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Research Article

Antimicrobial Effects of Medicinal Plant Species on *Salmonella typhimurium* Strains Isolated from Poultry Feces Samples

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Abstract

Background: It has been proven that plant extracts show great promise in fighting pathogenic microorganisms. This study aimed to evaluate the resistance of 20 strains of *Salmonella typhimurium* extracted from poultry feces against conventional antibiotics and the antibacterial activity of 10 medicinal plant extracts, including *Hibiscus sabdariffa* L., *Capparis spinosa* L., *Azadirachta indica* A. Juss., *Eryngium planum* L., *Rumex acetosa* L., *Calotropis procera* (Aiton) Dryand, *Psidium guajava* L., *Malva sylvestris* L., *Urtica dioica* L., and *Alcea setosa* Alef., against the extracted strains.
Methods: The susceptibility of *S. typhimurium* strains against tested antibiotics was determined using disk diffusion, and the antibacterial activity of medicinal plant extracts was evaluated using well diffusion and broth microdilution assays.
Results: The extracted *S. typhimurium* strains showed high resistance to cephalosporin (100%) and gentamicin (40%); however, all plant extracts examined in this study were influential in inhibiting the growth of the tested strains. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tested plant extracts ranged from 6.25 to 25 mg/mL and 12.5 to 50 mg/mL, respectively. The most effective plant extracts in inhibiting bacterial growth in the agar well diffusion method were *P. guajava, H. sabdariffa*, and *A. setosa*; nevertheless, the most potent bactericidal activity was recorded for *M. sylvestris* and *A. setosa* in the broth microdilution method. The examined strains showed 80% and 85% sensitivity to the MBC of alcoholic extracts of *M. sylvestris* and *A. setosa* (50 mg/mL), respectively, which is worthy of further exploration by scientists.
Conclusions: The results of this study represent the high potency of *M. sylvestris* and *A. setosa* extracts as appropriate medicinal and/or food supplements to replace ineffective antibiotics in bird breeding.

Keywords: Antibacterial Agent, Antibiotic Resistance, Plant Extract, *Salmonella typhimurium*

1. Background

Antibiotics are artificially or naturally synthesized organic substances that have been used for over 70 years in a wide variety of fields, including industrial production, agriculture, and medicine. Antibiotic use is the primary cause of the emergence of antibiotic-resistant microorganisms. Resistance to antibiotics is increasingly happening both in benign and pathogenic bacteria, giving rise to a growing global concern for humans, animals, and environmental health (1, 2). Antibiotic resistance is becoming more prevalent across various antibiotic classes, and some scholars argue that the threat and cost of antibiotic resistance are comparable to that of climate change (3). Global warming and antimicrobial resistance are closely intertwined; accordingly, rising temperatures raise the growth rate of bacteria and infections and horizontal gene transfer, which is a significant factor in the occurrence of antibiotic resistance (4-6).

Salmonella, named after the veterinarian Daniel Elmer Salmon, is a non-sporing Gram-negative bacillus genus in the family *Enterobacteriaceae*. *Salmonella* contains two species of *Salmonella enterica* and *Salmonella bongori*, with *S. enterica* being further divided into six additional subspecies and more than 2,600 serotypes (7). *Salmonella* species are abundantly represented in the environment and can cause a wide range of illnesses in both humans and animals. Generally, infection occurs via the ingestion of foods or water contaminated with the feces of infected humans or animals (7-9). The emergence of *Salmonella* serotypes resistant to multiple antibiotics is a major public health concern due to the remarkable food safety hazard that can happen (10). *Salmonella* is also able to form biofilms on a variety of biotic and abiotic surfaces, which might explain its survival in food production processes and clinical settings (11, 12).

Plant extracts and phytochemicals are considered promising new antimicrobial agents due to several reasons. They are cost-effective and readily available with no or negligible side effects in proper dosage. They also show great structural diversity and are less prone to produce antibiotic resistance than synthetic antibiotics. On the other hand, plant-based antimicrobial agents, unlike synthesized chemicals that pollute soil and water and move through ecological food chains, are environmentally friendly. Plants' secondary metabolites generally kill the bacteria by disruption of the cell envelope, metabolism, or intracellular communication (12, 13).

