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Dual Effect Against Foodborne *Salmonella* Isolates: Antibacterial and Antibiofilm Efficacy of Different Concentrations of Extracts from Cocos nucifera (L.) and Elettaria cardamomum (L.) Maton

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ABSTRACT

This study investigates the antibacterial and antibiofilm effect of ethyl acetate and methanolic extracts of Coconut kernel/testa and Cardamom seed pods against both typical Salmonella isolates that do not ferment lactose (Lac-) and the atypical group that can ferment lactose (Lac+). Both plant extracts, including ethyl acetate and methanolic fractions, demonstrated good antibacterial activity against typical and atypical resistant *Salmonella* isolates, with ethyl acetate fractions of both plants exhibiting the most effective results against planktonic cells, in which ethyl acetate fraction of Cardamom seed pods showed a minimum inhibitory concentration (MIC) of 5 mg/ml against potential Extended-spectrum β-lactamase (ESBL (CTX-M))-producing Lac+ isolate. While the ethyl acetate fraction of Coconut kernel/testa scored an MIC of 15 mg/ml against the multidrug-resistant (MDR), potential ESBL (CTX-M)-producing Lac- isolate.

The highest bactericidal activity was observed with the Cardamom methanolic fraction against the Lac- *Salmonella* sample with a minimum bactericidal concentration (MBC) of 25 mg/ml. Biofilm formation ability by the Lac+ isolate was significantly diminished by the methanolic fraction of Coconut kernel/testa at a sub-minimum inhibitory concentration (SMIC) of 25 mg/ml. Likewise the methanolic fraction of Cardamom also exhibited a noteworthy reduction in biofilm formation, with a significant impact observed at 5 mg/ml of SMIC.

Keywords: *Salmonella*, multidrug-resistant, lactose fermenter, Coconut kernel/test, Cardamom pods.

INTRODUCTION

Acknowledging the age-old connection between diet and disease, Foodborne illnesses arise from consuming food contaminated by pathogens that proliferate in the host or from toxins produced by microbes. Bacteria are often the primary offenders (Bintsis, 2017). *Salmonella* is a zoonotic bacterium and a significant global health concern that causes millions of cases of gastroenteritis and thousands of deaths worldwide each year. This bacterium thrives in the digestive systems of various animals, including humans (Galan-Relano *et al.*, 2023). *Salmonella enterica* subsp. *enterica* is a major cause of salmonellosis, affecting both humans and animals. Salmonellosis can involve a range of clinical symptoms, such as gastroenteritis and enteric fever to bacteremia and persistent carrier state. Transmission occurs primarily via consumption of contaminated food or water, particularly of poultry products (Ajmera and Shabbir, 2024; Bhat *et al.*, 2022).

Contrary to the classical bacteriological characterization of *S. enterica* members being non-lactose fermenters (Lac-), certain lactose fermenter (Lac+) variants have been inspected in geographically diverse foodborne outbreaks. Which suggests an adaptive divergence within the species, challenging the classical characterization. While Lac+ *Salmonella* reportedly constitutes less than 1% of *Salmonella* isolates, its true incidence may be underestimated due to the use of differential media in many laboratories that fail to distinguish Lac+ *Salmonella* from *Escherichia coli* (ALEXAN, 2017; Leonard *et al.*, 2015).

The emergence of pathogens resistant to clinically relevant antibiotics, necessitates the exploration and development of novel therapeutic strategies (Nair *et al.*, 2018). The isolation of bacteria characterized by the production of Extended-spectrum β-lactamase (ESBL) from food producing animals and their products is concerning, as these enzymes can lead to penicillin, 3rd and 4th generation Cephalosporins resistance, which are clinically important both to human and animals' health (Saechue *et al.*, 2024).

Biofilms, not just solitary cells, but more like microbial societies. Pathogenic bacteria typically exist as free-floating (planktonic) cells only briefly, transitioning to a sessile lifestyle in response to environmental signals, leading to the formation of multicellular, surface-attached communities, which mount a potent defense against environmental stressors. This biofilm formation is considered as one of the most effective approaches bacterial cells use to resist antibiotics, as this sophisticated architecture transforms a susceptible bacterial population into a persistent therapeutic obstacle (Desai and Kenney, 2019; Punchihewage-Don *et al.*, 2024; Younis, 2018). The ability of *Salmonella* spp. to form biofilms in foods and related environments has variable burdens, one of which is increasing microbial resistance. This biofilm formation, a virulence factor, varies among *Salmonella* serotypes due to differences in their specific properties and genetic makeup. Environmental factors like attachment substrate, temperature, and incubation period also impact biofilm formation and its quality (Akinola *et al.*, 2020).

