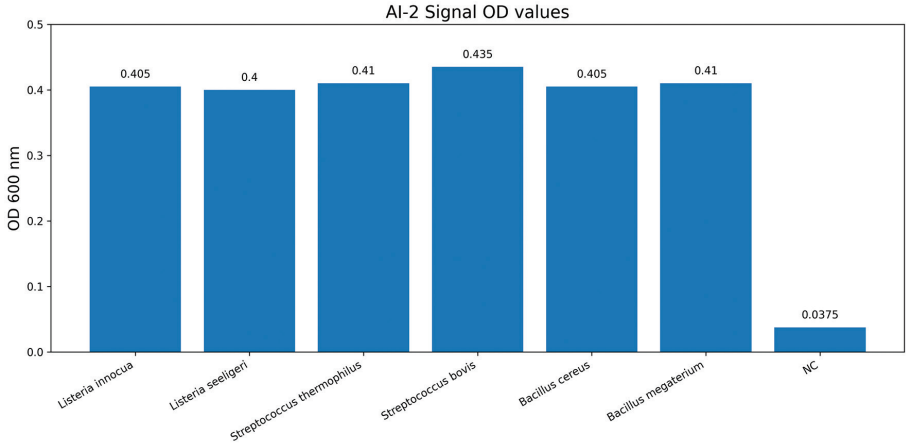

Figure 2. AI-2 signal detection using *Vibrio harveyi* BB170 for Gram-positive bacterial isolates (selected samples).
AI-2 = autoinducer-2; OD = optical density.

Table 1. Antimicrobial effects: inhibition zone values (mm) of natural compounds' extracts (n = 4).

Strains	***Calendula officinalis****	***Propolis****	***Resveratrol****	***Nisin****	***Hypericum perforatum****	***Thymus vulgaris****	***Cichorium intybus****	***Apple vinegar****
Listeria innocua (1)	9.0 (±0.0)	9.0 (±0.0)	10.0 (±0.0)	9.5 (±0.5)	8.5 (±0.5)	7.0 (±0.0)	<6.0	7.5 (±0.5)
Enterobacter cloacae (6)	8.0 (±0.0)	8.0 (±0.0)	7.5 (±0.5)	7.0 (±0.0)	7.0 (±0.0)	8.5 (±0.5)	<6.0	<6.0
Pseudomonas stutzeri (18)	9.0 (±0.0)	9.0 (±0.0)	< 6.0	7.0 (±0.0)	8.0 (±0.0)	<6.0	<6.0	7.0 (±0.0)
Streptococcus lactis (27)	7.0 (±0.0)	9.0 (±0.0)	7.5 (±0.5)	10.0 (±0.0)	9.0 (±0.0)	8.5 (±0.5)	<6.0	<6.0
Bacillus coagulans (32)	8.0 (±0.0)	8.0 (±0.0)	<6.0	7.5 (±0.5)	8.0 (±1.0)	7.0 (±0.0)	<6.0	<6.0
Pseudomonas aeruginosa ATCC 27853	10.0 (±0.0)	7.5 (±0.5)	<6.0	7.0 (±0.0)	7.5 (±0.5)	7.0 (±0.0)	7.0 (±0.0)	<6.0
Staphylococcus aureus ATCC 6538P	9.0 (±0.0)	10.0 (±0.0)	9.0 (±0.0)	9.0 (±1.0)	10.0 (±0.0)	10.0 (±0.0)	8.0 (±0.0)	<6.0
Micrococcus luteus ATCC 4698	9.0 (±0.0)	8.0 (±0.0)	8.0 (±0.0)	8.0 (±0.0)	7.0 (±0.0)	7.0 (±0.0)	<6.0	8.0 (±0.0)
Escherichia coli ATCC 25922	9.0 (±0.0)	8.5 (±0.5)	<6.0	7.0 (±0.0)	7.0 (±0.0)	< 6.0	8.0 (±0.0)	<6.0

*Mean (± standard deviation).

Table 2. AHL QQ Effects: Inhibition zone values (mm) of natural compounds' extracts.

