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Exploring the effects of phenolic compounds and essential oils in poultry: A sustainable strategy to combat *Salmonella* biofilm infections

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ABSTRACT

The rapid growth of the global chicken industry has raised concerns about food safety, particularly *Salmonella*, a significant zoonotic infection associated with poultry products. *Salmonella*'s capacity to form biofilms in farm and processing environments (slaughter, chilling, cutting, and packaging areas) makes it more resistant to disinfectants, antibiotics, and environmental stresses, complicating control attempts. Natural antibacterial agents are attracting renewed interest due to the drawbacks of traditional control strategies, such as the potential for antibiotic resistance development and harmful residuals. Plant-based phenolic compounds and essential oils (**EOs**) have emerged as safe, biodegradable, and environmentally friendly alternatives for inhibiting *Salmonella* biofilm formation during the various stages of infection and contamination in poultry, such as biofilm maturation, gut colonization, and eggshell adhesion, while preserving beneficial microbiota. These compounds also promote poultry health, performance, and gut immunity. This review examines the prevalence and causes of *Salmonella* biofilm development in chicken, assesses the antibiofilm and antibacterial characteristics of phenolics and EOs, and highlights their potential stage-specific applications along the poultry production chain. Furthermore, it addresses current obstacles, such as stability and delivery issues, and it also outlines future research priorities, with a focus on enhanced delivery methods, *in vivo* validation, and integrative approaches to sustainable biofilm control. Moreover, by focusing on stage-specific applications and resolving practical problems, this review connects laboratory discoveries to real-world chicken production, highlighting phenolic compounds and EOs as unique, residue-free *Salmonella* biofilm management agents with possible advantages but also potential human health safety concerns.

Introduction

Globally, chicken meat production has gradually increased over the last decade to meet growing consumer demand. In 2024, the world's broiler meat output amounted to around 103.7 million metric tons (**MMT**) and is predicted to rise to nearly 105.8 MMT by 2025 (Statista, 2024). The egg sector has also experienced significant growth, with production reaching 86.7 MMT in 2020, driven by key stakeholders. Eggs are now widely recognized as a high-quality protein source, and consumers are increasingly encouraged to incorporate eggs into their diets, with a growing range of options available, from shell eggs to processed egg products (Guyonnet, 2025). Looking ahead, the global poultry industry is projected to experience continued growth. A 2.5 % to 3.0 % growth in 2025 is forecast, driven by factors such as affordability, sustainability trends, and strong demand in emerging markets (Rabobank, 2025). However, the intensified production aimed at meeting growing demand has increased the risk of *Salmonella* contamination, a major public health concern.

Salmonella is both a zoonotic and foodborne pathogen that causes diarrhea in humans by contaminating water, animals, and plant products. It is the third greatest cause of death from diarrheal disorders worldwide (Mkangara, 2023). According to the Centers for Disease

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Control and Prevention (**CDC**), *Salmonella* causes around 1.35 million infections, 26,500 hospitalizations, and 420 fatalities in the USA each year (CDC, 2025). The estimated total combined expenses of medical care, loss of productivity, and early deaths caused by foodborne *Salmonella* infections in humans in the USA ranged from $4 to $11 billion per year (Gast et al., 2019). Non-typhoidal *Salmonella* (**NTS**) and invasive non-typhoidal *Salmonella* (**iNTS**) infections cause serious global health and economic issues (S. Kim et al., 2024). Although various sources contribute to human salmonellosis, chicken and poultry products remain among the most common, with NTS serotypes being the primary cause of salmonellosis in the poultry industry, which can be transmitted horizontally and vertically to chicks (Shaji et al., 2023). Therefore, controlling NTS in poultry has become an urgent global priority to reduce foodborne illnesses, underscoring the need for effective interventions.

Biofilms are significant contributors to the virulence of many pathogens. These organized microbial communities protect bacteria, increasing their resistance to antimicrobial agents, disinfectants, and various types of environmental stresses compared to their planktonic cells (Kang et al., 2023). *Salmonella* in biofilms are also known to be more tolerant to the host immune system, promoting their transmission to new hosts and contributing to increased mortality, chronic infection, and antibiotic resistance (Pradhan et al., 2023). As biofilm accumulation on food surfaces can cause contamination, post-processing contamination, and cross-contamination, resulting in food spoiling, product rejection, economic losses, and foodborne illnesses (Hossain et al., 2021), *Salmonella* can contaminate poultry and poultry products, such as meat and eggs, at several points of the food chain, including production, processing, distribution, retail marketing, handling, and cooking (Dookeran et al., 2012). It is crucial to control *Salmonella*'s ability to form biofilms under conditions found in farm environments, not just in the laboratory, given the significant food safety concern it poses. Early detection and management are the key to preventing and controlling salmonellosis (Peng et al., 2016).

Conventional *Salmonella* control tactics in poultry farming rely mainly on antibiotics and chemical disinfectants. However, their excessive use has led to concerning levels of antimicrobial resistance (**AMR**), regulatory constraints, and consumer concerns about chemical residues in chickens and eggs (Dlamini et al., 2024; Merino et al., 2019). This underscores the critical need for alternative, sustainable means to improve food safety without jeopardizing animal or human health.

Plant-derived phenolics and essential oils (**EOs**) are generally regarded as safe (**GRAS**), biodegradable, and environmentally friendly. Moreover, they can suppress biofilm development at several stages of poultry production and preserve commensal bacteria in poultry by selectively inhibiting pathogens (Álvarez-Martínez et al., 2021), which improves poultry health and performance (Gonçalves et al., 2020). Their versatility makes them valuable for use in feed, drinking water, surface sanitation, and even aerosolized administration in hatcheries (Hassid et al., 2025), while they may also supplement other natural or conventional therapies to improve disease control. Although large-scale cost-effectiveness has yet to be thoroughly assessed, with proper use, plant-derived phenolics and EOs could represent long-term, residue-free alternatives to antibiotics and chemical disinfectants.

Given the substantial research and burgeoning interest in natural substances with the potential to improve the health and wellbeing of poultry, especially in integrated production systems where the use of preventative measures and treatments becomes increasingly restrictive due to multi-drug resistance and the movement toward antibiotic-free and chemical-free practices, it is timely to provide a systematic review that comprehensively examines phenolic compounds' and EOs' functions in controlling *Salmonella* biofilms across the whole poultry production chain. This review presents them side by side, providing a thorough, stage-specific assessment of their antibiofilm activities, practical applications throughout the chicken production chain, and possible advantages and dangers to public health. Unlike previously published reviews that primarily focused on *in vitro* antimicrobial properties or feed additive effects, this work integrates molecular mechanisms, production-stage interventions, and real-world applicability, providing a practical and holistic resource for researchers, veterinarians, and poultry producers seeking innovative strategies to improve food safety and achieve sustainable poultry production.

Methods for searching papers

Relevant research articles, reviews, book chapters, and conference papers published in English up to September 2025 were identified on Google Scholar, PubMed, and Scopus. Among the search terms were "stage-specific *Salmonella* control," "natural antimicrobials in poultry," "phenolic compounds," "essential oils," "antibiofilm activity," and "*Salmonella* biofilm in poultry." Priority was given to studies that included grow-out farms, hatcheries, breeding flocks, and processing facilities. Studies unrelated to *Salmonella*, poultry, or biofilm management were removed, but papers on biofilm inhibition, antibacterial mechanisms, poultry health, and practical uses of phenolics and EOs were accepted. To provide a comprehensive overview of molecular mechanisms and stage-specific applications for residue-free, sustainable poultry production, each chosen study was carefully examined.

Salmonella in poultry

Prominent Salmonella serotypes in poultry

Salmonella species are facultative anaerobic, Gram-negative bacteria that can survive in various environments, including low moisture and a wide range of pH and temperature (Bhunia, 2018). Among the 39 distinct *Salmonella* serotypes discovered in U.S. broiler chickens at rehang, the five most common were Kentucky, Infantis, Typhimurium, Schwarzengrund, and Enteritidis, accounting for roughly 93 % of isolates (Williams et al., 2025). Similarly, in a study of South Africa, *Salmonella* was identified in 75 % of broiler chicks, with the most frequent serotypes being Enteritidis and Typhimurium. Prevalence of resistance isolates to tetracycline, nalidixic acid, and beta-lactams was high, raising concerns about treatment choices for both human and animal illnesses (Ramatla et al., 2024). Although many of these serotypes can be dangerous for humans and animals, their spread remains unclear, and serotypes such as Heidelberg and Kentucky often show antibiotic resistance, posing a threat to global health (Shah et al., 2017). *Salmonella enterica* ser. Kentucky, for example, has stronger AMR than *Sa.* Typhimurium, particularly to fluoroquinolones and cephalosporins, due to mutations in the *gyr*A and *par*C genes (Şık and Akan, 2024). The whole genome sequencing of *Sa.* Kentucky, Typhimurium, and Enteritidis isolates revealed serotype-specific differences, with *Sa.* Kentucky showing unique AMR genes and virulence factors that enhance persistence. In contrast, *Sa.* Typhimurium and Enteritidis carried key virulence plasmids but fewer AMR genes, highlighting the need for ongoing genomic surveillance in poultry production (Brenner and Wang, 2023).

Salmonella in chicken is not a one-size-fits-all threat, with severity, resistance, and persistence varying by serotype. Understanding these ecological differences is critical for anticipating epidemics and directing targeted research toward creative mitigation techniques. Given the complexity and diversity of *Salmonella* serotypes in chicken production, Table 1 lists the primary serotypes associated with poultry, along with their common sources and current outbreak patterns.