2. Objectives

Considering the above-mentioned issues, this study aimed primarily to determine the antibiotic resistance of *Salmonella typhimurium* isolates extracted from poultry feces in the Zabol region, Sistan and Baluchestan province, Iran, and evaluate the in vitro antimicrobial properties of 10 medicinal plant species on the *S. typhimurium* isolates.

3. Methods

3.1. Plant Sample Preparation

Plant organs of *Hibiscus sabdariffa* L., *Capparis spinosa* L., *Azadirachta indica* A. Juss., *Eryngium planum* L., *Rumex acetosa* L., *Calotropis procera* (Aiton) Dryand, *Psidium guajava* L., *Malva sylvestris* L., *Urtica dioica* L., and *Alcea setosa* Alef. were collected in 2021 from the Baqiyatallah Al-Azam Educational and Recreational Complex, belonging to the University of Zabol, Zabol, Iran (Table 1). The plant species were identified by the first author, a botanist at the Department of Biology of the University of Zabol, using regional and online floras. Additionally, the voucher specimens of each species were deposited at the University of Zabol Herbarium, Zabol, Iran. The plant materials were dried in a shade away from direct light before converting to a fine powder using an electric mill. The scientific, English, and local names, distribution ranges, and some characteristics of the studied plant species are presented in Table 1.

3.2. Preparation of Plant Extracts and Salmonella typhimurium Strains

In this study, 10 grams of each plant powder was soaked in 100 mL of 96% ethanol and mixed with a shaker machine (Azma Pars, Iran) at a speed of 130 rpm for 24 hours at room temperature. The obtained solution was filtered through Whatman No. 2 filter paper, and the filtrate was dried using a rotary device (Heidolph, Germany) and a vacuum pump (distillation in a vacuum) and stored in the dark at 4°C until needed for experiments. The dried extract was then dissolved in dimethylsulfoxide (DMSO) (10%) to prepare a stock solution at a concentration of 200 mg/mL from each plant extract, from which desired concentrations were made (14). In the Agar well diffusion method, the extract concentration of 50 mg/mL was used. For calculating minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), the extract concentration in the first row of the 96 well-plate was 200 mg/mL, which was subsequently diluted serially into 100, 50, 25, 12.5, 6.25, and finally 3.125 mg/mL in the last row. Additionally, 20 pure *Salmonella typhimurium* strains, isolated from fresh poultry feces from Zabol, were obtained from the microbiology section in the Central Laboratory of the University of Zabol.

3.3. Determining the Sensitivity of Bacterial Strains to Conventional Antibiotics

The sensitivity of bacterial strains to tetracycline, gentamicin, cephalosporin, and ciprofloxacin antibiotics was evaluated using the disk diffusion assay following the instructions of Bauer (15). Bacterial suspensions equivalent to a 0.5 McFarland turbidity were made from all bacterial strains in Mueller Hinton broth liquid medium and cultured on Muller Hinton's agar medium. Antibiotic disks were precisely placed at proper distances from one another on the surface of the previously inoculated cultures, followed by incubation for 24 hours at 37°C. The inhibition zones around the discs were measured to determine the resistance and sensitivity of 20 strains of *S. typhimurium* to the applied antibiotics. The absence of inhibition zones around the discs indicated complete resistance of the bacterial strain to the corresponding antibiotic.

3.4. Antibacterial Activity of Plant Extracts Against Salmonella typhimurium Assay

The sensitivity of bacterial isolates to the plant extracts was determined using the broth microdilution method with the help of 96-well microplates. An amount of 10

Table 1. Characteristics of Investigated Medicinal Plants, Including Names, Habitats, Distribution, and Parts Used in This Study

