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ANTIBIOTIC RESISTANCE OF *Salmonella* spp. ISOLATED FROM POULTRY MEAT AND THE ANTIMICROBIAL EFFECT OF PLANTS EXTRACTS

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ABSTRACT

***Salmonella* spp. is one of the major pathogens causing foodborne disease outbreaks worldwide. It is a priority public health concern due to antimicrobial resistant strains. Poultry is considered to be a major source of transmission of *Salmonella* spp. The objective of this study was to determine the prevalence and susceptibility of *Salmonella* spp. strains isolated from poultry to antibiotics and to oregano essential oil (*Origanum vulgare* L.), chestnut shell extract (*Castanea sativa*) and grape pomace extract (*Vitis vinifera*). Samples were collected from chickens, hens, and broilers (cloacal swabs) (n=120), poultry meat (n=98) and eggs (n=138). *Salmonella* spp. were isolated by selective enrichment and culture method and confirmed by polymerase chain reaction (PCR) (identification of *hilA* gen). ERIC fingerprinting PCR was used to select strains according to their genetic diversity. Susceptibility of selected strains to antibiotics and plant extracts was determined by disk diffusion method and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by dilution method. The total prevalence of *Salmonella* spp. was 6.53% (23/356) and only meat samples were positive, with a higher prevalence in packaged meat ($P \leq 0.05$). All selected strains were multi-drug resistant (i.e., resistant to three or more classes of antibiotics).**

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Oregano essential oil and chestnut shell extract had antibacterial activity against resistant *Salmonella* spp. isolates. However, grape pomace extract showed no inhibitory activity. Therefore, the use of oregano essential oil and chestnut shell extract at some stages of the poultry production chain should be evaluated in future studies to identify potential solutions for the industry.

Keywords: Antimicrobial resistance (AMR), poultry chain, essential oils, plant extracts, by-products.

INTRODUCTION

Antimicrobial resistance (AMR) is a global public health threat that caused 1.27 million deaths in 2019. Antibiotic resistance results from the misuse and overuse of antibiotics, causing the death of susceptible bacteria and allowing resistant bacteria to persist and multiply (FAO and WHO, 2023; AGDOH, 2023). Antibiotic-resistant pathogens have a poor response to treatment, leading to complications (Chiș et al., 2022).

The main bacterial agents associated with intestinal infections related to poultry products are *Salmonella* spp. and *E. coli* (López et al., 2021). Contamination of poultry meat with *Salmonella* spp. may occur during processing, handling, marketing, and storage, primarily from fecal origin, and may led to human illnesses such as salmonellosis (López et al., 2021). *Salmonella* spp. live naturally in the gastrointestinal tract of a wide variety of animals and can act as an opportunistic pathogen, causing various infections in humans and animals, including gastroenteritis, typhoid fever mainly in humans, and septicemia (Shen et al., 2020). The control of salmonellosis is a major challenge, especially given the emergence of new serotypes, the diversity of existing serotypes, and the ability of these bacteria to persist in colonized animals and the environment, where they thrive under favorable conditions (Poudel and Adhikari, 2024). *Salmonella* spp. are typically found in soil, water, and the digestive tracts of animals and humans. Such interactions with antibiotics can lead to selective pressure in the environment, facilitating genetic exchange with other microorganisms (Fernandez et al., 2021). Poultry and poultry-related products are known to serve as potential reservoirs for antimicrobial resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) (Moulana and Asgharpour, 2022).

In recent years, significant progress has been made in the search for alternatives to classical antibiotics. Various natural alternatives, such as probiotics, bacteriocins, antioxidants, and plant-derived chemical compounds have been studied in the food and veterinary industries. Among these options, plant extracts have demonstrated benefits, such as their antioxidant and, in some cases, antimicrobial properties (Heredia et al., 2022).

Studies suggest that plant phytochemicals may be a promising alternative against multi-drug resistant (MDR) *Salmonella* spp. These compounds target resistance factors such as efflux pumps, membrane proteins and biofilms (Almuzaini, 2023). In addition, plant phytochemicals have economic benefits by reducing the cost of synthesis and purification of antimicrobial compounds. Their versatility in acting against multiple bacterial targets may also delay the emergence of resistance (Khare et al., 2021).