Transmission route

Salmonella spreads through vertical transmission from breeder flocks to chicks, through horizontal transmission via contaminated feed, water, and farm equipment, and via cross-contamination during processing. The consumption of *Salmonella*-contaminated food can lead to foodborne illnesses in humans, ranging in severity from mild gastroenteritis

Table 1
Prominent *Salmonella* serotypes in poultry linked to outbreaks: classification and recent trends.

Salmonella serotype	Common source in poultry	Associated outbreaks/notes	References
Enteritidis	Contaminated eggs, chicken kebab, chicken meat	Major outbreak in the USA in 2010 traced to eggs from Iowa. Multi-country outbreak in 2023 with 335 cases; connected to tainted chicken products from Poland; 1 death recorded.	((EFSA), 2023; Foley et al., 2011)
Typhimurium	Broilers in Egypt, USA poultry	Among the most commonly reported in the USA; prevalent on Egyptian poultry farms; outbreaks vary by geographic region.	(Allam et al., 2024)
Kentucky	Contaminated poultry meat, particularly broilers in Europe and Asia.	The emergence of multidrug-resistant (**MDR**) ST198 strains resistant to cefotaxime, ciprofloxacin, and tigecycline in Asia. ST198.2-2 subclade has predominated since 2019.	(Z. Wang et al., 2023)
Thompson	Chicken shawarma, chicken	Outbreak in Canada in 2014: 59 cases (16 in Quebec), most likely associated with contaminated chicken. Outbreak in Quebec in 2016: cluster associated with fast food chicken shawarma.	(Gaulin et al., 2017)
Heidelberg	Poultry, eggs, egg-containing foods	Analysis of outbreaks in the USA from 1973 to 2001: there were 184 *S.* Heidelberg outbreaks (3 % of the total), with 53 of them linked to poultry or eggs.	(Chittick et al., 2006)
Infantis	Poultry meat and poultry products	In 2016, 108 *Salmonella* isolates were recovered from 1,321 poultry samples; 29 (26.85 %) were *S.* Infantis. These showed 100 % resistance to amoxicillin and nalidixic acid.	(Kalaba et al., 2017)
Schwarzengrund	Chicken meat (also found in dry pet food)	Associated with poultry-related human infections in the USA, Denmark, and Thailand. In 2016, an outbreak in China was connected to contaminated spiced donkey meat, as proven by next-generation sequencing (**NGS**) and serotyping.	(Du et al., 2018)
Hadar	Backyard poultry (chickens, turkeys, ducks), ground turkey	Outbreaks in 2020–2021 were linked to contact with backyard poultry and the intake of ground turkey. Genetic similarity (0 – 16 core genome multilocus typing [**cgMLST**] alleles) discovered between strains from both sources.	(Brandenburg et al., 2024)

to severe systemic infections, and even death (Fig. 1) (Oastler et al., 2022). Therefore, to minimize the incidence of human salmonellosis cases, it is imperative to understand the transmission dynamics and decrease the prevalence of *Salmonella* at the breeder and hatchery levels.

Vertical transfer of gut microbiota in commercial poultry is minimal because chicks are kept independently from hens. A few bacterial strains detected in hens were present in chicks, indicating effective transmission, most likely via the reproductive tract and eggshell. Disrupting this transmission lowered the predominance of particular bacterial groups in chicks, showing that artificial exposure to adult microbiota may improve gut microbiota function in commercial chickens (Shterzer et al., 2023). *Sa.* Enteritidis can colonize the reproductive tract of infected hens and be transmitted vertically through the eggs, resulting in direct contamination of the yolk, albumen, and eggshell before laying. This prolonged infection promotes *Sa.* Enteritidis deposition on the yolk membrane, and immunological responses may aid in reproductive tract colonization and egg contamination (Liu et al., 2023). Another emerging foodborne pathogen, *Sa.* Reading, can also efficiently colonize reproductive tissues, move to the ceca, and contaminate eggs, further indicating the possibility of vertical transmission in chickens (Isah et al., 2024).

Sa. Enteritidis can spread rapidly horizontally within flocks due to factors such as direct contact with hens, intake of contaminated feed or feces, movement of personnel and equipment, and airborne contamination (Thomas et al., 2009). Horizontal transmission occurs primarily via the fecal–oral route and contaminates eggs by eggshell penetration or contact with infected feces during or after laying. The risk of contamination is highest after oviposition, with high specific gravity shells, hence, thicker shells, providing better resistance to *Sa.* Enteritidis invasion than shells with low specific gravity (Shaji et al., 2023). The horizontal transmission of *Sa.* Enteritidis in laying flocks is influenced by the length of illness and the frequency with which individual hens shed. Newly hatched chicks are extremely sensitive and can shed the pathogen for months; older chickens normally clear the infection faster, although persistent shedding can still occur (Gast et al., 2014).

Cross-contamination occurs when bacteria or viruses are transferred from a contaminated product to a non-infected product, whether directly or indirectly (Pérez-Rodríguez et al., 2008). Other terminology, such as recontamination, is used to describe bacterial translocation, specifically referring to contamination of food after it has undergone an inactivation treatment. Within slaughterhouses, body parts can become contaminated with *Salmonella* by cross-contamination within the same flock or from previously killed flocks. Water, utensils, and equipment can all cause cross-contamination (Rouger et al., 2017). To minimize cross-contamination, it is crucial to maintain high hygienic and sanitary standards in the processing environment, particularly in areas where food contact surfaces, equipment, and utensils are present (Osimani et al., 2014). Although governments and industry attempt to control *Salmonella*, there is no industrial technique that can completely prevent

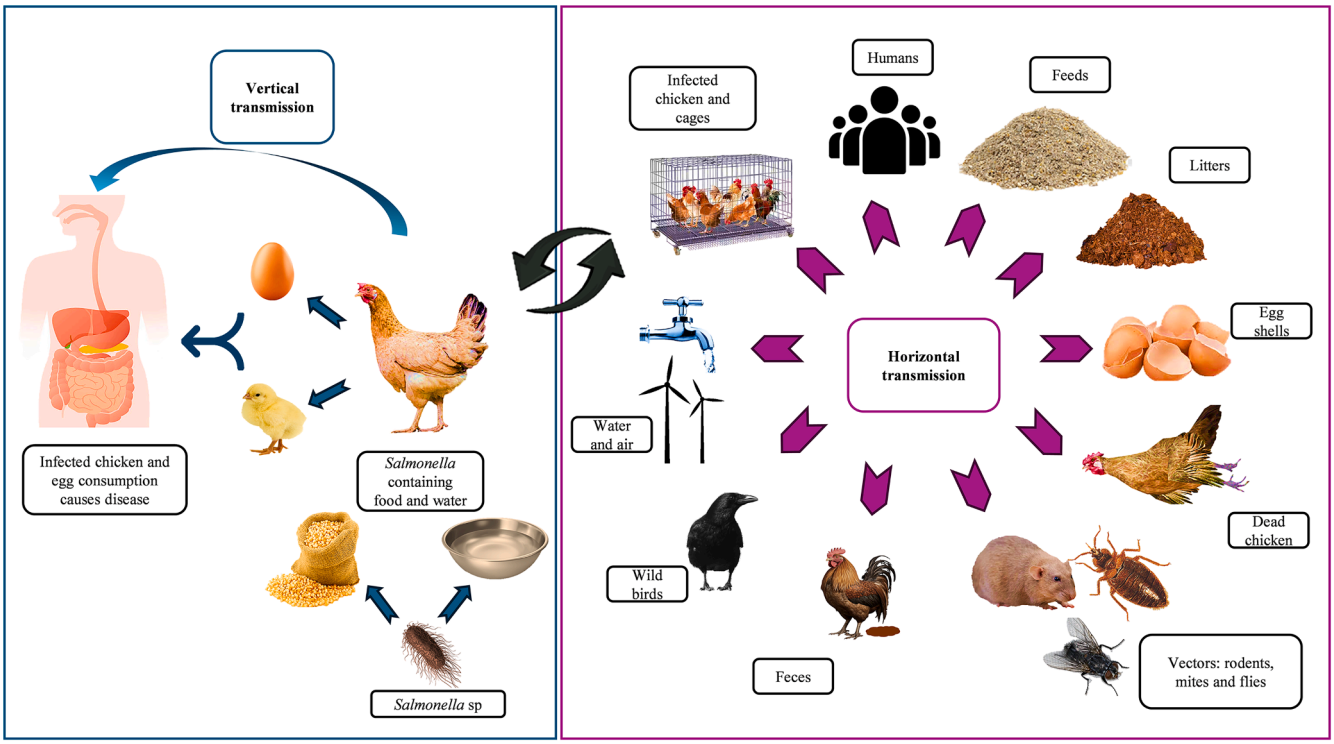


Fig. 1. *Salmonella* spreads in poultry via vertical and horizontal pathways. Following intake of contaminated feed, water, or other sources, the bacteria can colonize the host and spread vertically from infected hens to their chicks and eggs. Horizontal cross-contamination occurs due to sharing cages, contaminated settings (feed, litter, water, equipment), human movement, contact with diseased excrement or carcasses, and vectors, such as mice, flies, and wild birds. Humans may become infected after eating contaminated chicken meat or eggs.

or eliminate the bacteria from chicken carcasses. While washing and disinfection can remove *Salmonella* from surfaces, it is impossible to eliminate it from birds that arrive at the facility already contaminated. No procedure can lower the pathogen to an acceptable level (i.e., absent in 25 g of the sample) (Reta et al., 2023). Despite the high frequency of foodborne outbreaks, information on cross-contamination and recontamination in scientific literature is scarce. Review papers on outbreaks often remain unpublished because they do not satisfy prerequisites such as a large number of affected patients, an emerging pathogen, uncommon serotypes, or new food matrices. Furthermore, the lack of residual food samples makes source attribution difficult (Carrasco et al., 2012).