Natural compounds	**Small-chain AHL* (*Chromobacterium violaceum* CV026)**	**Middle/long-chain AHL* (*Agrobacterium tumefaciens* A136)**
Vanillin	11.5 (±0.5)	9.0 (±1.0)
Panax ginseng	11.0 (±1.0)	8.5 (±0.5)
Curcumin	9.0 (±0.0)	8.5 (±0.5)
Camellia sinensis	17.5 (±0.5)	10.0 (±0.0)
Propolis	8.0 (±1.0)	9.0 (±1.0)
Apple vinegar	13.0 (±0.5)	12.0 (±0.5)
Calendula officinalis	7.5 (±0.0)	13.5 (±0.5)
Thymus vulgaris	7.5 (±0.0)	9.5 (±0.5)
Hypericum perforatum	7.0 (±0.0)	8.0 (±0.5)
Resveratrol	8.5 (±1.0)	10.5 (±0.5)
Nisin	6.5 (±0.0)	26.5 (±0.5)
Blank	<6.0	<6.0

*Mean (± standard deviation); [v]alues are expressed as mean ± standard deviation. *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* A136 were used as indicator microorganisms for the detection of short-chain and medium-/long-chain AHLs, respectively.
Inhibition zones of *Cichorium intybus*, *Ganoderma lucidum*, *Prunella vulgaris*, and *Sambucus nigra* were <6 mm in both indicator systems.
AHL = N-acyl homoserine lactone.

Juárez et al., 2015; Si & Quan, 2017). In contrast, AI-2 signaling functions as a broader interspecies communication pathway and is frequently detected in Gram-positive bacteria via LuxS-dependent mechanisms (Sun et al., 2014). The coexistence of both systems in dairy environments may enhance multispecies biofilm coordination and resilience.

The inhibitory profiles of the tested agents differed between signaling systems. *C. officinalis* and propolis reduced both bacterial growth and QS-associated responses, suggesting interference with signal perception or receptor interaction, as previously described for plant-derived phytochemicals (Bouyahya et al., 2022; Shariati et al., 2024; Truchado et al., 2015). In some cases, AHL inhibition occurred without proportional antimicrobial activity, supporting a QQ effect independent of direct bactericidal action.

A notable finding was the pronounced inhibition of long-chain AHL signaling by nisin. Although nisin is primarily recognized for its bactericidal activity against Gram-positive bacteria through membrane pore formation, membrane destabilization may indirectly affect signal diffusion or receptor function, thereby influencing QS responses. While this interaction has not been fully elucidated, it is consistent with reports describing indirect QS modulation via membrane-active compounds (Hegstad et al., 2010).

The differences observed between Gram-negative and Gram-positive isolates likely reflect structural divergence in QS architectures. Gram-negative bacteria mainly rely on LuxI/LuxR-type AHL circuits, whereas Gram-positive species and mixed communities utilize AI-2–mediated communication (Castillo-Juárez et al., 2015; Sun et al., 2014). These regulatory differences may partly explain the strain-dependent variability observed in inhibition responses.

Given the environmental and health-related concerns associated with conventional disinfectants (Hegstad et al., 2010; Huang et al., 2012), natural extracts capable of targeting both microbial growth and communication pathways represent a promising alternative strategy for sustainable biofilm control in dairy processing systems.

This study is limited by its *in vitro* design and single-concentration screening approach, which does not permit dose–response analysis or detailed mechanistic clarification. Future studies should focus

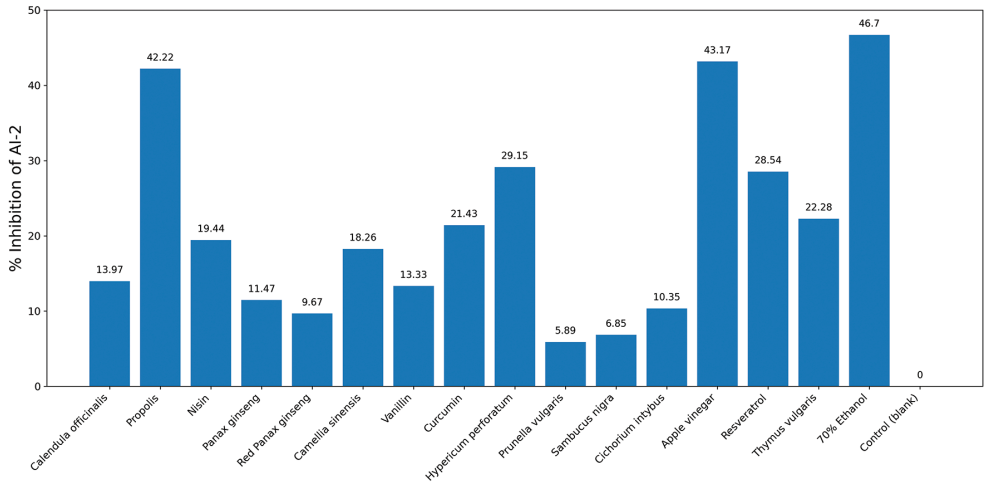

Figure 3. AI-2 signal inhibitory activity of natural compound extracts against the high AI-2–producing isolate Streptococcus bovis.
AI-2 = autoinducer-2.

Table 3. MIC, MBC, and inhibition ratio of natural compounds.