Some plants can produce phenolic compounds as secondary metabolites in response to environmental stress or as a defense mechanism against pathogens. In addition, upon injury or stress, plants rapidly increase synthesis of these compounds to promote wound healing and repair (Hu et al., 2022). Several types of phytochemicals, including alkaloids, phenolics, coumarins, and terpenes, have been shown in previous studies to be effective inhibitors against MDR pathogens (Suganya et al., 2022).

The safety of poultry products is a major concern. Pre-harvest food safety measures are critical to controlling foodborne pathogens in poultry, reducing the prevalence of flocks contaminated with a specific pathogen and reducing the concentration of a pathogen in contaminated flocks (Pessoa et al., 2021). In addition, the poultry industry faces significant challenges, including the need to ensure the safety of poultry products following the COVID-19 outbreak (Hafez and Attia, 2020). It is also important to educate poultry producers, handlers, and consumers about the potential risks of *Salmonella* spp. transmission and to promote proper hygiene, biosecurity measures, and safe food handling practices (Poudel and Adhikari, 2024).

Therefore, the aim of this study was to determine the prevalence and susceptibility of *Salmonella* spp. strains of poultry origin to antibiotics, and to plant extracts: essential oil (EO) of oregano, chestnut shell extract and grape pomace extract.

MATERIALS AND METHODS

Sampling

A total of 356 samples were collected from the poultry chain (animals, eggs, and meat) between December 2021 and January 2022 in the regions of Ñuble and Biobío, Chile. The sample size (Supplementary Data S1) was determined using equation 1 (Moore, 2007).

$$n \geq \left(\frac{z}{m}\right)^2 \times \hat{p}\left(1-\hat{p}\right) \quad (1)$$

Where n is the sample size, z is the standard value in the normal distribution frequency, m is the margin of error and $\hat{p}$ is the prevalence of antimicrobial resistant (AR) pathogens obtained from other studies. The confidence level of the interval is 95%, with z=1.96 and m= 0.05 (considering an interval not greater than 10%).

Cloacal swab samples were collected from laying hens and broiler chickens on farms (n = 120, from 3 farms, multiple visits); whole and cut-up chicken meat samples were purchased from supermarkets, butcher shops, and retail stores (n = 98, from 5 stores, multiple visits); and egg surface swab samples were collected on farms and from eggs purchased from supermarkets and retail stores (n = 138, from 6 stores and 2 farms, multiple visits). Legal and ethical approval was obtained from the Ethics, Bioethics and Biosafety Committee of the Universidad de Concepción, Chile, prior to sampling (Supplementary Data S2). Samples were transported at 4 °C and analyzed immediately.

Salmonella isolation

Isolation of *Salmonella* spp. was performed using selective enrichment and culture methods. Briefly, swab samples were immersed in 10 mL of sterile buffered peptone water (BPW), and meat samples (25 g) with BPW (225 mL) were placed in a sterile filter bag and homogenized using a laboratory blender (BagMixer400 P; Interscience, St. Nom, France) at 8 strokes per second for 90 seconds. The enrichment was incubated at 35 ± 2 °C for 24 h. After incubation, 0.1 mL of the enrichment was inoculated into 10 mL Rappaport-Vassiliadis (RVB) selective broth and incubated at 35 ± 2 °C for 24 h. A loopful was then plated on xylose-xysine-deoxycholate agar (XLD) and incubated at 35 ± 2 °C for 48 h. Presumptive positive colonies (black color with red halo) were selected and streaked onto trypticase soy agar (TSA), and incubated at 35 ± 2 °C for 24 h. Presumptive *Salmonella* spp. colonies were stored at -80 °C in cryotubes containing brain heart infusion (BHI) broth with 20% glycerol.

DNA extraction

DNA from presumptive *Salmonella* spp. strains was extracted using the boiling the method proposed by Ngamwongsatit et al. (2008). Briefly, each strain was plated on TSA and incubated at 35 ± 2 °C for 24 h. Then, 1 to 3 colonies were inoculated into 1 mL of trypticase soy broth (TSB) and centrifuged at 5,000 × g for 2 min (Prism R™ Refrigerated MicroCentrifuge; Labnet; USA). The pellet was resuspended in 500 µL of DNA/RNAase-free H_2O and centrifuged at 5,000 x *g* for 2 min. The pellet was then resuspended in 100 µL of DNA/RNAase-free H_2O and kept at 100 °C for 10 min (AccuBlock™ digital dry baths; Labnet; USA) followed by centrifugation at 1,000 × g for 5 min. The resulting supernatant was stored at -20 °C. DNA concentration was adjusted to A260/A280 = 1.8 – 2.0 (10 – 100 ng mL^{-1}).