These dynamics collectively show that vertical transmission seeds early infection but is limited in commercial settings, whereas cross-contamination and horizontal dispersion dominate later phases, resulting in rapid flock-wide dissemination. Because the proportionate impact of each mode of infection varies by serotype and production stage, *Salmonella*'s adaptive persistence cannot be addressed by a one-size-fits-all control strategy.

Biofilm formation: mechanisms and consequences

Salmonella excreted by livestock can infect food workers and form biofilms on biotic (e.g., eggshell, meat) and abiotic surfaces (e.g., stainless steel, plastic, glass), contaminating processing plants, packaged foods, wastewater, and commercial and residential environments (Flemming et al., 2016; Lamas et al., 2018; Peng et al., 2016). Biofilm formation by bacteria, including *Salmonella*, is a multistep process that includes (a) reversible attachment, (b) irreversible adhesion via adhesins or exopolysaccharides, (c) microcolony formation, (d) biofilm maturity, and (e) dispersion under favorable conditions (Fig. 2). The initial reversible attachment is dependent on the motility of the bacteria, which enables planktonic (free-swimming cells) to reach appropriate surfaces. Flagella serve a dual purpose, aiding mobility and functioning as adhesion elements for attachment. Stringent regulation of flagella rotation and function is therefore necessary for optimal biofilm formation; slow motility causes bacterial aggregate formation, resulting in structured biofilms, whereas cells exhibiting high motility tend to spread across surfaces, resulting in flat biofilms (Yaron and Römling, 2014).

In addition, *Salmonella* produces curli fimbriae, which are proteinaceous amyloid fibrils that are common across serotypes. These structures bind fibronectin and laminin, major components of the host extracellular matrix, and facilitate auto-aggregation, allowing cells to clump together into a biofilm and thereby increasing environmental persistence and resistance to diverse stressors. Curli production is activated by low temperatures, low osmolarity, and nutrient scarcity, which aids survival beyond host habitats (MacKenzie et al., 2017). Following irreversible attachment, the bacteria proliferate to form microcolonies and release extracellular polymeric substances (**EPS**), which enhance interactions with the surface, other cells, and matrix components, resulting in biofilm formation. Proteins within the biofilm matrix perform structural and physiological activities, whereas exopolysaccharides improve mechanical stability, water retention, and nutrient availability (Yaron and Römling, 2014).

Cells further proliferate, and over time, a complex, three-dimensional polymer matrix is formed around the microcolonies, resulting in a mature biofilm. Biofilms formed on equipment surfaces, water lines, and even poultry skin or eggshells in poultry production and processing environments allow the bacteria, including *Salmonella*, to persist (Arnold, 2009). In addition to being a source of contamination, biofilm bacteria can detach actively or passively, spreading as single cells or clumps to new settings, thus spreading contamination. Environmental signals, quorum sensing (**QS**), and cyclic dimeric guanosine monophosphate (**di-GMP**) signaling all play important roles in regulating biofilm formation (Ahmad et al., 2013). QS is a population density-dependent process by which bacteria communicate, cooperate, and control their response to environmental changes that cause specific

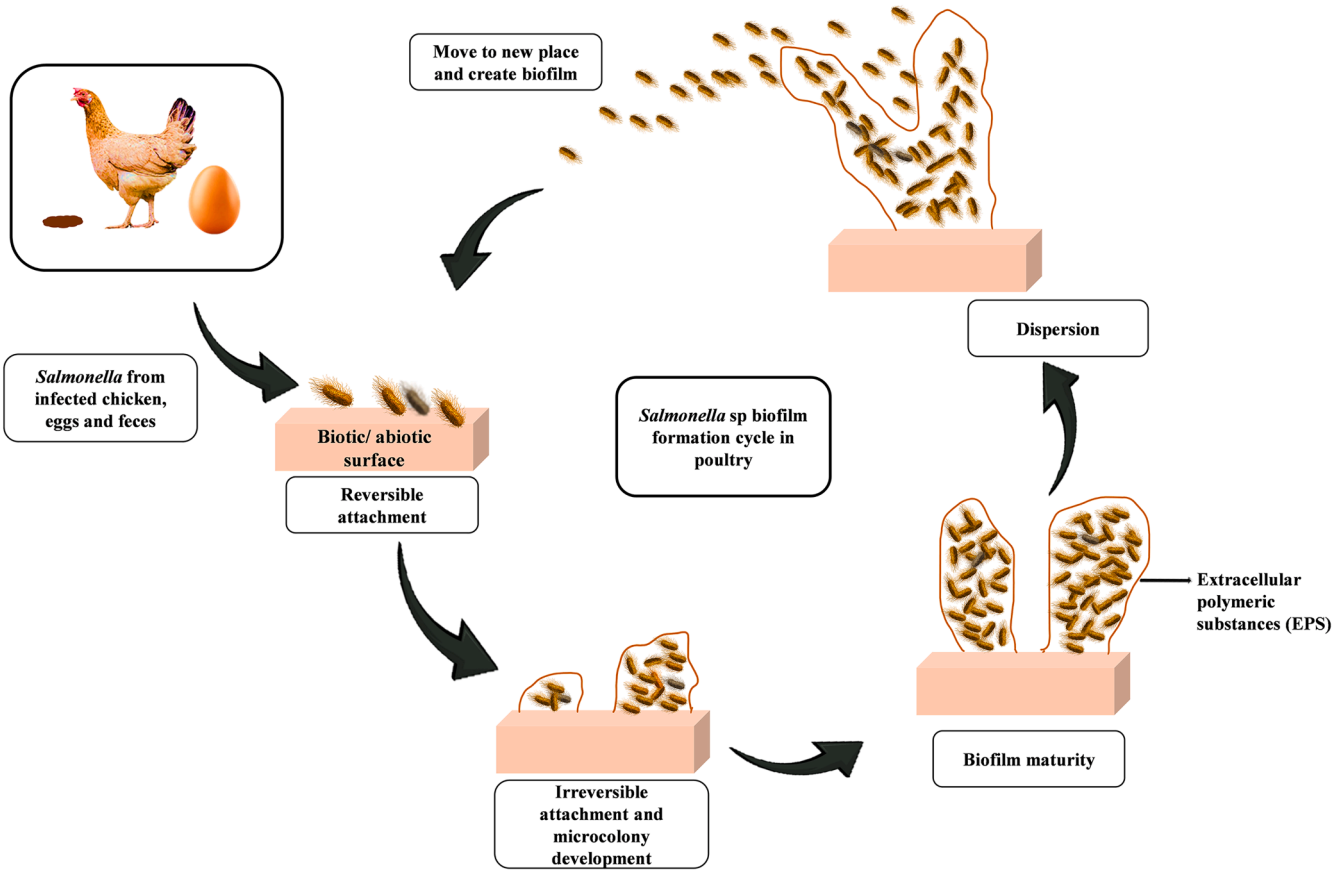


Fig. 2. Stepwise process of *Salmonella* biofilm development in poultry environments. Initially, planktonic cells attach reversibly to biotic or abiotic surfaces, such as eggshells, water lines, or machinery. This is followed by irreversible adhesion, aided by fimbriae and adhesins. Bacteria subsequently multiply, establishing microcolonies and producing extracellular polymeric substances (**EPS**) composed of proteins, polysaccharides, and extracellular DNA, which provide structural support and protection. Over time, a sophisticated, three-dimensional biofilm is formed. In the last stage, cells actively or passively disperse from the biofilm due to stress or lack of nutrients, allowing them to colonize new surfaces and cause long-term contamination in poultry production systems.

behaviors, such as biofilm formation, nutrition uptake, and defense mechanisms. It relies on the production, detection, and response to autoinducers, which are extracellular signaling molecules. Several types of QS systems can be distinguished: the type 1 autoinducer (**AI-1**) QS system, represented by *N*-acyl homoserine lactones (**AHLs**), exists only in Gram-negative bacteria; the type 2 autoinducer (**AI-2**) QS system is found in both Gram-positive and Gram-negative bacteria; the type 3 autoinducer (**AI-3**) QS system is used for interaction with mammalian host signaling; and the auto-inducing peptide (**AIP**) system is exclusive to Gram-positive bacteria (Sholpan et al., 2021). Therefore, biofilms protect *Salmonella* from a variety of environmental challenges, including antibiotics, extreme pH, heat, freezing, osmotic changes, metallic cations, oxidative stress, UV radiation, desiccation, and biocides. They also improve tolerance to disinfectants, sanitizers, and cleaning agents, thus limiting the effectiveness of traditional cleaning procedures. Furthermore, the self-produced biofilm matrix improves bacterial survival by delivering nutrients and providing space for growth (Bai et al., 2021). Thus, biofilm development increases *Salmonella*'s persistence and resistance, resulting in reservoirs that complicate eradication efforts and raise the risk of contamination and disease transmission across the poultry supply chain.

Overview of phenolic compounds and EOs

Phenolic compounds

Phenolic compounds are abundant, diverse, and widely distributed in nature. These bioactive secondary metabolites play a significant role in plant-based foods, contributing to the color of red fruits, juices, and wines; acting as substrates for enzymatic browning; and enhancing flavor qualities (Cheynier, 2012). Moreover, phenolic molecules are known to have a variety of other biological actions, including antibacterial, antioxidant, and anti-inflammatory (Al Mamari, 2021).