Phytochemicals present a promising solution to microbial resistance. Their historical use in herbal medicine, combined with potent antibacterial properties and the ability to disrupt essential microbial processes like efflux pump activity, membrane proteins' functions, makes them a viable alternative to conventional antibiotics. Phytochemicals are cost-effective and eco-friendly. The diversified chemical structures allow them to depict antimicrobial effects by disturbing bacterial cell envelopes, energy metabolism, and intracellular communication. The usage of phytochemicals as natural additives in animal feed further demonstrates their capacity to combat bacterial infections and reduce antibiotic dependence (Al-Noamy, 2020; Almuzaini, 2023; Khare *et al.*, 2021; Sakarikou *et al.*, 2020). Additionally, phyto-derived bioactive compounds show promising antibiofilm agents that can act through various mechanisms. These compounds which involve flavonoids, terpenes, phenolics, fatty acids, oligosaccharides typically inhibit biofilms in a concentration-dependent manner. Their key advantage is that they can be effective at a sub-lethal concentration, which reduces the likelihood of inducing antibiotic resistance (Pompilio *et al.*, 2023). Coconut (*Cocos nucifera* (L.)), the "*Tree of Heaven*", is a prized plant for its diverse nutritional and medicinal applications. The different parts are widely utilized for different purposes, offering natural products and therapeutic

potential. Both the kernel and its testa (the thin brown covering) are known to contain variable bio-active compounds, which make them retain defensive properties against different microorganisms (Mat *et al.*, 2022; Ramesh *et al.*, 2023).

The crowned "*Queen of Spices*", (*Elettaria cardamomum* (L.) Maton) commonly known as Cardamom, is considered as the third most expensive spice, it is known by its unique flavor and aroma, widely used as food flavoring. Traditionally, Cardamom has been employed for medicinal purposes. Similarly, Cardamom is rich in wide variety of secondary metabolites which contribute to their bioactivity in different aspects of life (Ramadan, 2023).

This study aimed to investigate the antibacterial and antibiofilm effect of plant extracts to combat resistant *Salmonella* isolates, with a particular focus on different concentrations of extracts from Coconut kernel/testa and Cardamom seed pods. To the best of our knowledge, this is the first study to examine the impact of these extracts against potential ESBL (CTX-M)-producing Lac+ and Lac- *Salmonella* isolates in Erbil city-Kurdistan Region-Iraq.

MATERIALS AND METHODS

Food samples collection and processing

A total of 61 hens' eggs were collected aseptically from local markets. Samples were processed following a slightly modified version of (Long *et al.*, 2017). Eggshell samples were either swabbed or crushed and subsequently immersed in buffered peptone water (AcuMedia, Michigan), vortexed and eventually incubated at 37°C/24 hours. Following pre-enrichment, 1 ml of each broth culture was transferred to 9 ml of *Salmonella* selective enrichment broth base (HIMEDIA, India), these cultures were then incubated for 24 hours/37°C. Enriched cultures were streaked on selective and differential media for *Salmonella* including Hektoen Enteric Agar (HEA) (HIMEDIA, India), Xylose Lysine Deoxycholate agar (XLD) (Scharlau, Spain), Salmonella-Shigella agar (S-S) (Scharlau, Spain), and on MacConkey agar (MacC) (Scharlau, Spain), then incubated for 24-48 hours/37°C.

Suspected *Salmonella* colonies were characterized biochemically using triple sugar iron agar (Scharlau, Spain) and Simmon`s citrate agar (LABM, United Kingdom) (Ahmed *et al.*, 2024; Bahjat *et al.*, 2019). Typical Lac- and atypical Lac+ colonies were further confirmed to be *Salmonella* via conventional polymerase chain reaction (PCR) targeting the *invA* gene (cycling conditions are detailed in (Table 1). The VITEK 2 Compact system (bioMérieux, France), in conjunction with GN AST-N417 cards involving the following antimicrobial panel (Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Cefazolin, Cefuroxime, Cefuroxime Axetil, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Fosfomycin, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole), were employed for antibiotic susceptibility testing for confirmed *Salmonella* isolates (Salman *et al.*, 2021). The 2023 Clinical and Laboratory Standards Institute guidelines (Global CLSI-based 2023) were followed for data analysis. Two potential ESBL (CTX-M)-producing *Salmonella* isolates (one having moderate resistance from the Lac+ group, while the other being multidrug-resistant (MDR) from the Lac- group), along with a *Salmonella* Typhimurium ATCC 14028 (*S.* Typhimurium) standard were further utilized to conduct the antibacterial and antibiofilm assays in this study.