Identification/confirmation of *Salmonella*

The *hilA* gene (transcriptional activator of invasion genes, 784 bp) was identified by PCR to confirm *Salmonella* spp. The primers (sequences) were *hilA* 1 (5′ CGG AAC GTT ATT TGC GCC ATG CTG AGG TAG 3′) and *hilA* 2 (5′ GCA TGG ATC CCC GCC GGC GAG ATT GTG 3′). PCR reactions were performed in a volume of 25 µL containing 2 µL of DNA template (<250 ng), 8.6 µL 0.7X GoTaq Master Mix (200 µM dNTPs, 3 mM MgCl2), 1 µL primers (0.5 µM) (Integrated DNA Technologies Inc., Coralville, IA, USA). PCR conditions were adjusted according to the protocol described by Velásquez et al. (2018) using a thermocycler (Labnet™ Thermocycler MultiGene™ OptiMax TC9610-230, Hamburg, Germany). Subsequently, 10 µL of the resulting products were loaded onto 1.5% agarose gels in 1X TAE with 10 µL of SafeView as a DNA-intercalating dye. A 100-bp molecular weight marker ladder (Maestrogen, Inc., Las Vegas, NV, USA) was included in each gel.

Positive control-*Salmonella* ATCC 13076, negative control-*S. aureus* ATCC 25923 were included. Electrophoresis was carried out at 100 V in TAE 1X for 1.5 h. Bands were visualized using a UV transilluminator at 312 nm (BIOTOP TU1002; Shangai Bio-Tech Co. Ltd. Shangai, China).

Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC- PCR) Fingerprinting for *Salmonella*

The genetic diversity of the confirmed *Salmonella* spp. isolates was assessed at the strain level by ERIC-PCR molecular fingerprinting using the primers Eric1R and Eric2F (ERIC1R: 5′ ATGTAAGCTCCTGGGGATTCAC 3′; ERIC2F: 5′ AAGTAAGTGACTGGGGTGAGCG 3′) (Versalovic et al., 1991). *Salmonella enterica* subsp.

enterica ATCC 13076 was used as a positive control, and *Staphylococcus aureus* ATCC 43300 as a negative control. Genomic DNA of the strains was obtained using the E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek) according to the manufacturer's instructions.

The ERIC PCR mix consisted of 2.0 µL of 50 ng DNA template, 1.25 µL of forward and reverse primers 10 µM, 12.5 µL of GoTaq® Colorless Master Mix (Promega), and 9.5 µL of nuclease free grade water (Promega) for a total of 25 µL. The reaction mixture was initially held at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 25 °C for 1 min, and extension at 72 °C for 4 min, followed by a final hold at 72 °C for 5 min (Lamas et al., 2016).

The PCR products were electrophoresed on 2% (w/v) agarose gels after standardization of the DNA content for each sample. Each lane was loaded with a mixture of 15 µL of PCR product and 3 µL of loading dye, supplemented with SafeView Plus DNA gel stain 20,000X (Fermelo Biotec). The marker used was 100 bp Plus DNA Ladder (Maestrogen). Electrophoresis was carried out in tanks buffered with 1xTAE (40mM Tris-Acetate, 1mM EDTA, pH 8.0) at 100V for 3 h. Bands were visualized in a UV transilluminator at 320 nm.

The banding patterns were analyzed for the presence of PCR products of specific molecular sizes, as described by Gerding et al. (2013). A binary matrix was constructed with a score of 1 or 0 for the presence or absence of a band at each molecular size. The matrix was then analyzed using AFPL SURV version 1.0 (Vekemans et al., 2002) to calculate the genetic distance between isolates. The distance matrix was then subjected to unweighted pair group method with arithmetic mean (UPGMA) cluster analysis using the NEIGHBOR application of the PHYLIP software package. The cladograms were visualized in MEGA7 (Kumar et al., 2016), and strains were selected according to their genetic diversity (Supplementary Data S3).