Phenolic compounds can range from simple phenolic molecules, which consist of a single phenol unit with various possible substitutions, to highly polymerized compounds called polyphenols. Simple phenolic compounds have a C6 skeleton with functional groups (e.g., alkyl, hydroxy, amino) positioned at *ortho* (1,2-), *meta* (1,3-), or *para* (1,4-) locations on the aromatic ring. Polyphenols feature multiple phenol units, each with at least one hydroxyl group. They are most commonly found in nature as esters or glycosides rather than as free phenolic acids. While flavonoids, a major class of polyphenols, have a C15 skeleton, other specialized phenolic compounds, such as lignans, stilbenes, and phenolic acids, do not share this same basic structure (Al Mamari, 2021). Table 2 summarizes the classification of phenolic compounds, their biological activities, and recent research trends related to the utilization of these compounds in poultry, with a focus on their application in the control of *Salmonella* in chicken.

EOs

EOs play an important role in nature. They protect plants from microorganisms and herbivores, partly by reducing herbivore appetite through unpleasant smell or taste. Conversely, many insects are drawn to the aroma of EOs, aiding in the spread of seeds and pollen. Countries with moderate to warm climates host various fragrant plants whose

Table 2
Phenolic compounds, classification, and targeted effects on *Salmonella* biofilm stages relevant to poultry control.

Class	Subclasses/ Example	Source plants	Biological activities	Effect on *Salmonella* biofilm stages	Application in the production of poultry (stage and method)	Relevance to poultry *Salmonella* biofilm control	References
Simple phenols	Phenolic acids (coumaric acid, gallic acid)	Fruits, vegetables, beverages	Antimicrobial and antioxidant activities	Initial adhesion, extracellular polymeric substances (**EPS**) formation, early biofilm development	Grow-out farms (drinking water, feed additives)	Reduced *Sa.* Typhimurium in chicken cecum; evaluated as feed additives to promote gut microbiota and minimize infections.	(Rashid et al., 2021; Alvarado-Martinez et al., 2023; Fontana et al., 2023; Goławska et al., 2023; Kadhim et al., 2025; Xu et al., 2022)
Flavonoids	Flavonols: quercetin, rutin; flavones: luteolin, apigenin	Tea, cocoa, fruit, vegetables	Anticarcinogenic, antioxidative, antimutagenic, anti-inflammatory	Initial adhesion, EPS production, biofilm maturation, regulatory gene expression	Grow-out farms, processing surfaces, storage surfaces, hatchery (drinking water, feed additives, spraying, dipping)	Quercetin and luteolin prevented *Salmonella* biofilms on surfaces and downregulated quorum sensing (**QS**) genes, suggesting potential for processing surfaces and poultry intestines.	(Goławska et al., 2023; Kim et al., 2022; Mahamud et al., 2024; Martinengo et al., 2021; Nath and Kumar.k, 2021; Roy et al., 2022; Wen et al., 2021)
Tannins	Hydrolysable or condensed	Vegetables, fruits, cereals, seeds	Anti-aging, anti-inflammatory, antiproliferative	Initial adhesion, early colonization, EPS matrix production, biofilm maturation	Breeder folks, grow-out farms (drinking water, feed additives)	Tannic acid disrupted *Sa.* Typhi EPS and biofilms; potential for limiting contamination in poultry.	(Choi and Kim, 2020; Long et al., 2024; Melo et al., 2023; Sivasankar et al., 2020; Tong et al., 2022; Trentin et al., 2013; Xu et al., 2025)
Lignans	Solanaceae, Moraceae, Annonaceae	Plant's roots and seeds	Anti-inflammatory, anticancer, antioxidative, anti-neuroprotective	Initial attachment, biofilm matrix production, biofilm maturation	No application reported in poultry	Limited poultry data: maple syrup lignans demonstrated general antibiofilm capability, motivating poultry biosecurity studies.	(Elbakush et al., 2024, 2023; Sun et al., 2024)
Stilbenes	Resveratrol	Grapes, peanuts, berries	Anti-obesity, anti-inflammatory, anticancer, antimicrobial, antioxidant	Initial adhesion, biofilm maturation, virulence factor suppression	Slaughterhouse, hatchery, storage surfaces (spraying)	Anti-virulence and antibiofilm effects of stilbene-related compounds against pathogens were suggested for inclusion in poultry feed to reduce pathogen load.	(Kasiotis et al., 2013; Kluska et al., 2023; Lee et al., 2014; Matencio et al., 2021; Navarro-Orcajada et al., 2022)

extracts consist of EOs (Kar et al., 2018). EOs are complex mixtures of volatile secondary metabolites, including terpenes, ketones, aldehydes, esters, ethers, alcohols, and phenols, which give the plant its particular fragrance, flavor, or both. These oils can be found in various plant parts, including buds, stems, roots, seeds, fruits, flowers, leaves, twigs, wood, and bark, and are stored in canals, secretory cells, cavities, epidermal cells, or glandular trichomes. EOs are generated by around 17,500 species of plants from many angiosperm families, including Lamiaceae, Rutaceae, Myrtaceae, Zingiberaceae, and Asteraceae, although only about 300 EOs have been commercialized (Mérillon and Riviere, 2018). EOs have been used throughout history for a variety of purposes, including flavoring food, cosmetics, and medicine, due to their biological activities. Their antimicrobial and antioxidant effects make them useful in industries such as medicine, agriculture, and cosmetology (Wińska et al., 2019).

EOs are typically colorless or pale yellow, liquid at room temperature, and less dense than water. They are composed of low molecular weight molecules (less than 300 Da), some of which are optically active and soluble in organic solvents such as ether, alcohol, and acetone, but insoluble in water (de Sousa et al., 2023). Thousands of compounds belonging to the family of terpenes have so far been identified in EOs, such as functionalized derivatives of alcohols (geraniol, α-bisabolol), ketones (menthone, *p*-vetivone), aldehydes (citronellal, sinensal), esters (γ-tepinyl acetate, cedryl acetate), and phenols. The composition of EOs determines their antibacterial action; phenols and aldehydes, in particular, are key elements that drive efficacy, whereas esters, ketones, and terpene hydrocarbons contribute slightly (Chraibi et al., 2020; Dhifi et al., 2016; El-Tarabily et al., 2021). Table 3 lists the kinds of EOs, their modes of action, and stage-specific uses for controlling *Salmonella* biofilms in chicken production.

Effects of phenolic compounds and EOs on *Salmonella* biofilm

Phenolic compounds and EOs inhibit *Salmonella* biofilms by disturbing biofilm structure, inhibiting gene expression, reducing metabolic activity, decreasing cell motility, reducing EPS production, affecting membranes, and suppressing QS, as illustrated in Figs. 3 and 4, respectively. These mechanisms are discussed in greater detail in the sections below.

Disruption of biofilm integrity

Phenolic substances such as luteolin, cinnamaldehyde, carvacrol, ferulic acid (**FA**), and *p*-coumaric acid (***p*-CA**) efficiently inhibit *Salmonella* biofilm development on both biotic (e.g., eggshell) and abiotic (e.g., stainless steel) surfaces in poultry habitats. They reduce bacterial adhesion and hydrophobic interactions, disturb biofilm integrity, and enhance bacterial susceptibility through processes such as oxidative stress, membrane damage, and metabolic dysfunction (Cetin-Karaca and Newman, 2015; Chen et al., 2024; Mahamud et al., 2024; Xu et al., 2022). Even at low minimum inhibitory concentrations (**MICs**; <15–20 ppm), several plant-derived phenolics, such as epicatechin, thymoquinone, rutin, and myricetin, exhibit potent antibacterial activity and impair the structural integrity of bacteria and biofilms. In *Sa.* Enteritidis, FA and *p*-CA specifically downregulate important biofilm-related genes that impact EPS secretion (*adr*A, *gcp*A, *csg*D), flagella assembly (*mot*A, *mot*B, *fl*D), and chemotaxis (*che*A, *che*B) (Xu et al., 2022).

The EO from *Lippia origanoides* chemotype thymol-carvacrol II

Table 3
Essential oils classification and targeted effects on *Salmonella* biofilm stages relevant to poultry control.