No.	Taxa	Family	Habitat	Local Name	English Name	Distribution	Part Used
1	*Hibiscus sabdariffa* L.	Malvaceae	Annual, up to 2 m tall	Chaye Torsh	Roselle	Native to tropical Africa	Sepals
2	*Capparis spinosa* L.	Capparaceae	Shrubs, prostrate or hanging, up to 100 cm	Kabar	Caper	S. Europe eastward to Australia	Fruit
3	*Azadirachta indica* A. Juss.	Meliaceae	Tree, up to 15 m tall	Derakhte Azad	Neem	Native to the Indian subcontinent	Flower, Fruit, Leaf
4	*Eryngium planum* L.	Apiaceae	Perennial to 1 m	Shishagh	Blue eryngo	Europe and Central Asia	Leaf
5	*Rumex acetosa* L.	Polygonaceae	Perennial up to 120 cm high	Torshak	Sorrel	Eurasia	Leaf
6	*Calotropis procera* (Aiton) Dryand.	Apocynaceae	Subshrubs to 2 m or more high	Estabragh	Sodom apple	Native to dry tropical Asia and Africa	Flower
7	*Psidium guajava* L.	Myrtaceae	Tree	Govava	Common guava	Native to the Caribbean, Central, and South America	Fruit
8	*Malva sylvestris* L.	Malvaceae	Biannual up to 1.5 m	Panirak	Common mallow	Eurasia and Africa	Leaf
9	*Urtica dioica* L.	Urticaceae	Perennial herb 50-150 cm	Gazaneh	Common nettle	Europe, Asia, and North Africa	Leaf
10	*Alcea setosa* Alef.	Malvaceae	Perennial up to 2 m	Khatmi	Bristly hollyhock	Western Asia	Flower

microliters of 0.5 McFarland microbial suspension was added to Mueller Hinton broth nutrient liquid medium (MHB) and incubated at 37°C for 24 hours. Bacterial growth or inhibition was determined by the visual evaluation of turbidity, and the lowest concentration of the plant extract that inhibited the bacterial growth was considered the MIC. For the determination of MBC, 10 μL of the content of each clear well was transferred to Mueller Hinton agar medium and incubated for 24 hours at 37°C. The plant extract concentration (corresponding well) at which 99.9% of the bacteria were eliminated was regarded as the MBC.

3.5. Agar Well Diffusion Method

The entire surface of the Mueller Hinton agar culture medium was inoculated by 50 microliters of 0.5 McFarland concentration of bacterial suspension. Wells of 5 $\times$ 4 mm were created on the medium, and 50 μL of each extract solution (the concentration of 50 mg/mL) was added to the wells (16). Then, the plates were kept at 37°C for 24 hours, and the inhibition zone of the extracts was measured.

3.6. Statistical Analyses

All tests were performed in three repetitions. The data obtained from the agar well diffusion method were analyzed statistically using two-way analysis of variance (ANOVA) and Tukey post hoc tests at a significance level of 0.05 via SPSS statistical software (version 16).

4. Results

The in vitro evaluation of the efficacy of tested antibiotics in *S. typhimurium* strains showed the highest antibiotic resistance to cephalosporin (100%), followed by gentamicin (40%), tetracycline (20%), and ciprofloxacin (5%) respectively; however, the most sensitivity was observed to ciprofloxacin (90%) and tetracycline (70%) (Table 2).

The two-way ANOVA analysis indicated that different plant species showed a significant impact on the inhibition zone diameter (f = 5.760, P = 0.000); nevertheless, the bacterial strains and species bacterial interaction revealed no significant differences (P = 0.378 and P = 0.078, respectively). To look for differences between the groups, a Tukey post hoc test was run with a total alpha of 0.05. The pairwise comparisons revealed significant differences between *P. guajava* and all other species except for *A. setosa*. Additionally, *A. setosa* was significantly effective in inhibiting bacterial growth in comparison to *C. spinose* (P = 0.003). There were no significant differences among other species based on the pairwise comparisons using the Tukey post hoc test.

The results of the antibacterial assay of ethanolic extracts of tested plant species on 20 isolated strains of *S. typhimurium* based on the agar well diffusion method are summarized in Table 3. All alcoholic plant extracts were more or less influential in inhibiting the growth of the tested strains except for *C. procera*, which was ineffective

Table 2. Percentage of Sensitivity and Resistance of *Salmonella typhimurium* Strains to Tested Antibiotics

Resistance Level	Tetracycline	Gentamicin	Cephalosporin	Ciprofloxacin
Sensitive	70	35	0	90
Intermediate	10	25	0	5
Resistant	20	40	100	5