Susceptibility to antibiotics

Selected *Salmonella* spp. strains were subjected to antibiotic susceptibility testing using the disk diffusion method. Briefly, 0.1 mL of each bacterial solution (0.85% saline NaCl solution, McFarland $0.5 \approx 10^8$ CFU mL^{-1}) was plated on Mueller-Hinton agar (MHA) using a sterile cotton swab. After the discs were placed on the agar surface, the samples were incubated at 35 ± 2 °C for 24 h. The Clinical and Laboratory Standard Institute (CLSI, 2020) criteria were used for interpretation of the results.

Seven antibiotics from seven different classes were tested: ampicillin, ceftaroline, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, and oxitetracycline.

Characterization of plant extracts

The following extracts were evaluated: oregano essential oil (EO) (carvacrol chemotype, 99.9% carvacrol; R.C. Treatt & Co Ltd, Suffolk, UK), chestnut shell extract (chestnut shell from El Carmen, Ñuble region, Chile), grape pomace extract (Carmenere, Merlot and Syrah from the Maule region, Chile). Quantification of total phenolic compounds in chestnut shell and grape pomace extracts was performed according to Singleton et al. (1999), a modification of the Folin-Ciocalteu method. In this procedure, the Folin-Ciocalteu reagent and a 20% (w/v) sodium carbonate solution were used. The absorbance of the samples was measured at 765 nm using a microplate multilector. The results were expressed as gallic acid equivalents per gram of extract (GAE g^{-1}).

Susceptibility to plant extracts

Concentrations of plant extracts were adjusted according to preliminary tests. Oregano EO was prepared in absolute ethanol (99.8%; Merck, Darmstadt, Germany) to obtain a concentration of 5.0%. Chestnut shell extract was prepared with absolute ethanol to obtain a concentration of 40%. Grape pomace extract was tested using an ethanol:distilled water concentrations at a ratio of 60:40 v/v. Ethanol (Emsure®, Merck) was used as a control.

Plant extracts were sterilized using a 0.22 µm filter (EDLAB CA Syringe Filter). The antimicrobial activity of these products was evaluated by the disk diffusion method using sterile paper discs (Whatman No. 1, 6 mm diameter) according to the procedure described by Rota et al. (2008).

Bacterial suspensions were prepared in trypticase soy broth (TSB) with an optical density (OD) of 0.1 (OD_{600} = 0.1) measured in a UV/VIS spectrophotometer (BioTek Epoch Microplate Spectrophotometer, Epoch Life Science, Inc., USA). A volume of 0.1 mL of this suspension was then inoculated onto TSA plates. Sterile discs impregnated with 15 µL of each plant extract solution were placed on the inoculated plates and incubated at 35 ± 2 °C for 24 h.

Inhibition criteria (including disc diameter) were as follows: an inhibition zone ≥20 mm indicated strong inhibition; between <20 and 12 mm, moderate/slight inhibition; and <12 mm, no inhibition (Rota et al., 2008). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the dilution method according to Rota et al. (2008).

Isolates were inoculated onto TSA agar plates and incubated at approximately 35 ± 2 °C for 24 h. The colonies were then transferred to trypticase soy broth (TSB) in 2 mL Eppendorf tubes (OD_{600}=0.1). An aliquot of this solution was inoculated into serial dilutions of each plant extract in triplicate. Ethanol and distilled water were used as controls to validate the results. After inoculation, the plates were incubated at 35 ± 2°C for 24 h. After incubation, the OD was read and samples with an OD equal to or greater than the initial OD were selected and inoculated onto TSA agar plates and incubated at 35 ± 2 °C for 24 h to allow growth in triplicate.

Statistical analysis

The chi-square method or Fisher's exact test was used to determine the differences in the prevalence of *Salmonella* spp. between different types of samples from the poultry production chain ($P \leq 0.05$) (Moore, 2007). The sizes of the inhibition zones were evaluated using descriptive statistics.