Classification	Major chemical constituents	Sources	Biological Activities	Effect on *Salmonella* biofilm stages	Application in the production of poultry (stage and method)	Relevance to poultry *Salmonella* biofilm control	References
Phenolic essential oils (EOs)	D-Carvone, D-limonene, camphor, α-pinene	Fruits of aniseed, caraway, and coriander	Antibiofilm, antioxidant	Extracellular polymeric substances (**EPS**) production, impairs bacterial colonization, disrupts quorum sensing (**QS**) signaling.	Storage and packaging (edible films, coatings)	Inhibited *Sa.* Enteritidis biofilm and microbial spoilage on chicken; maintained sensory quality and reduced pH, total volatile basic nitrogen (**TVB-N**), and moisture loss.	(Ju et al., 2018; Kanekar et al., 2021; Stojićević et al., 2017; W. Wang et al., 2023)
Aldehyde-rich EOs	Cinnamaldehyde	Cinnamon bark	Antibacterial, antioxidant, antiviral, anticancer, and involved in controlling gut microbiota	Initial adhesion, biofilm maturation, peptidoglycan synthesis, energy metabolism disruption	Poultry breeders, grow-out farms, storage and packaging (feed additives, dipping)	Inhibited *Sa.* Enteritidis biofilm and decreased total aerobic plate count in chicken meat, preserving chicken quality and reducing spoilage. Inhibited Type I fimbriae (*fim*A, *fimZ*, *fim*Y, *fim*H, *fim*W), decreased adhesion and invasion, and modulated the inflammatory response.	(Albano et al., 2019; Wang et al., 2025; W. Wang et al., 2023; Yang et al., 2021, 2025; Yin et al., 2022)
Terpene EOs	Carvone, carvacol, thymol, geraniol	Present in different parts of the plant (e.g., leaves, stems, flowers, seeds, fruit, bark, roots)	Antimicrobial activity against both antibiotic-susceptible and antibiotic-resistant bacteria (caused cell rupture, impeded protein and DNA synthesis)	Initial adhesion, biofilm maturation, overall biofilm inhibition	Slaughterhouse, hatchery, poultry breeders (spraying, dipping, food additives)	Applied to chicken feed and equipment surfaces to minimize *Salmonella* colonization and biofilm persistence; effectively inhibited *Salmonella* and *Escherichia coli* biofilms at sub-minimum inhibitory concentration (sub-**MIC**) levels; potential as a dietary supplement for preventing and managing chicken illnesses.	(Bibow and Oleszek, 2024; El Abed et al., 2011; Masyita et al., 2022; Salinas et al., 2022; Seyedtaghiya et al., 2021)
Alcohol EOs	Eugenol, menthol	Clove, Peppermint	Antimicrobial, antibiofilm, anti-virulence, anti-QS	Initial adhesion, early biofilm formation, biofilm maturation	Hatchery (spraying)	Lowered *Sa.* Typhimurium pathogenicity in poultry by downregulating adhesion factors (TIF) and type III secretion system (**T3SS**) genes, reducing bacterial colonization and inflammation; potential utility in minimizing *Salmonella* infections in chickens.	(Ashrafudoulla et al., 2020; Gatea et al., 2023; Palomares-Navarro et al., 2025; Ribeiro et al., 2024; Zhao et al., 2022)
Oxide EOs	Cineole	Eucalyptus oil	Anti-inflammatory, antioxidant, antimicrobial	Early and mature stages	Hatchery, slaughterhouse, storage (spaying, dipping)	Antibiofilm and anti-QS activities against methicillin-resistant *Staphylococcus aureus* (**MRSA**). Although not yet researched, especially against *Salmonella* in poultry, it has proven antibiofilm potential on farm surfaces.	(Baird Real Estate, 2019; Merghni et al., 2018; Y. Wang et al., 2022)
Ketone EOs	Menthone, carvone	Plant species: *Mentha* spp., *Origanum*	Suppression of exoprotein	Initial attachment, matrix development,	Poultry breeders, grow-out farms, storage and	Investigated for antimicrobial vapor and direct contact	(Bouyahya et al., 2021; Porfírio et al., 2017; Samber et al., 2015;

(*continued on next page*)

Table 3 (*continued*)

Classification	Major chemical constituents	Sources	Biological Activities	Effect on *Salmonella* biofilm stages	Application in the production of poultry (stage and method)	Relevance to poultry *Salmonella* biofilm control	References
		spp., *Rosmarinus* spp., *Thymus* spp.	production linked to virulence	biofilm maturation	packaging, slaughterhouse (feed additives, vapor)	activity against *Sa.* Typhimurium on chicken skin.	Vepštaitė-Monstavičė et al., 2023)

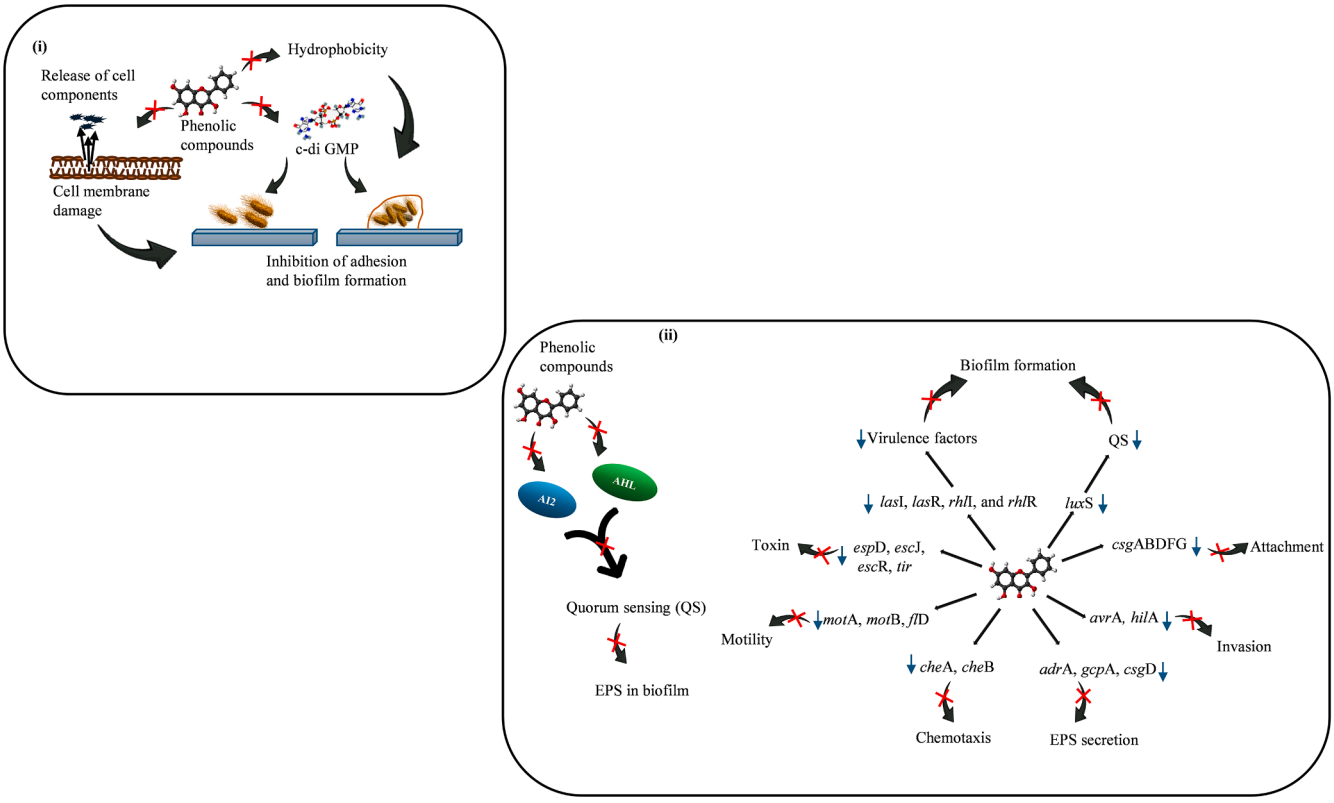


Fig. 3. Schematic representation of the inhibitory mechanisms of phenolic compounds against *Salmonella* biofilm formation. (**i**) Phenolic compounds can inhibit mature biofilm formation by disrupting the bacterial cell membrane, resulting in cellular leakage. They also reduce cell surface hydrophobicity and lower the intracellular levels of cyclic di-GMP (**c-di-GMP**), a key signaling molecule involved in biofilm regulation. This results in decreased adhesion and impaired biofilm development. (**ii**) Phenolic compounds interfere with quorum sensing (**QS**) signaling molecules, such as autoinducer-2 (**AI-2**) and *N*-acyl homoserine lactones (**AHLs**), leading to decreased extracellular polymeric substances (**EPS**) production. They also downregulate key QS and virulence-associated genes, including *las*I, *las*R, *rhl*I, *rhl*R, *lux*S (QS, virulence), *csg*ABDFG, *adr*A, *csg*D, *gcp*A (attachment, EPS), *avr*A, *hil*A (invasion), and *esp*D, *esc*J, *esc*R, and *tir* (toxin secretion). Motility and chemotaxis are also impaired due to suppression of *mot*A, *mot*B, *flh*D, *che*A, and *che*B. These combined effects result in reduced biofilm formation and virulence in poultry-associated *Salmonella*.

exhibited strong antibiofilm activity against *Sa.* Enteritidis and Typhimurium, significantly downregulating biofilm-associated genes (*csg*A, *csg*B, *csg*D, *flh*D, *fliZ*, *mot*B). By suppressing gene expression, the development and structural integrity of the biofilm were compromised, as demonstrated by scanning electron microscopy (**SEM**) (Guillin et al., 2021). Such thymol- and carvacrol-rich EOs are especially useful in poultry systems, where they can destroy *Salmonella* biofilms on processing surfaces while also supporting chicken health under production stress circumstances (Señas-Cuesta et al., 2023).

Similarly, clove essential oil (**CEO**) and oregano essential oil (**OEO**) significantly inhibited *Salmonella* biofilm formation at sub-MIC levels, with no major difference between concentrations. Transcriptomic analysis showed that these two EOs downregulated genes related to energy metabolism, including fatty acid degradation, the tricarboxylic acid (**TCA**) cycle, and oxidative phosphorylation. Meanwhile, CEO also suppressed the expression of ribosomal and amino acid biosynthesis genes (Liu et al., 2023). These outcomes indicate that CEO and OEO affect both gene expression and metabolism, disrupting crucial cellular mechanisms required for biofilm growth and maintenance. In poultry systems, these EOs may also help to impair *Salmonella* biofilm integrity on intestinal and processing surfaces, decreasing pathogen persistence and increasing overall flock resilience (Park et al., 2025).

These findings show that phenolics and EOs rarely operate via a single mechanism, but rather affect signaling, structural assembly, and cellular metabolism all at the same time. This multi-targeted action may explain their robust antibiofilm effect and point to the possibility of strategic combinations that take advantage of complementary processes.