Table 1: Primer sequences and thermal cycling program for *Salmonella* confirmation.

Primer code	Sequence (5'-3') of *invA* Gene	Amplicon size	Primers' source
S139- F	GTGAAATTATCGCCACGTTCGGGCAA	284 bps	(Dawood Saleem *et al.*, 2022)
S141- R	TCATCGCACCGTCAAAGGAACC		
PCR amplification conditions	95˚C/5 min (initial denaturation), 35 cycles, (95˚C/40 sec denaturation, 58˚C/40 sec annealing, 72˚C/1 min elongation), ending extension 72˚C/10 min		

(F: forward; R: reverse)

Obtaining bioactive compounds from coconut and cardamom

Utilized plant material and pre-processing steps

Two plants were obtained from Erbil city markets: powdered Cardamom seed pods and mature Coconuts, in which the latter were manually de-shelled, the water discarded, and the white meat (kernel) with its outer thin cover (testa) were retained for the study as (kernel/testa) combination.

Defatting of Coconut kernel/testa was performed as a preliminary step to facilitate efficient extraction of bioactive compounds, which was carried out based on a previously described method (Adeloye *et al.*, 2020) with some modifications. The Coconut kernel/testa was washed, shredded, and air-dried at 35°C. Defatting of the 300 g material was performed via sequential extraction with n-hexane 96% (Scharlau, Spain) using both sonication bath (Anonkia DP360, China) and maceration methods. The resulting semi-powder form was then dried and ground to a finer powder texture for subsequent analyses.

Sequential extraction

Phytochemical extraction was conducted sequentially, employing solvents of increasing polarity (Ethyl acetate 99.5+% (CHEM-LAB, Belgium), then Methanol ≥ 99.9% (Fisher Chemical, Belgium)). A combined method from (Gunarathne *et al.*, 2021; Nurcholis *et al.*, 2022) with certain modifications was utilized. Two successive sonication cycles were performed per solvent.

Defatted Coconut kernel/testa powder (80 g) underwent sequential extraction via sonication (40°C, 40 mins) and maceration (30 mins) in 600 ml ethyl acetate. Following decantation, residual material was re-extracted with a fresh batch of ethyl acetate, repeating the process. Combined extracts were filtered twice with filter paper (Double Rings 15.0cm, China) and concentrated by rotary evaporation (Heidolaph Laborota 4000 rotovap - Germany). The Coconut kernel/testa material was dried, and the procedure was repeated using methanol as the solvent.

An analogous protocol was applied to Cardamom seed pods (100 g), employing ethyl acetate (250 ml, then 150 ml) and methanol (250 ml, then 150 ml) in successive extraction cycles to each (50 g) powder. All filtrates were concentrated and stored.

Preparing a concentration range and determination of minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs), and sub-minimum inhibitory concentrations (SMICs)

Stock solutions of crude plant extracts were prepared by dissolving 1 g of each substance in 5 ml of dimethyl sulfoxide (DMSO) (CHEM-LAB, Belgium), resulting in a final concentration of 200 mg/ml. Different concentrations of the plant extracts were prepared from stock in the range of (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 mg/ml) (Ismaeil and Saleh, 2019).

The MICs of plant extracts against *Salmonella* isolates were determined using the broth microdilution method after applying slight alterations to the protocol mentioned in (Ahmed *et al.*, 2023). Overnight *Salmonella* cultures were standardized to 0.5 McFarland (OD550). In 96-well microtiter plates (MTPs), the first column with 200 µl nutrient broth (NB) (TMMEDIA. REF TM 350, India) served as control, inoculated with 15 µl of the standardized bacteria. Subsequent columns contained 200 µl of plant extracts at various concentrations, also inoculated with 15 µl of bacteria.

Initial absorbance (630 nm) was measured using (BioTek ELX800, U.S.) microplate reader, followed by incubation at 37°C/24 hours and a repeated absorbance reading. The MIC was the lowest concentration inhibiting visible growth. The streaking method on nutrient agar was used to determine MBCs. SMICs were subsequently estimated from values below MICs for later assay.