RESULTS AND DISCUSSION

Table 1 shows the prevalence of *Salmonella* spp. strains isolated from the poultry production chain. The total prevalence of *Salmonella* spp. was 6.53% (23/356). *Salmonella* spp. positive samples were found only in meat samples, in packaged meat (n=22) and in unpackaged meat (n=1). These results highlight the presence of *Salmonella* spp. in the poultry chain, which is a major food safety and public health concern. Contamination of packaged meat may have occurred at prepackaging stages, such as in farms from animals, during slaughtering, meat handling or due to inadequate storage and transportation conditions. During handling, meat can be exposed to various sources of contamination, such as contaminated equipment, surfaces, or food handlers. In addition, if proper hygiene and temperature control conditions are not maintained during storage and transportation of products, there is a significant risk of bacterial proliferation. These inadequate conditions could allow pathogens to contaminate meat.

There are local and international regulations that guarantee food safety in poultry production. According to the Chilean Food Safety Regulations, DS Nº 977/96 (Ministerio de Salud, 2016), a maximum total plate count (TPC) of up to 10^7 colony forming units per gram (CFU g^{-1}) is allowed in raw poultry meat. However, existing regulations do not specifically address the presence of different types of strains, including those that are antibiotic-resistant or capable of producing toxins. FAO and WHO (2023) suggested that no single measure is sufficient to effectively control *Salmonella* spp. in broilers and poultry meat, and emphasized the importance of multi-faceted control strategies. These strategies, supported by the revised Codex guidelines (CXG 78-2011), are key to ensuring the safety of poultry products.

Several studies have identified *Salmonella* spp. in different types of food. For instance, a study conducted by Corredor et al. (2021) reported the prevalence of *Salmonella* spp. as 0.1% in fruits, 0.2% in leafy vegetables, 13.7% in ready-to-eat (RTE) salad-related mixed vegetables, 0.1% in root vegetables, 0% in tomatoes, and 17.41% in chicken. Fourteen different serotypes of *Salmonella* spp. were identified in chicken meat, with the most common being *S. paratyphi*, *Salmonella enterica*

Table 1. Prevalence of *Salmonella* spp. isolated from poultry production chain.

Type of sample	No. of samples	Positive samples of *Salmonella* (prevalence)
Animals	120	0
Conventional	50	0
Cage-free	70	0
Meat	98	23 (23.47%)
Packaged	73	22 (30.14%) a
Non-packaged	25	1 (4.00%) b
Eggs	138	0
Conventional	102	0
Cage-free	36	0
Total	356	23 (6.53 %)

Different letters in the same type of sample indicate significant differences (Chi-square / Fisher tests, $P \leq 0.05$).

serovar Hvittingfoss, and *S. muenster* (López et al., 2023). Additionally, a study conducted in Bogota, Colombia, reported a prevalence of *Salmonella* spp. of 9.4% in eggs. A significant variability in the prevalence of *Salmonella* in eggs has been observed, with studies from countries such as Uruguay and India documenting values ranging from 5.5 to 9.4% (Castañeda et al., 2017). In the present study, the absence of *Salmonella* spp. on eggshells may be attributed to the fact that *Salmonella* spp. are not well adapted to dry environments (Shaji et al., 2023), which could limit their survival on eggshells. Moreover, eggs have natural antimicrobial compounds on their surface that may inhibit the growth of *Salmonella* spp. (Whiley and Ross, 2015; Ricke et al., 2018). As *Salmonella* spp. can cause disease in chickens, strict management protocols should be implemented on farms (Shaji et al., 2023). It is important to note that *Salmonella* spp. were not detected in the cloaca of the animals in this study. *Salmonella* spp. can colonize various parts of the gastrointestinal tract of birds, including more internal sections, and the cloaca is considered a representative sampling site for the detection of *Salmonella* spp. in birds. This is because it serves as one of the main routes of excretion of the bacterium. Therefore, the absence of *Salmonella* spp. in hens and chickens can be attributed to effective management measures implemented in these farms to control this pathogen.

Three *Salmonella* spp. strains were selected according to the genetic diversity determined by ERIC fingerprinting (Supplementary Data S3). These strains detected in meat samples were MDR, i.e., resistant to three or more types of antibiotics (Table 2). Other studies have also identified the presence of MDR *Salmonella* strains in the poultry production chain. Significant rates of MDR were detected in *Salmonella* spp. isolated from chickens, reaching 81.1%. This resistance included sulfisoxazole (76.1%), tetracycline (75.3%), ampicillin (48.0%), and ofloxacin (44.7%) (Castro et al., 2020). Similarly, a study conducted in Ethiopia found that 96.77% of *Salmonella* spp. strains isolated from poultry products were AMR (Asefa and Duga, 2022). These findings confirm the concerning prevalence of MDR observed in *Salmonella* spp. isolated from chickens (Rodríguez, 2019).