Inhibition of QS and gene expression

Polyphenols are potential quorum sensing inhibitors (**QSIs**) that disrupt bacterial communication, pathogenicity, and biofilm formation in poultry-associated diseases (Xu et al., 2023). Green tea polyphenols, carvacrol, soybean isoflavones, and quercetin (a polyphenolic

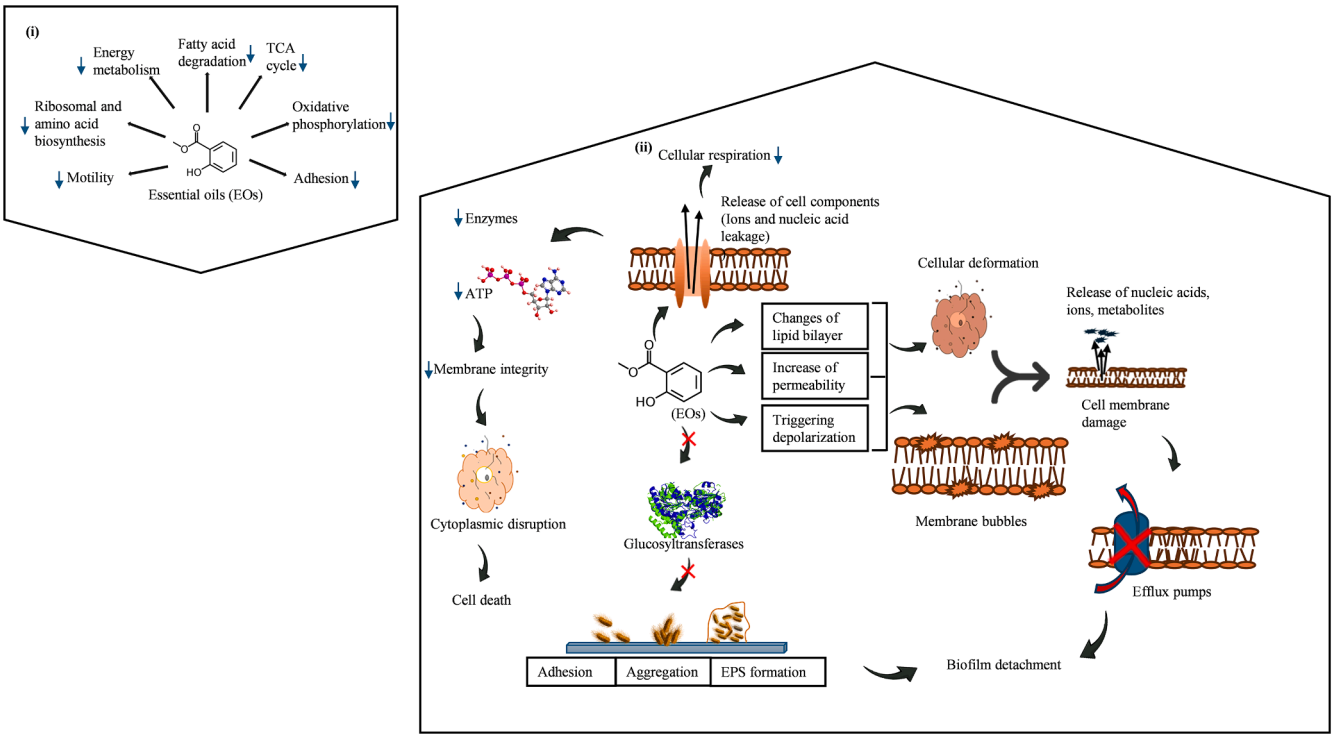


Fig. 4. Mechanism of essential oil-mediated inhibition of biofilm formation in *Salmonella*. (**i**) Treatment with essential oils (**EOs**) suppresses biofilm formation by downregulating genes associated with multiple physiological processes, including energy metabolism (e.g., oxidative phosphorylation), fatty acid degradation, and the tricarboxylic acid (**TCA**) cycle, thereby limiting energy production. Additionally, the biosynthesis of amino acids and ribosomal proteins is reduced, impairing protein synthesis. EOs also interfere with bacterial adhesion by downregulating genes responsible for fimbriae and flagella formation, crucial for surface attachment and motility. Collectively, these effects lead to impaired biofilm development and maintenance. (**ii**) EOs have strong antibiofilm properties through both physical and chemical processes. They destabilize the lipid bilayer, increasing membrane permeability and resulting in depolarization, wrinkling, and intracellular content leakage such as ions and nucleic acids. This disruption also affects efflux pumps, respiration, and ATP synthesis, resulting in enzyme inactivation, membrane damage, and cell lysis. Furthermore, EOs inhibit glucosyltransferase (**GTF**) expression, lowering EPS synthesis and bacterial aggregation and inhibiting persistent biofilm development.

flavonoid) all target the AI-2/AHL pathways to prevent QS in *Sa.* Typhimurium. Quercetin specifically reduces the production of AI-2, downregulates *lux*S, and suppresses the expression of virulence genes (*avr*A, *hil*A), which together reduce EPS production, biofilm formation, and pathogenicity (Deryabin et al., 2023; Liu et al., 2020; Zhang et al., 2021). Cinnamaldehyde inhibits bacterial resuscitation, emphasizing its role as a QSI in stress reactions (Ayrapetyan et al., 2014; Chen et al., 2024). Moreover, methyl gallate, the methyl ester of gallic acid (**GA**), downregulates QS genes (*las*I, *las*R, *rhl*I, *rhl*R) and reduces biofilms without impacting growth (Hossain et al., 2017; Huang et al., 2021). Likewise, esculetin inhibits AI-2 trafficking and downregulates *lux*S, *lsr*B, *adr*A, and *rpo*S by binding competitively to the LsrB receptor. This reduces biofilm formation, EPS production, motility, and pathogenicity (Xu et al., 2023).

EOs can inhibit biofilm formation by downregulating QS-related genes (*lux*R, *lux*S, *qse*B, *sdi*A) (Gaulin et al., 2017). For instance, ginger essential oil (**GEO**) contains bioactive components, such as allicin, which have been found to hinder biofilm formation in *Sa.* Typhimurium by interfering with QS and lowering the expression of genes linked to motility and adhesion, such as those regulating fimbriae and flagella (dos Santos et al., 2023; Robinson et al., 2022). Black cardamom essential oil may have an antibiofilm effect not just by harming cells, but also by regulating QS pathways, limiting the microbial communication required for biofilm development (Abdullah et al., 2021).

These natural substances not only destroy the biofilm structure, but also prevent bacterial transmission, hence reducing virulence and persistence. Their chemical diversity, ranging from hydroxyl-rich polyphenols to lipophilic EO elements, enables a multi-layered attack that might be strategically used for poultry biofilm reduction; however, knowing how these processes work in real-world production settings remains an essential future direction.

Reduction of EPS production

According to a study, eugenol decreased the exopolysaccharide contents of *Sa.* Typhimurium biofilms by 24 % and 27 % at 0.3 and 0.7 mg/mL after 12 h, reducing biofilm stability and potentially improving disinfection (Palomares-Navarro et al., 2025). It also reduced glucan (a key EPS) production and downregulated biofilm-and adhesion-related genes, including curli (*csg*ABDFG), type I fimbriae (*fim*CDH), and Ler-regulated toxin genes (*esp*D, *esc*J, *esc*R, *tir*) (Li et al., 2012; Palomares-Navarro et al., 2025). Likewise, EPS components (proteins, extracellular DNA, polysaccharides) in poultry-associated biofilms were considerably decreased by vanillin and syringic acid (Karre et al., 2013; Minich et al., 2022). Tannic acid reduced EPS generation by 35−50 % in *Sa.* Typhi and Paratyphi A, with Fourier transform infrared (**FTIR**) analysis suggesting decreased protein−carbohydrate interactions and altered biofilm formation (Sivasankar et al., 2020).

In poultry habitats, EPS reinforces *Salmonella* biofilms, which can be disturbed by EOs. CEO, OEO, and components such as myrtenol decrease EPS production and metabolic activity, affecting biofilm structure and adherence (Ganić et al., 2025; Liu et al., 2023). Propolis EO microemulsion inhibits glucosyltransferase (**GTF**), decreasing EPS production and bacterial colonization and providing antioxidant advantages in poultry (Mahmoud et al., 2016; F. Wang et al., 2023).

The findings indicate that phenolic compounds may modulate EPS formation and gene expression. Meanwhile, EOs might impair biofilm construction and metabolism. Together, phenolic compounds and EOs provide a tiered, mechanism-based method for poultry biofilm control that may also benefit the host.

Disruption of cell membrane integrity

In poultry, phenolic substances can play an important role in regulating *Salmonella* by attacking bacterial membranes in the gut. These compounds disrupt bacterial membranes by altering lipid bilayer properties and membrane protein function, increasing permeability, causing leakage, and enhancing antibiotic efficacy, while also potentially shielding host cells from oxidative damage (Berecka-Rycerz et al., 2025). Among the polyphenols tested, resveratrol significantly suppressed *Sa.* Typhimurium by destroying bacterial cell walls and membranes, as evidenced by electron microscopy. Therefore, it affected essential metabolic processes, including amino acid metabolism and the TCA cycle, which impaired energy production and prolonged the bacterial lag phase (Wang et al., 2024). Therefore, resveratrol's membrane-targeting activity in poultry reduces *Salmonella* survival and persistence in the gut, hence improving the efficacy of natural defenses and dietary treatments (He et al., 2022).

EOs damage *Salmonella* membranes by altering lipid bilayers, increasing permeability, depolarizing membranes, collapsing the membrane potential, and depleting ATP; therefore, reducing biofilm formation and bacterial viability in poultry settings (Hu et al., 2023; Kumar et al., 2024; Martínez et al., 2021; Yammine et al., 2022). The EPS matrix and biofilm structure are disrupted by carvacrol EO, allicin, and other EOs such as *Citrus limon, Helichrysum italicum, Cinnamomum zeylanicum*, and *Pinus sylvestris*. They also interfere with ion transport and efflux pumps, inhibit enzymes, lower respiration, and cause cytoplasmic deformation, nucleic acid and ion leakage, and lipopolysaccharide (**LPS**) disruption, all of which ultimately result in bacterial death (Lira et al., 2020; Qumsani, 2025; Touati et al., 2025; Zhang et al., 2022). Overall, these findings suggest that while EOs rupture membranes, inhibit ATP production, and cause biofilm collapse, phenolic compounds destabilize membranes and disrupt microbial metabolism.