Antibiofilm activity determination of phyto-extracts at SMICs

The protocol described by (Ismaeil *et al.*, 2024) was adapted with slight alterations to observe the effect of phyto-extracts on biofilm formation ability post-treatment. Biofilm formation was quantified using a crystal violet assay in 96-polystyrene MTPs. Activated cultures of *Salmonella* isolates were standardized to a turbidity equivalent to 0.5 McFarland standard (OD550 nm). 10 µl of the standardized cultures were then added to each SMIC of plant extracts, in small Eppendorf tubes.

These mixtures were incubated for 24 hours/37°C. 15 µl of each inoculated mixture were re-suspended in 200 µl of tryptone soya broth (Accumix, India) with 0.5% glucose in quadruplicates of each extract. The plates were incubated for 48 hours/37°C.

Following incubation, planktonic cells were discarded, the plates were rinsed thrice with sterilized distilled water, and dried at 60°C. Biofilms were stained with 1% crystal violet for 20 minutes at room temperature, rinsed with sterilized distilled water for another three times, and dried. The stained biofilms were then dissolved in 96% ethanol for 20 minutes. Absorbance was measured at 630 nm using an ELISA BioTek ELX800 microplate reader.

Statistical analysis

GraphPad Prism Version 8.0 was utilized for data analysis. Multiple comparisons were conducted via one-way analysis of variance (ANOVA), and data are illustrated as the mean ± SEM.

RESULTS AND DISCUSSION

Recently, it has been noticed that MDR *Salmonella* strains are spreading from animals to humans via the food chain (Adel *et al.*, 2021). In our study, *Salmonella* was detected in 13.11% of raw egg samples. A total of 9 isolates were recovered, with two isolates from a single egg exhibiting different antibiotic resistance profiles (one MDR, one non-MDR) via the two isolation methods (shell swab and shell crush). All isolates were confirmed to be *Salmonella* using PCR via detection of *invA* gene as illustrated in Fig. (1). The *invA* gene is a widely recognized and utilized diagnostic target for *Salmonella enterica* detection, serving as an international standard. It is recommended by the U.S. Food and Drug Administration for PCR validation of *Salmonella* isolates (AHMAD and MUSTAFA, 2023). Five isolates, including the ATCC standard, displayed moderate, non-MDR resistance, while the remaining five exhibited an MDR profile. MDR state is expressed as an isolate displaying non-susceptibility to at least one agent in three or more antimicrobial classes (Adel *et al.*, 2021). VITEK 2 AST interpretations are shown in (Table 2).

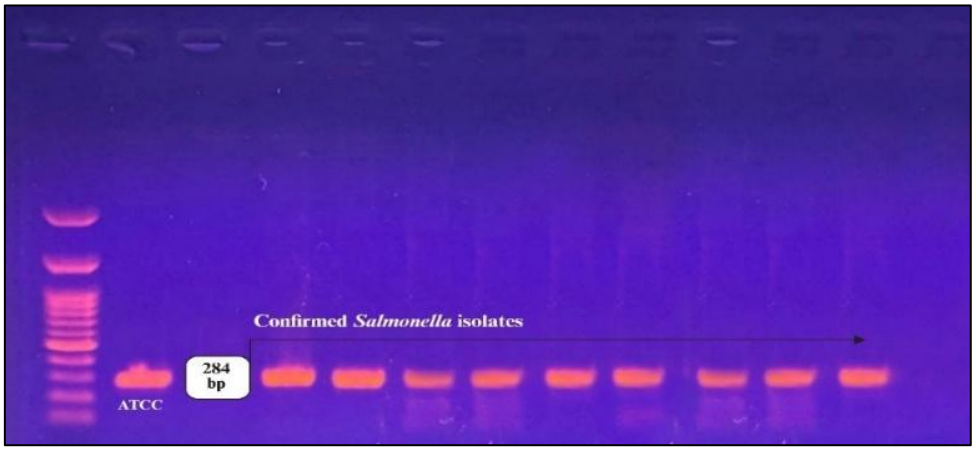

Fig. 1: Gel electrophoresis showing positive detection of *invA* gene in *S.* Typhimurium ATCC 14028 reference strain and the nine Lac+ and Lac- *Salmonella* isolates.