Some plant-derived alternatives could be used to control ARB in the food chain. The compound with the highest concentration in oregano EO was carvacrol (99.9%). Chestnut shell extract had a concentration of phenolic compounds of 181.93 ± 13.10 mg GAE g^{-1}. A significantly higher concentration was found in grape pomace extracts, Cabernet Sauvignon extract had the highest content of phenolic compounds (316.97 ± 22.57 mg GAE g^{-1}), followed by Carmenere (280.31 ± 8.84 mg GAE g^{-1}) and Merlot (259.75 ± 5.74 mg GAE g^{-1}). Oregano EO showed inhibitory activity against all *Salmonella* spp. strains with halo diameters ≥20 mm, which was classified as strong inhibitory activity. Chestnut shell extract showed moderate inhibitory activity against two *Salmonella* spp. strains. Grape pomace extracts showed no biological activity against *Salmonella* spp. (Table 2).

Oregano EO contains mainly carvacrol and thymol, with other compounds such as linalool, p-cymene and γ-terpinene in smaller amounts (Sakkas and Papadopoulou, 2017; Walasek-Janusz et al., 2023). In the present study, a carvacrol chemotype of oregano EO was used.

Leyva-López et al. (2017) found that the strong antimicrobial activity of oregano EO against pathogenic bacteria is due to the abundance of phenolic compounds, including carvacrol, eugenol and thymol. In this study, the MIC and MBC of oregano EO were 0.015 µg mL^{-1} in all MDR *Salmonella* spp. strains (Table 3), which is lower than the MIC and MBC values of 0.250 mg mL^{-1} and 0.300 mg mL^{-1} reported by Luna-Solorza et al. (2023) and 0.125 mg mL^{-1} reported by Walasek-Janusz et al. (2023) in *Salmonella typhimurium* strains. Liu et al. (2022) explain that carvacrol and thymol functional groups influence

Table 2. Antibiotic-resistance profile and inhibitory activity (cm) of plant extracts against *Salmonella* spp. strains from poultry production chain.

			Inhibition halo diameter (mm)	
Strains	**Origin**	**Resistance profile**	**Oregano EO**	**Chestnut shell extract**
1 (meat)	Packaged	CEF-AMP-CIP-TET-CLO	≥20	Not observed
2 (meat)	Packaged	CEF-TSX-AMP-CIP-TET-CLO	≥20	9.69 ± 1.87
3 (meat)	Non-packaged	CEF-TSX-AMP-TET-CLO	≥20	8.39 ± 1.09

CEF - Ceftaroline, TSX - Trimethoprim sulfamethoxazole, AMP - Ampicillin, CIP – Ciprofloxacin, TET - Tetracycline, CLO - Chloramphenicol.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts in *Salmonella* spp. strains from poultry production chain.

	MIC		MBC	
Strains	Oregano EO (μg mL^{-1})	Chestnut shell extract (mg mL^{-1})	Oregano EO (mg mL^{-1})	Chestnut shell extract (mg mL^{-1})
1 (meat)	0.015	75.00	0.015	150
2 (meat)	0.015	75.00	0.015	75.0
3 (meat)	0.015	37.50	0.015	75.0

hydrophobicity and antimicrobial activity by altering cell membrane permeability, thereby inducing cell death. These findings support the understanding of how specific components of oregano EO contribute to the antimicrobial activity determined in this study.