Table 3. Average Diameter of Inhibition Zone (mm) and Corresponding Standard Deviation of Ethanolic Extracts of Investigated Medicinal Plants Against 20 Strains of *Salmonella typhimurium* Based on the Agar Well Diffusion Method

Strain	*H. sabdariffa*	*C. spinose*	*A. indica* flower	*A. indica* leaf	*A. indica* fruit	*E. planum*	*R. acetosa*	*C. procera.*	*P. guajava*	*M. sylvestris*	*U. dioica*	*A. setosa*
1	5 ± 0.7	1 ± 0.2	1 ± 0.1	2 ± 0.4	1 ± 0.1	3 ± 0.5	4 ± 0.5	0 ± 0.0	4 ± 0.6	1 ± 0.2	1 ± 0.1	5 ± 0.7
2	4 ± 0.5	2 ± 0.3	3 ± 0.4	2 ± 0.2	1 ± 0.3	2 ± 0.3	1 ± 0.1	7 ± 0.6	5 ± 0.5	1 ± 0.1	3 ± 0.4	3 ± 0.4
3	8 ± 0.5	3 ± 0.3	3 ± 0.4	1 ± 0.3	1 ± 0.3	2 ± 0.3	5 ± 0.7	0 ± 0.0	6 ± 0.5	3 ± 0.4	3 ± 0.4	4 ± 0.6
4	3 ± 0.6	1 ± 0.1	3 ± 0.4	1 ± 0.3	1 ± 0.1	1 ± 0.3	6 ± 0.5	5 ± 0.7	8 ± 0.6	2 ± 0.2	3 ± 0.5	5 ± 0.7
5	2 ± 0.2	1 ± 0.2	3 ± 0.4	1 ± 0.3	1 ± 0.1	3 ± 0.4	6 ± 0.6	4 ± 0.4	7 ± 0.6	1 ± 0.1	2 ± 0.3	13 ± 1.1
6	2 ± 0.2	1 ± 0.2	1 ± 0.3	3 ± 0.6	1 ± 0.2	4 ± 0.6	2 ± 0.5	1 ± 0.1	5 ± 0.5	1 ± 0.2	1 ± 0.2	7 ± 0.8
7	3 ± 0.6	1 ± 0.3	1 ± 0.3	1 ± 0.1	1 ± 0.2	1 ± 0.3	3 ± 0.6	1 ± 0.2	6 ± 0.4	1 ± 0.2	2 ± 0.4	1 ± 0.2
8	6 ± 0.6	1 ± 0.2	3 ± 0.4	1 ± 0.3	1 ± 0.1	2 ± 0.3	2 ± 0.3	1 ± 0.1	5 ± 0.7	2	3 ± 0.5	4 ± 0.6
9	7 ± 0.6	1 ± 0.1	3 ± 0.4	2 ± 0.3	1 ± 0.3	4 ± 0.6	5 ± 0.7	1 ± 0.2	6 ± 0.6	3 ± 0.6	2 ± 0.3	1 ± 0.1
10	10 ± 1.1	1 ± 0.3	5 ± 0.7	1 ± 0.2	1 ± 0.1	2 ± 0.3	2 ± 0.3	1 ± 0.1	8 ± 0.6	5 ± 0.6	2 ± 0.3	4 ± 0.5
11	5 ± 0.7	1 ± 0.1	3 ± 0.5	1 ± 0.2	1 ± 0.2	1 ± 0.2	2 ± 0.2	3 ± 0.5	5 ± 0.7	1 ± 0.1	1 ± 0.3	1 ± 0.1
12	3 ± 0.6	1 ± 0.2	1 ± 0.3	2 ± 0.4	1 ± 0.3	2 ± 0.5	1 ± 0.3	1 ± 0.3	7 ± 0.7	2 ± 0.5	2 ± 0.2	4 ± 0.5
13	5 ± 0.6	2 ± 0.2	2 ± 0.3	2 ± 0.2	2 ± 0.4	2 ± 0.2	2 ± 0.2	1 ± 0.3	7 ± 0.6	3 ± 0.6	1 ± 0.3	6 ± 0.5
14	6 ± 0.5	1 ± 0.3	1 ± 0.3	1 ± 0.3	2 ± 0.4	2 ± 0.2	1 ± 0.3	2 ± 0.4	5 ± 0.5	2 ± 0.3	5 ± 0.5	1 ± 0.3
15	2 ± 0.5	3 ± 0.6	3 ± 0.6	1 ± 0.3	5 ± 0.7	1 ± 0.2	3 ± 0.5	1 ± 0.2	8 ± 0.6	2 ± 0.2	4 ± 0.4	3 ± 0.6
16	1 ± 0.3	1 ± 0.3	4 ± 0.6	5 ± 0.5	1 ± 0.3	1 ± 0.2	2 ± 0.4	1 ± 0.2	7 ± 0.5	2 ± 0.3	1 ± 0.1	4 ± 0.4
17	3 ± 0.5	4 ± 0.4	1 ± 0.2	4 ± 0.5	2 ± 0.2	3 ± 0.6	1 ± 0.3	1 ± 0.2	5 ± 0.6	1 ± 0.1	1 ± 0.1	1 ± 0.1
18	5 ± 0.6	2 ± 0.2	1 ± 0.2	3 ± 0.6	1 ± 0.3	4 ± 0.4	2 ± 0.4	1 ± 0.2	3 ± 0.6	3 ± 0.3	4 ± 0.6	4 ± 0.4
19	5 ± 0.6	1 ± 0.2	5 ± 0.6	5 ± 0.6	1 ± 0.3	1 ± 0.3	1 ± 0.3	2 ± 0.4	4 ± 0.5	4 ± 0.6	2 ± 0.3	3 ± 0.6
20	2 ± 0.2	1 ± 0.2	2 ± 0.2	5 ± 0.5	1 ± 0.3	2 ± 0.2	1 ± 0.3	1 ± 0.1	2 ± 0.4	2 ± 0.3	3 ± 0.6	1 ± 0.1