Plant extracts were evaluated for their antibacterial efficacy against the ATCC 14028 standard and the two isolates phenotypically flagged for ESBL production by VITEK 2 system, which is a rapid and effective system for identifying bacteria with antimicrobial resistance (Tshabuse *et al.*, 2022).

Table (2): Antibiotic susceptibility/resistance profiles of *Salmonella* isolates based on VITEK 2 MIC result

Card: GN AST-N417	***Salmonella* spp. (n=10 / including ATCC 14028 standard)**		
	VITEK 2 system interpretations		
Antimicrobials	**Susceptible %**	**Intermediate %**	**Resistant %**
Amoxicillin/Clavulanic acid	60 %	10 %	30 %
Piperacillin/Tazobactam	100 %	-	-
Cefazolin	-	-	100 %
Cefuroxime	10 %	-	90 %
Cefuroxime Axetil	10 %	-	90 %
Ceftazidime	100 %	-	-
Ceftriaxone	100 %	-	-
Cefepime	100 %	-	-
Ertapenem	100 %	-	-
Imipenem	100 %	-	-
Meropenem	100 %	-	-
Fosfomycin	100 %	-	-
Amikacin	10 %	-	90 %
Gentamicin	10 %	-	90 %
Nitrofurantoin	80 %	-	20 %
Ciprofloxacin	70 %	-	30 %
Trimethoprim/Sulfamethoxazole	80 %	-	20 %

The plant extracts' inhibitory and bactericidal effects were observed within the concentration range of 5-50 mg/ml. Despite the antibiotic resistance profiles of the three tested subjects, the consistency in activity of tested substances across the samples was notable. The ethyl acetate fraction of Cardamom seed pods exhibited the most potent bacteriostatic activity with MIC of (5 mg/ml) against the (1 Lac+) *Salmonella* isolate, while the methanolic fraction of the same plant showed the most potent bactericidal activity with MBC of (25 mg/ml) against the MDR (2 Lac-) sample. Full results are listed in (Table 3). A study on both Gram-positive and Gram-negative bacteria stated that the methanolic fraction of Coconut testa showed significant antibacterial properties, displaying a notable zone of inhibition against all the tested samples. Conversely, ethyl acetate fraction exhibited no impact at a concentration of 10 mg/ml (Ojha *et al.*, 2019). In another study, even though the ethanolic fraction at 0.2% concentration showed wider spectrum of inhibition on the bacterial isolates tested, the methanolic fraction of defatted Coconut meal demonstrated maximum inhibition activity against *S.* Typhimurium at the same concentration, using the disc diffusion method. It was also shown how the two fractions had the same bactericidal effect on *S.* Typhimurium after 8 hours of incubation.

The same study also proposed that the phenolic content of the defatted Coconut meal caused deformation in the bacterial cells, suggesting the ability of the extracts to act as food preservatives against spoilage (Sengar and Madkour, 2024). Our Cardamom seed pod extracts exhibited more potent antibacterial activity than Coconut kernel/testa, with methanolic fraction having the same MIC value among the three tested samples. The observation that Cardamom extracts can be effective against resistant food poisoning pathogens is supported by a study conducted by Yassin and colleagues in 2022, which assessed different extracts of Cardamom seeds indicating that the ethyl acetate extract scored the highest bioactivity against *S.* Typhimurium. Furthermore, due to the efficacy of the Cardamom ethanolic extract, the same study suggested that these extracts can be valuable in developing safe and efficient food bio-preservatives (Yassin *et al.*, 2022). In another research, When Cardamom pods' extract was tested on an anaerobic, Gram-negative bacteria, the study exhibited low

MIC and MBC values of 0.0039 mg/ml (Nouri *et al.*, 2023), this finding substantiates the claim that the antibacterial activity of *Elettaria cardamomum* varies according to the type of extract and the organism tested (Kaushik *et al.*, 2010).

Table (3): Obtained MIC and MBC values from the antibacterial tests, and determined SMICs for antibiofilm assay.