Synergistic effect with other antimicrobials

In one study, the use of a ceftiofur−GA combination decreased the proliferation, biofilm formation, and motility of *Sa.* Typhimurium in intestinal cells without causing cytotoxicity, indicating that it is safe for oral use in chickens (Hossain et al., 2020). Even in the presence of antioxidants, FA and *p*-CA demonstrated potent synergistic antibacterial actions with UV-A light radiation, decreasing multidrug-resistant (**MDR**) *Sa.* Typhimurium by >6–7 log CFU/mL, disrupting bacterial membranes, increasing permeability, and causing DNA damage. This combination effectively reduced pathogens burden on chicken meat while maintaining quality, providing a natural way to control infections in poultry applications (de Oliveira et al., 2021; Liu et al., 2025). Thymol greatly improved biofilm eradication, solubility, penetration, and efficacy against MDR *Salmonella* when coupled with aminoglycosides or loaded into thymol-loaded poly (lactic-*co*-glycolic acid) (**PLGA**) nanoparticles (Kuaté Tokam et al., 2023; Tokam Kuaté et al., 2021).

One of the studies evaluated the synergy between phage endolysin LysPB32 with selected EOs against *Sa.* Typhimurium. Carvacrol and eugenol combined with LysPB32 lowered the MICs by 2 − 4 times and achieved complete inhibition in 12 h, confirming that EOs enhance membrane permeability and restore endolysin activity, leading to peptidoglycan cleavage (Kim et al., 2024). Similar to this, EPS matrices were broken down when treated with hydrolytic enzymes followed by aromatic phenolic EOs, which also improved EO penetration and decreased biofilms on plastic and stainless steel by up to 4.6 log CFU/-coupon (Olaimat et al., 2024). In order to lower the MICs against *Sa.* Enteritidis and *Staphylococcus aureus*, EOs from *Drypetes gosswelleri, Echinops giganteus*, and *Melaleuca leucadendron* worked in concert with antibiotics (ciprofloxacin, ceftriaxone), with *D. gosswelleri* EO being the most successful (Feudjieu et al., 2023). Similarly, *Cinnamomum cassia* EO and *trans*-cinnamaldehyde increased enzyme-mediated biofilm disintegration by permeating membranes, depleting ATP, and decreasing intracellular pH (Cervantes-Huamán et al., 2025; Feudjieu et al., 2023). In a recent study, food supplementation with EOs and organic acids dramatically reduced the bacterial burden of *Sa.* Heidelberg, Minnesota, and Typhimurium in experimentally infected broiler chickens at days 7 and 14 post-infection (Stingelin et al., 2023). By reducing *Salmonella*'s ability to remain in the stomach and on meat, these combinations applied to chicken could improve food safety and promote antibiotic-free practices.

The antibiofilm efficacy of phenolics and EOs against *Salmonella* is frequently dependent on their interactions with complementary antimicrobials, with outcomes determined by strain, environment, and delivery, underscoring the significance of mechanistic understanding for optimal application.

Combined effect of phenolic compounds and EOs on *Salmonella* biofilm in poultry

The combination of phenolic compounds and EOs is a promising method for increasing antibiofilm efficacy against *Salmonella* in poultry production while also overcoming their individual limitations, such as strong odor, instability, or the necessity for high concentrations when used alone. EOs, including phenols and aldehydes, disturb bacterial membranes by increasing permeability and inducing cellular leakage, disrupting critical functions such as ATP balance, protein synthesis, DNA integrity, and QS. EO components can act synergistically, and when combined with phenolic compounds that target biofilm formation and bacterial communication, this multi-target approach enhances antibiofilm effects and is especially effective against *Salmonella* biofilms in poultry environments (El-Tarabily et al., 2021).

Studies have revealed that combinations, such as cinnamaldehyde and 2-hydroxycinnamic acid, have considerable additive effects against foodborne pathogens, including *Sa.* Enteritidis and *Listeria monocytogenes* (Corrêa et al., 2021). Similarly, cinnamaldehyde and eugenol, individually degraded over 50 % of mature biofilms of *L. monocytogenes* and *Sa.* Typhimurium and showed synergistic antibiofilm activity when combined, with fractional inhibitory concentration index (**FICI**) values of 0.24 for *L. monocytogenes* and 0.40 for *Sa.* Typhimurium (Purkait et al., 2020). This synergy enables lower effective doses, making them attractive candidates for combating infections. Additionally, synergistic interactions between EOs and phenolic extracts from plants, such as *Artemisia* sp. and *Citrus aurantium*, have resulted in increased antibacterial activity (Benamar-Aissa et al., 2024b). Similarly, rosemary-based formulation enriched with EOs and phenolic compounds improved broiler growth performance, reduced mortality, and enhanced antioxidant activity. Treated birds showed higher body weight, better feed conversion, and elevated catalase levels compared to controls (Filali Samouh et al., 2024). These combinations boost efficacy while also lowering toxicity and formulation costs, making them promising options for natural antibacterial methods in poultry production (Benamar-Aissa et al., 2024a). The relationship between the phenolic substances and EOs in poultry systems is still conceptually ambiguous, despite its potential. According to available data, their combined biochemical effects may change the microbial ecology in the gut and processing environment. However, a major problem is striking a balance between antimicrobial potency and metabolic and sensory stability. Such dual-acting systems may affect microbial homeostasis and host physiology in addition to targeting pathogens like *Salmonella*; this effect necessitates careful formulation rather than straightforward additive application.

Effects of phenolic compounds and EOs on *Salmonella* biofilm across poultry production stages

Breeding flocks

Breeding flocks, which serve as the basis of the chicken production chain, are crucial to guaranteeing downstream food safety. Controlling

Salmonella at this stage is critical for preventing vertical transmission to eggs. Phenolic compounds from *Laetiporus sulphureus* can increase efficiency, growth, and nutrient absorption while decreasing oxidative stress, inflammation, and mortality and boosting mucosal immunity (Lin and Lee, 2020; Mahfuz et al., 2021; Mountzouris et al., 2011). Similarly, EOs, which are **GRAS**, also have potential benefits in poultry diets. EOs are promising antibiotic alternatives in poultry, providing broad-spectrum antimicrobial activity against pathogens, such as *Salmonella*, while improving gut health by reducing pathogens, toxins, and oxidative stress and promoting villi growth, digestive enzyme activity, nutrient absorption, and overall performance (Clavijo-Romero et al., 2019; Isabel and Santos, 2009; Rouger et al., 2017; Salinas-Chavira and Barrios-García, 2024; Toso et al., 2023). This was supported by research showing that OEO (100 mg/kg) increased *Bifidobacterium* and *Lactobacillus* while decreasing *Escherichia coli* and *Salmonella* in laying hens (He, 2017). Additionally, oral carvacrol (300–400 μL) or dietary clove and camphor EOs (0.1–1.2 %) decreased *Salmonella* and *Campylobacter* in chicken guts by as much as 50 % in just 2 weeks (Abd El-Hack et al., 2022; Liu et al., 2018; Shaji et al., 2023). Moreover, incorporating phenolic substances, specifically GA and vanillic acid, into feed or water would help prevent *Sa*. Typhimurium colonization, while also promoting the growth of beneficial gut bacteria, such as *Bifidobacterium*, resulting in a healthier microbial balance (Alvarado-Martinez et al., 2023).

Therefore, by limiting *Salmonella* colonization and fostering commensal gut microbiota, phenolic compounds and EOs in breeding flocks improve flock health and productivity, laying the groundwork for subsequent broiler and layer production stages.

Hatchery application

In hatchery environments, phenolic compounds and EOs can be used as disinfectants to sanitize hatching eggs, effectively minimizing microbial contamination on egg surfaces. In particular, they can reduce *Salmonella* adhesion (Baek et al., 2025; Hu et al., 2021; Oliveira et al., 2024), which is the first step of biofilm formation. As an example, EOs from *Cymbopogon nardus, Syzygium aromaticum*, and *Cinnamomum cassia* reduced enterobacteria on eggshells by 2 and $\geq$3 $\log_{10}$ CFU/mL, respectively, providing a safer substitute for formaldehyde fumigation (Vale et al., 2024). Moreover, EOs from *Citrus aurantifolia, Ocimum basilicum*, and *Allium sativum* can also be applied to eggshells to reduce bacterial load, improve hatchability, and promote healthy embryo development (Oliveira et al., 2024). Phenolic compounds can also be provided *in ovo*, providing benefits like better hatchability and antioxidant status in developing embryos. Supporting this hypothesis, research employing natural bioactive extracts, such as black cumin delivered *in ovo*, exhibited improved hatchability, increased antioxidant enzyme activity, and superior post-hatch growth, suggesting that phenolic compounds and EOs may impart similar benefits (Oke et al., 2021). Furthermore, phenolic compounds and EOs can be used to sterilize hatchery equipment and surfaces, such as incubators, setters, and work tables, to decrease biofilm formation and contamination, thus contributing to safer production settings (Kim et al., 2022; Oliveira et al., 2024). Given the sensitivity of eggs and hatchlings to early-stage *Salmonella* infection, tailored strategies are necessary. Phenolics and EOs suppress infection most effectively when administered *in ovo* or supplemented in feed during post-hatch, together with thorough sanitation and controlled incubation, negotiating microenvironmental and egg-surface challenges.