on strains number 1 and 3. However, the plant extracts affected different strains inconsistently. The best result was obtained with *A. setosa* on strain number 5 (13 mm) and *H. sabdariffa* on strain number 10 (10 mm). The most effective plant extract in inhibiting bacterial growth was *P. guajava* (Table 3).

The MIC and MBC of the ethanolic extracts of 10 investigated medicinal plants in 20 isolated strains of *S. typhimurium* from poultry feces are shown in Table 4. The results showed that the lowest and highest inhibitory concentrations of *H. sabdariffa* L. were 12.5 and 100 mg/mL, respectively, where one strain of *S. typhimurium* (strains number 9 and 19) was inhibited by the ethanolic extract of the flowers.

The lowest and highest inhibitory concentrations of *C. spinosa* L. were at 25 and 100 mg/mL, inhibiting 9 and 1 strains in these concentrations, respectively. The lowest inhibitory concentration of *A. indica* flower extract was 6.25 mg/mL, which inhibited one strain growth; nevertheless, its highest inhibitory concentration was 50 mg/mL, in which 12 strains were inhibited. The lowest and highest inhibitory concentrations of *A. indica* leaf extract were recorded at 6.25 and 50 mg/mL, where 1 and 5 strains were inhibited, respectively. On the other hand, the lowest and highest inhibitory concentrations of fruit extract of *A. indica* were observed to be 12.5 and 50 mg/mL, and 1 and 9 strains were inhibited consecutively (Table 4). The *E. planum* leaf extract showed the lowest and highest inhibitory concentrations of 12.5 and 100 mg/mL, in which 3 strains were inhibited. The lowest and highest inhibitory concentrations of *R. acetosa* were also shown to be 12.5 and 100 mg/mL, and the growth of 3 and 6 strains was inhibited accordingly (Table 4).