		ATCC 14028 (mg/ml)			1 Lac+ (mg/ml)			2 Lac- (mg/ml)		
Treatment	**Solvent**	**MICs**	**SMICs**	**MBCs**	**MICs**	**SMICs**	**MBCs**	**MICs**	**SMICs**	**MBCs**
Cardamom seed pods	**Eth.**	10	1	35	5	1	ND	10	1	35
	Met.	15	5	30	15	5	30	15	5	25
Coconut kernel/testa	**Eth.**	20	10	ND	20	10	ND	15	5	50
	Met.	40	30	50	35	25	50	35	25	50

Eth.: ethyl acetate, Met.: methanol, ND: not detected

Phytochemicals can weaken virulence by interrupting the bacterial attachment and essential processes like protein and nucleic acid synthesis. They can also compromise bacterial survival by electrochemical gradients disruption. Moreover, they exhibit immunomodulatory effects by enhancing immune response (Almuzaini, 2023). The extraction of plants' secondary compounds, particularly the polyphenolic ones, is inherently complex, as they are found in association with other compounds, they are diverse, and they have varying solubilities in different solvents. The extraction yield is further influenced by the extraction method, and the nature of the plant material (Ojha *et al.*, 2019), which explains the variability in the activity of the extracts even when the same plant part is used. Upon examination of the literature, it was found that variable types of phytochemicals are found in each extract of Coconut kernel/testa and Cardamom pods, of which, phenolics and flavonoids were detected in both plants (Dhanyakrishnan *et al.*, 2018; Gunarathne *et al.*, 2021; Sengar and Madkour, 2024; Tarfaoui *et al.*, 2022). Which exhibit antimicrobial properties against various microorganisms, including those relevant to food safety and clinical settings. These molecules, differing in structure and composition, have diverse effects that contrast with traditional antibiotics, this makes them promising alternatives in the fight against drug-resistant strains (Tako *et al.*, 2020).

In *vitro* analysis, SMICs ranging from 1 to 25 mg/ml effectively impaired the ability of *Salmonella* to form biofilms, which was shown mostly with the (1 Lac+) isolate Fig. (2), in which significant prevention was noticed (**p = 0.0026) after incubation with methanolic fraction of Coconut kernel/testa as compared to the control. Likewise, Cardamom methanolic fraction gave significant ability (*p = 0.0134) in reducing the capacity of biofilm formation. Other fractions were also found to have the reduction impact. Despite the limited amount of literature testing the antibiofilm activity of Coconut kernel/testa and Cardamom seed pods, a study exhibited the broad-spectrum antibiofilm activity of Coconut essential oil, effectively preventing the ability to form biofilms by various microorganisms, as well as disrupting those biofilms that had already been formed (Mezher *et al.*, 2022). When antibiofilm activity of *Amomum tsao-ko* (Black Cardamom) was tested, the extract displayed highest biofilm inhibition capacity at 51.96% against *S.* Typhimurium (Rahman *et al.*, 2017).

The slight increase in biofilm formation noticed after exposing (2 Lac-) sample to SMIC of methanolic fraction of Cardamom extract as seen in Fig. (2) was found to be a common phenomenon when using SMICs to eradicate biofilms, especially at ½ or ¼ MICs, as this situation is observed with plant extracts (Mohammed, 2019), biocides, cleaners, and antibiotics, which is claimed to arise as a rapid, temporary, non-specific response, as an urgent protection for the population against stressful conditions, as mentioned in (Ranieri *et al.*, 2018) which was found to offer a more in depth understanding of the mechanisms leading to this state.

Quorum Sensing (QS) is a bacterial communication system that regulates virulence and biofilm formation. Natural bioactive compounds have shown potential as anti-QS agents. These substances can act by downregulating the expression of genes involved in biofilm development, which are regulated by QS (Khan *et al.*, 2023; Pompilio *et al.*, 2023). The ability of the studied extracts to reduce the capacity for biofilm construction after bacterial incubation with SMICs hints to the possibility of our used natural products in having bio-active compounds that can impact *Salmonella* behavior at a molecular level by targeting internal communication systems and genes responsible for biofilm production, calling for further investigations.