Grow-Out farms/broiler production

During this stage, broilers are grown in high-density conditions where *Salmonella* can survive in litter, water lines, and feeders, encouraging biofilm formation and horizontal microbial transmission. Bioactive phenolic compounds and EOs can help limit biofilm growth and lower the risk of contamination (Machado Junior et al., 2020). While some benefits, such as promoting growth and improving feed utilization, are similar to those found in breeder flocks, grow-out farms prioritize minimizing environmental contamination, limiting pathogen spread among birds, and improving production performance over preventing vertical microbial transmission to eggs. Phenolic compounds and EOs help to achieve these objectives by boosting feed efficiency, increasing bird resilience, and minimizing microbial accumulation in the rearing environment. For example, fogging broiler houses with thyme or peppermint oils has been proven to reduce bacterial counts in air, litter, walls, and drinkers, suggesting their potential for stage-specific environmental cleanliness (Witkowska and Sowińska, 2013).

Slaughterhouse

The slaughterhouse is a major control point where *Salmonella* can transform from a hidden resident in processing equipment to a direct contaminant of poultry products. Biofilms operate as invisible shields within drains, de-feathering machines, and cutting blades, allowing the pathogen to live, propagate despite routine cleaning efforts, and spread via cross-contamination of carcasses during processing (Reta et al., 2023). Unlike earlier stages, where the focus is on animal health, actions here have a direct bearing on consumer safety.

Salmonella biofilms frequently persist on stainless steel surfaces in slaughterhouses, including cutting tables, blades, and conveyors, serving as sources of cross-contamination. Phenolic compounds have been proven to inhibit biofilm formation in processing settings (Soni et al., 2013). Notably, eugenol inhibited bacterial adhesion to stainless steel and glass at sub-inhibitory levels, lowering biofilm biomass and altering its structure (Palomares-Navarro et al., 2025). Furthermore, when mixed with other natural compounds, GEO reduced *Salmonell*a on chicken skin by up to 99 % and hindered its growth in simulated scalding tank conditions, indicating a promising strategy to reduce cross-contamination in slaughterhouses (Robinson et al., 2022). Although promising, the practical implementation of these natural compounds necessitates careful integration with existing sanitation methods, adherence to regulatory standards, and consideration of operational practicality to achieve consistent and successful *Salmonella* biofilm control.

Storage and packaging

Salmonella can remain on chicken products after processing by forming biofilms on packaging materials or cold-storage surfaces, creating a risk of post-processing contamination (Thames and Sukumaran, 2020). Temperature variations, moisture, and handling habits can all help *Salmonella* biofilm survive and grow (Krüger et al., 2025).

Phenolic compounds and EOs can reduce *Salmonella* adherence and biofilm formation on packaging and meat surfaces. For example, quercetin destroys *Sa. enterica* biofilms at sub-MIC levels, as evidenced by SEM and confocal laser scanning microscopy (**CLSM**) imaging, while also increasing broiler meat quality through pH, softness, moisture retention, muscle development, and lipid stability without causing cytotoxicity (Kim et al., 2022; Park et al., 2025; M. Wang et al., 2022). Similarly, these phytochemicals can reduce *Salmonella* biofilms on packaging, while pomegranate peel extracts successfully suppressed *Sa*. Kentucky and Enteritidis growth on chicken during refrigerated storage (Wafa et al., 2017).

The true power of phenolic compounds and EOs to combat biofilms is revealed when they are strategically combined with stage-specific interventions. These interventions include limiting early biofilms in hatcheries, improving gut health and immunity in breeding flocks, controlling environmental *Salmonella* during grow-out, reducing equipment and carcass contamination at slaughter, and suppressing biofilms in storage (freezer). These actions turn chemical activity into

effective, production-ready *Salmonella* control.

Challenges in application

Many natural bioactive compounds (phenolics and EOs) have a variety of biological actions, including antibacterial, antioxidant, anticancer, and anti-inflammatory properties, making them attractive for poultry production. However, their low water solubility and stability limit both their efficient distribution within the complex fowl gastrointestinal tract, as well as their bioavailability, making them impractical for use as feed additives or in gut health management (Movahedi et al., 2024).

The quality of plant phenolic compounds can be influenced by a variety of factors, including plant age, geographical location, seasonal fluctuations, and collection time and methodology. This variation hampers the systematic evaluation and standardization of raw ingredients used in chicken feed supplements or treatments, increasing costs and creating difficulty in guaranteeing safety and efficacy (Ginsburg and Deharo, 2011).

The sensory influence of EOs on food products is a major source of concern. Depending on the type and concentration, EOs can contribute strong or unpleasant aromas and flavors to poultry meat or eggs (Ferrentino et al., 2020; Meira et al., 2017; Zhai et al., 2018). The necessity for appropriate dosing and additional research is further highlighted by the fact that EOs can have varying effects on gut microbiota and that *in vivo* results may differ from *in vitro* results due to host characteristics, infection pressure, and dose (Girard et al., 2024; Laptev et al., 2021; Zoroaster et al., 2025). These data indicate that gut dysbiosis may occur depending on the EO composition and environment. Furthermore, concerns about EOs' toxicity and a lack of long-term safety evidence demand additional research. Overall, although EOs have great potential, their use must be carefully planned to minimize their shortcomings and provide continuous benefits.

Finally, utilizing these natural chemicals in large-scale chicken production might be expensive due to seasonal supply changes, a paucity of high-quality raw materials, and difficult extraction and purification methods. Maintaining consistent quality for large-scale chicken feed or processing necessitates investments in controlled cultivation, harvesting, and distillation procedures, which may raise production costs.

Potential benefits and risks to public health

The use of phenolic compounds and EOs in poultry can reduce *Salmonella* biofilm contamination, antibiotic use, and chemical residues in chicken meat and eggs, contributing to lower disease incidence in humans. They may also provide additional human health benefits due to their antioxidant, anti-inflammatory, antimicrobial, and cardioprotective properties. Phenolic compounds, in particular, can alter enzymes and transcription factors associated with chronic illnesses (e.g., COX, NF-κB, Nrf2, ACE), perhaps leading to minor health advantages for consumers (Rahman et al., 2021; Ramsey et al., 2020). Their presence in poultry products may have subtle health-promoting effects for consumers, including potential psychological benefits such as stress alleviation, increased mood, and improved sleep quality (Ramsey et al., 2020).

In contrast, the use of phenolic compounds and EOs in chicken production may pose certain adverse effects on human health. From a human health perspective, the bioavailability of phenolic compounds varies widely, which means their beneficial properties are not always achieved, and some compounds might produce side effects or toxicity. Polyphenols, while typically beneficial, can limit iron uptake, slow digestive enzymes, change the intestinal bacteria composition, interfere with drug metabolism, upset hormonal balance, and even have prooxidative, mutagenic, or genotoxic effects under some circumstances (Duda-Chodak and Tarko, 2023).

EOs have recently been recognized as more complex than previously assumed, with increased use associated with allergic contact dermatitis and possibly endocrine-disruptive effects, such as early breast growth in adolescents. While they provide advantages, these risks underscore the need for increased awareness of their implications for human health (Ramsey et al., 2020; Sindle and Martin, 2021). Moreover, misuse of EOs can result in off-flavors or residues in meat and eggs, compromising both safety and consumer appeal (Ghaderi-Ghahfarokhi et al., 2016). This underlines practical problems in implementing EO-based therapies on a large scale, emphasizing the importance of clear rules and quality control. Such risks highlight the importance of cautious application, proper supervision, and public awareness, especially among vulnerable groups, such as pregnant women, children, and older adults, as well as individuals with immunocompromised status.

Future direction

To address the limitations of phenolic compounds and EOs in poultry farming, future research should focus on enhanced delivery technologies (nanoencapsulation, liposomes, biopolymer carriers), *in vivo* validation across poultry models, and the investigation of synergistic interactions with other feed additives. Choosing high-bioactive plant cultivars, standardizing extraction processes, and utilizing omics technology can all increase efficacy and mechanistic knowledge. Additionally, addressing environmental destiny, safety, and regulatory frameworks is crucial for long-term utilization.

Future research from the standpoint of public health should concentrate on the metabolite safety, long-term toxicological assessments, and elucidation of human metabolism of these compounds (EOs, phenolic compounds), as these elements will dictate actual exposure and possible hazards like genotoxicity, allergenicity, or endocrine disruption. To ensure responsible and safe use, it will be equally vital to build clear policy frameworks and broaden consumer acceptability. In order to transform research on these natural antimicrobials into practical and sustainable solutions that enhance poultry health while safeguarding public health, interdisciplinary cooperation between microbiologists, animal scientists, chemists, toxicologists, and legislators will be crucial.

Conclusion

Phenolic compounds and EOs present a viable, residue-free strategy for reducing *Salmonella* biofilms and decreasing reliance on antibiotics and chemical disinfectants in poultry production. In an era of rising antibiotic resistance, phenolic compounds and EOs can play an important role in long-term poultry health measures by combining molecular understanding with practical applications. While major obstacles remain, notably in terms of distribution, standardization, and safety, these natural substances offer a long-term solution with potential benefits ranging from poultry health to consumer safety. With continuing innovation and responsible application, they have the potential to become critical tools in promoting both food safety and public health.

CRediT authorship contribution statement

Syeda Roufun Nesa: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Conceptualization. **Md Furkanur Rahaman Mizan:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Nigar Sultana Meghla:** Writing – review & editing, Visualization. **IkSoon Kang:** Writing – review & editing, Visualization. **Sang-Do Ha:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Disclosures

Manuscript has been 'spell checked' and 'grammar checked'

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A competing interests statement is provided, even if the authors have no competing interests to declare

Journal policies detailed in this guide have been reviewed

Referee suggestions and contact details provided, based on journal requirements.

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