The lowest and the highest inhibitory concentrations of the alcoholic extracts of both *C. procera* and *P. guajava* were 12.5 and 50 mg/mL; nonetheless, the first species inhibited the growth of 3 and 5, and the second species inhibited 4 and 7 strains serially (Table 4). Table 4 also shows that the lowest and highest inhibitory concentrations were 25 and 50 mg/mL for *M. sylvestris* and *A. setosa*, respectively, where the first species inhibited the growth of 16 and 4 strains, and the latter inhibited

Table 4. Minimum Inhibitory Concentration (MIC, mg/mL) and Minimum Bactericidal Concentration (MBC, mg/mL) of Ethanolic Extracts of 10 Medicinal Plants Against 20 *Salmonella typhimurium* Strains Showing Quantitative Antimicrobial Activity

	H. sabdariffa		*C. spinose*		*A. indica* Flower		*A. indica* Leaf		*A. indica* Fruit		*E. planum*		*R. acetosa*		*C. procera*		*P. guajava*		*M. sylvestris*		*U. dioica*		*A. setosa*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	50	100	25	50	25-50	50	25	50	25	50	25	50	100	200	50	100	25	50	25	50	25	50	25	50
2	50	100	25	50	25-50	50	50	100	50	100	25	50	100	200	25	50	12.5	25	25	50	6.25	12.5	25	50
3	50	100	50	100	50	100	50	100	25	50	25	50	50	100	25	50	25	50	25	50	25	50	25	50
4	50	100	50	100	25-50	50	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100	50	100
5	50	100	50	100	25-50	50	25	50	50	100	25	50	100	200	50	100	50	100	25	50	100	200	25	50
6	50	100	25	50	25-50	50	25	50	50	100	12.5	25	50	100	12.5	25	25	50	25	50	25	50	25	50
7	50	100	50	100	50-100	100	25	50	25	50	25	50	100	200	25	50	25	50	25	50	25	50	25	50
8	25	50	25	50	50	100	50	100	25	50	25	50	50	100	25	50	50	100	25	50	25	50	25	50
9	100	200	25	50	50	100	25	50	25	50	25	50	100	200	25	50	50	100	25	50	25	50	25	50
10	50	100	50	100	50	100	25	50	50	100	25	50	50	100	50	100	25	50	50	100	50	100	50	100
11	25	50	50	100	50	100	25	50	50	100	100	200	100	200	50	100	25	50	50	100	50	100	25	50
12	25	50	50	100	50	100	50	100	25	50	25	50	50	100	25	50	25	50	25	50	50	100	50	100
13	25	50	25	50	50	100	12.5	25	50	100	100	200	25	50	12.5	25	12.5	25	25	50	50	100	25	50
14	25	50	25	50	6.25	12.5	25	50	25	50	25	50	50	100	25	50	12.5	25	25	50	50	100	25	50
15	25	50	50	100	12.5	25	25	50	25	50	25	50	50	100	25	50	12.5	25	25	50	25	50	25	50
16	25	50	50	100	25	50	25	50	12.5	25	25	50	25	50	12.5	25	12.5	25	25	50	25	50	25	50
17	25	50	25	50	50	100	6.25	12.5	25	50	12.5	25	25	50	25	50	25	50	25	50	25	50	25	50
18	25	50	100	200	50	100	25	50	25	50	12.5	25	12.5	25	25	50	12.5	25	25	50	25	50	25	50
19	12.5	25	50	100	50	100	25	50	50	100	25	50	25	50	25	50	12.5	25	25	50	50	100	25	50
20	25	50	25	50	50	100	25	50	50	100	25	50	25	50	25	50	ng	Ng	50	100	25	50	25	50

Abbreviations: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

the growth of 18 and 2 strains successionally. *U. dioica* in concentrations of 100 and 6.25 mg/mL exhibited the highest and lowest inhibitory concentrations and inhibited the growth of 1 strain each (Table 4).

The lowest MBC of the alcoholic extracts of all examined species was 12.5 mg/mL for *A. indica* flower and leaf and *U. dioica*, and only one strain was inactivated in each case. The lowest MBC of 25 mg/mL was observed in *H. sabdariffa*, *A. indica* fruit, *E. planum*, *R. acetosa*, *C. procera*, and *P. guajava*, in which 1, 1, 2, 1, and 3 strains were destroyed serially. The highest MBC among all tested species was observed to be 50 mg/mL in *C. spinose*, *M. sylvestris*, and *A. setosa*, where 9, 16, and 17 strains were eliminated, respectively.