The circular economy is fundamental to achieving sustainable agro-industrial waste management. In this context, the use of chestnut shell, an agro-industrial by-product, for the extraction of antimicrobial compounds exemplifies how waste can be reused in accordance with the principles of the circular economy (Khanna et al., 2022). Chestnut (*Castanea sativa*) shells contain bioactive molecules such as phenolics, flavonoids, triterpenes, sugars, and tannins. The brown pigment in these shells has antioxidant, antibacterial, and anti-inflammatory properties, making it a valuable source of bioactive compounds (Shao et al., 2023). Chestnut shell extract has been reported to have antimicrobial activity against various bacterial species, including both Gram-positive and Gram-negative foodborne bacteria. Sensitive strains include *Staphylococcus aureus*, *Bacillus cereus* and *S. typhimurium* (Lee et al., 2016). Other authors, such as Rodrigues et al. (2023), reported MIC values ranging from 1.25 to 10 mg mL^{-1} and MBC of >10 mg mL^{-1} in *Salmonella enterica*. Ciríaco et al. (2023) determined MIC values between 0.015 and 0.021 mg mL^{-1} in *Staphylococcus aureus*, *Enterococcus faecalis* and *Listeria monocytogenes*, with no effect against *Salmonella* spp. In this study, MIC values were higher, ranging from 37.5 to 75 mg mL^{-1} and MBC ranged from 75 to 150 mg mL^{-1} (Table 3). These results suggest a potential of chestnut shell extract as an antimicrobial agent. The presence of phenolic acids in chestnut shells may be responsible for these observed antimicrobial effects. Previous studies have shown that phenolic acids, particularly p-coumaric acid, have antibacterial properties against Gram-negative bacteria. Mechanisms such as DNA binding or modification of cell membrane permeability have been identified as possible contributors to this antibacterial activity (Silva et al., 2020). These findings support existing evidence and underscore the promising ability of chestnut shell extract against Gram-negative bacteria.

Despite the presence of phenolic compounds in grape pomace extract, no antimicrobial effect against *Salmonella* spp. was observed. This viticultural by-product is characterized as a rich source of flavonoid phenolics, such as anthocyanins, flavanols, flavonols, and tannins, as well as non-flavonoid phenolics, including phenolic acids, according to the classification proposed by Ramirez and DeWitt (2014). Although the literature highlights the antibacterial potential of grape pomace extracts against various foodborne pathogenic bacteria (Sanhueza et al., 2014), several studies indicate a lack of biological activity against Gram-negative bacteria. For example, Filocamo et al. (2015) demonstrated the efficacy of white grape juice extracts against various Gram-positive bacteria; however, these extracts did not affect the growth of Gram-negative bacteria, including *Salmonella typhi*. Similar results were reported by Xu et al. (2015) when evaluating the antimicrobial activity of grape pomace. All extracts showed antibacterial activity against *Listeria monocytogenes* and *Staphylococcus aureus* (Gram-positive bacteria), but no antibacterial activity was detected against *E. coli* O157:H7 and *S. typhimurium* (Gram-negative bacteria). This may be due to the cell wall composition of Gram-negative bacteria as they have an additional protective outer membrane that may make them more resistant to phenolic compounds (Tirado et al., 2021). In fact, phenolic compounds have membrane-active properties against bacteria, causing leakage of cellular components, including nucleic acids, proteins, and inorganic ions such as potassium or phosphate (Lobiuc et al., 2023). However, this activity may be less effective in Gram-negative bacteria due to the presence of lipopolysaccharides in their membrane, which may repel or slow the interaction with polyphenols (Tirado et al., 2021). The complex composition of grape pomace

and the variability in the distribution of these antimicrobial compounds among parts of the pomace, such as skin, seeds and blends, makes it difficult to understand the precise mechanism of microbial inhibition (Hassan et al., 2019).

CONCLUSIONS

Salmonella spp. and multidrug-resistant (MDR) strains are present in the poultry production chain, primarily in raw chicken meat in this study.

Plant extracts have an antimicrobial activity against MDR *Salmonella* spp. isolated from poultry meat, such as oregano essential oil with a high inhibitory activity (MIC/MBC = 0.015 µg mL^{-1}) and chestnut shell extract with a moderate inhibitory activity on some strains (MIC = 37.5 – 75 mg mL^{-1}, MBC = 75 – 150 mg mL^{-1}).

Conflict of interest

No conflict of interest declared.

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Author Contributions

The authors declare active participation in the bibliographic review: Franco Guzmán, Valeria Velasco and Ana María Bonilla; in the development of the methodology: Franco Guzmán, Valeria Velasco, Ana María Bonilla, Felipe Medina, Macarena Gerding, Christian Folch and Pía Oyarzúa; in the discussion of the results: Franco Guzmán, Valeria Velasco, Pamela Williams, Macarena Gerding and Christian Folch; in review and approval of the final version of the article: Valeria Velasco and Ana María Bonilla.

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