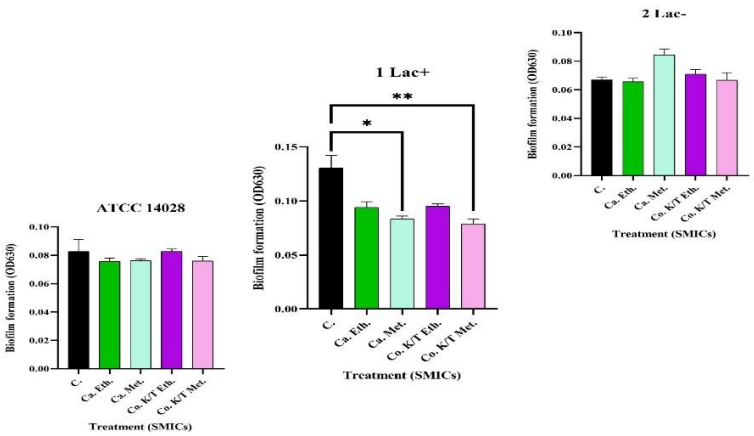

Fig. 2: Quantitative measurement of biofilm formation after treatment as compared to untreated control (C.: control; Ca. Eth.: Cardamom ethyl acetate; Ca. Met.: Cardamom methanol; Co. K/T Eth.: Coconut kernel/testa ethyl acetate; Co. K/T Met.: Coconut kernel/testa methanol). Data are illustrated as (mean ± SEM).

CONCLUSION

The rise of antibiotic-resistant *Salmonella* in food poses a growing threat, diminishing the effectiveness of new antibiotics. To recap, this study demonstrates the effect of Coconut kernel/testa and Cardamom seed pod extracts as natural sources of antibacterial and antibiofilm agents against both typical and atypical *Salmonella* isolates. The observed efficacy of specific solvent fractions, particularly ethyl acetate against planktonic cells and methanolic fractions against biofilms, points out the presence of distinct bioactive compounds with targeted mechanisms of action. The ability of such extracts to interfere with the behavior of *Salmonella*, including potential ESBL-producers and MDRs, highlights their promise in addressing the growing challenge of antibiotic resistance. Further research is warranted to indicate the specific compounds in charge of the observed activities, to elucidate their mechanisms of action, specifically their impact on intracellular communication and QS, and to evaluate their potential for in *vivo* applications. These findings support the exploration of plant-based extracts as a complementary strategy to combat *Salmonella* infections.

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التأثير المزدوج ضد عزلات *Salmonella* المنقولة بالغذاء: الفعالية المضادة للبكتريا والمضادة للأغشية الحيوية لتراكيز مختلفة من مستخلصات *Cocos nucifera* (L.) و Elettaria *cardamomum* (L.) Maton

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الملخص

تبحث هذه الدراسة التأثير المضاد للبكتريا والمضاد للأغشية الحيوية لمستخلصات إيثيل أسيتات وميثانول من لب/قشرة جوز الهند والكبسولة البذرية للهيل ضد كل من عزلات السالمونيلا النمطية التي لا تخمر اللاكتوز (−Lac) والمجموعة غير النمطية التي يمكنها تخمير اللاكتوز (+Lac). اظهرت كلتا المستخلصات النباتية، بما في ذلك أجزاء إيثيل أسيتات وميثانول، نشاطًا جيدًا مضادًا للبكتريا ضد عزلات السالمونيلا المقاومة النمطية وغير النمطية، مع اظهار أجزاء إيثيل أسيتات من كلا النباتين نتائج أكثر فعالية ضد الخلايا الحرة، حيث أظهر جزء إيثيل أسيتات من الكبسولات البذرية للهيل تركيزا مثبطا أدنى (MIC) بمقدار ٥ ملغم/مل ضد العزلة المشتبه في إنتاجها لإنزيمات البيتا لاكتاميز الممتدة الطيف ((ESBL (CTX-M) من النوع +Lac. في حين سجل جزء إيثيل أسيتات من لب/قشرة جوز الهند MIC بمقدار ١٥ ملغم/مل ضد العزلة المقاومة للأدوية المتعددة، المشتبه في إنتاجها لإنزيم ESBL من النوع −Lac. لوحظ أعلى نشاط مبيد للبكتريا مع الجزء الميثانولي من الهيل ضد عينة السالمونيلا من النوع −Lac بتركيز مبيد أدنى للبكتريا (MBC) قدره ٢٥ ملغم/مل. تم تقليل قدرة العزلة من النوع +Lac على تكوين الأغشية الحيوية بشكل كبير بواسطة الجزء الميثانولي من لب/قشرة جوز الهند بتركيز دون مثبط (SMIC) قدره ٢٥ ملغم/مل. وبالمثل، أظهر الجزء الميثانولي من الهيل أيضًا انخفاضًا ملحوظًا في تكوين الأغشية الحيوية، مع ملاحظة تأثير كبير عند تركيز ٥ ملغم/مل SMIC.

الكلمات الدالة: السالمونيلا، مقاومة للأدوية المتعددة، مُخمِّر اللاكتوز، لب/قشرة جوز الهند، كبسولات الهيل.