Strains	Comp ound*	MIC and MBC	Inhb.%	Comp ound*	MIC and MBC	Inhb.%	Comp ound*	MIC and MBC	Inhb.%
Listeria innocua (1)	1	500 µg/mL	21.2%	3	500 µg/mL	7.14%	5	500 µg/mL	18.9%
Enterobacter cloacae (6)	1	500 µg/mL	49.4%	3	500 µg/mL	7.5%	5	500 µg/mL	53.04%
Pseudomonas stutzeri (18)	1	500 µg/mL	38.8%	3	500 µg/mL	7.6%	5	500 µg/mL	43.4%
Streptococcus lactis (27)	1	500 µg/mL	33.6%	3	500 µg/mL	6.5%	5	500 µg/mL	48%
Bacillus coagulans (32)	1	500 µg/mL	21.7%	3	500 µg/mL	8.4%	5	500 µg/mL	26.2%
Staphylococcus aureus ATCC 6538 P	1	500 µg/mL	47.7%	3	500 µg/mL	7.6%	5	500 µg/mL	12.5%
Pseudomonas aeruginosa ATCC 27853	1	500 µg/mL	22.6%	3	500 µg/mL	11.7%	5	500 µg/mL	39.2%
Micrococcus luteus ATCC 4698	1	500 µg/mL	24%	3	500 µg/mL	8.9%	5	500 µg/mL	47.5%
Escherichia coli ATCC 25922	1	500 µg/mL	72.4%	3	500 µg/mL	6.5%	5	500 µg/mL	37.2%
L. innocua (1)	2	500 µg/mL	87.7%	4	500 µg/mL	21.6%	6	500 µg/mL	11.8%
E. cloacae (6)	2	500 µg/mL	44.2%	4	500 µg/mL	12.4%	6	500 µg/mL	17.01%
P. stutzeri (18)	2	500 µg/mL	53.6%	4	500 µg/mL	18.8%	6	500 µg/mL	16.5%
S. lactis (27)	2	500 µg/mL	97.2%	4	500 µg/mL	10.2%	6	500 µg/mL	8.5%
B. coagulans (32)	2	500 µg/mL	24.3%	4	500 µg/mL	15.1%	6	500 µg/mL	11.3%
S. aureus ATCC 6538 P	2	500 µg/mL	66.7%	4	500 µg/mL	8.7%	6	500 µg/mL	16.5%
P. aeruginosa ATCC 27853	2	500 µg/mL	28.6%	4	500 µg/mL	18.2%	6	500 µg/mL	10.5%
M. luteus ATCC 4698	2	500 µg/mL	27.5%	4	500 µg/mL	14.1%	6	500 µg/mL	34%
E. coli ATCC 25922	2	500 µg/mL	14.8%	4	500 µg/mL	16.5%	6	500 µg/mL	8.8%

*(1) *Calendula officinalis* L.; (2) Propolis; (3) Resveratrol; (4) *Thymus vulgaris* L.; (5) Nisin; and (6) *Hypericum perforatum* L.
Inhb. = inhibition; MBC = minimum bactericidal concentration; MIC = minimum inhibitory concentration.

on identifying active constituents and validating their efficacy under industrial conditions.

Overall, the results support the potential of selected plant-derived extracts and natural-origin compounds as dual-function agents capable of disrupting both bacterial viability and QS-regulated biofilm persistence in dairy environments.

Conclusion

This study revealed that biofilm formation is widespread among bacterial isolates from dairy process lines, with *Bacillus* and *Pseudomonas* species being the most dominant. Selected plant-derived extracts and natural-origin compounds, particularly *C. officinalis* and propolis, showed strong antimicrobial and QQ activities by inhibiting both AHL- and AI-2-mediated signaling

pathways. These findings highlight the potential of natural products as eco-friendly alternatives to chemical disinfectants for maintaining hygiene and controlling biofilm-associated risks in the food industry.

Future research should focus on identifying the specific active constituents responsible for QQ activity and evaluating their effectiveness under real industrial conditions. Integration of natural products into sanitation protocols could provide sustainable and consumer-acceptable strategies to improve food safety and reduce environmental impact.

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Ethics

Ethics Committee Approval: Since the article does not contain any studies with human or animal subjects, ethics committee approval was not required.

Data Sharing Statement: All data are available within the study and in the Supplementary Material.

Footnotes

Authorship Contributions: Conceptualization: D.İ., N.N.D.Z., J.H.K., and H.L.; Design/methodology: D.İ., N.N.D.Z., J.H.K., and H.L.; Execution/investigation: D.İ. and N.N.D.Z.; Resources/materials: N.N.D.Z. and J.H.K.; Data acquisition: D.İ. and H.L.; Data analysis/interpretation: D.İ., N.N.D.Z., and H.L.; Writing – original draft: D.İ. and H.L.; Writing – review & editing/critical revision: D.İ., N.N.D.Z., and J.H.K.

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