5. Discussion

The results of this study showed that the 20 isolated strains of *S. typhimurium* from the poultry feces of Zabol chickens are 100% and 40% resistant to cephalosporin and gentamicin, respectively. Cephalosporin and gentamicin are widely used to treat human and animal bacterial infections worldwide. In line with the current study's results, there are several reports on *Salmonella* resistance to these vital antibiotics (17-20). However, high sensitivity to antibiotics was expected due to the traditional way of breeding poultry and the limited use of drugs and antibiotics in this region. It is recommended to use ciprofloxacin as the best option against *S. typhimurium* infection among the other tested antibiotics. The susceptibility of *S. typhimurium* isolates to ciprofloxacin has already been reported (21).

Despite the absolute resistance of *S. typhimurium* strains against cephalosporin and relatively high resistance against tetracycline, almost all tested strains were inhibited by the ethanolic extracts of examined medicinal plants. The most effective plant extracts in inhibiting *Salmonella* growth in the disk diffusion method were those of *P. guajava* and *A. setosa*. The lowest MIC of the alcoholic extracts of tested medicinal plants varies from 6.25 (*U. dioica* and *A. indica* flower and leaf extracts) to 25 mg/mL (*C. spinosa*, *M. sylvestris*, and *A. setosa*); nevertheless, the lowest MBC ranged from 12.5 (*U. dioica* and *A. indica* flower and leaf extracts) to 50 mg/mL (*C. spinose*, *M. sylvestris*, and *A. setosa*). Although *A. setosa* and *M. sylvestris* showed higher MIC and MBC than some other examined plants in this study (*A. indica*, *P. guajava*, *H. sabdariffa*, *E. planum*, *R. acetosa*, *U. dioica*, and *C. procera*), they manifested the best efficacy against various strains with different levels of drug resistance (Table 4). *A. setosa* and *M. sylvestris* were capable of eliminating 16 and 17 out of 20 *S. typhimurium* strains, respectively, at concentration of 50 mg/mL. Then, the tested *S. typhimurium* strains were 80% and 85% sensitive to alcoholic extracts of *M. sylvestris* and *A. setosa*, respectively, which candidate them as appropriate medicinal and/or food supplements in bird breeding in the Zabol region.

Numerous studies have been conducted to discover effective medicinal plants on *Salmonella* species and strains and their mechanism of action. The effectiveness of *P. guajava* leaf extract was shown against the clinical isolates of *S. Typhi* with a much higher zone of inhibition (15 mm) than the results of this study. The reported MIC and MBC were also much lower than the present study's results (3.13 and 6.25, respectively) (22). These results validate the traditional use of *P. guajava* as anti-diarrheal and anti-typhoid fever in tropical countries (23, 24). *A. indica* also showed a broader inhibition zone (11 mm), lower MIC (1.56), and higher MBC (25) in comparison to the results of this study (22).

The *H. sabdariffa* calyx extract efficacy against *Salmonella* strains in this study coincides with previous studies in which *H. sabdariffa* calyx extracts exhibited antimicrobial activity against 13 multidrug-resistant *Salmonella* strains extracted from raw carrots (25). In addition, acetone extract and hibiscus acid extracted from *H. sabdariffa* calyces exhibited potent antimicrobial activity against multidrug-resistant *Salmonella* strains (26). In another study, *H. sabdariffa* ethanolic extract was employed successfully as a natural preservative to extend the shelf-life of beef by removing foodborne bacteria (27). However, the ethanolic leaf extract of *H. sabdariffa* was reported to be ineffective against the clinical isolates of *S. typhi* (28).

The potent antibacterial potential of *M. sylvestris*, as witnessed in the present study, agrees with the reports on it against various bacteria, including *Salmonella* (29, 30). Moreover, the MIC/MBC of *M. sylvestris* extract against the standard and clinically isolated *Salmonella enterica* from diarrheic lambs in Urmia, Iran, were reported to be 50/100 and 42/80 mg/mL, respectively (31). Additionally, *M. sylvestris* contains various chemical ingredients, such as carbohydrates, tannins, flavonoids, phenolic compounds, and ascorbic acid, denoting its multiple pharmaceutical properties. Additionally, Malvone (a phytoalexin) is found in *M. sylvestris* with a potent antimicrobial effect and might be a candidate for its prominent action against *Salmonella* (32, 33).

To the best of our knowledge, there is no scientific report on the effects of *A. setosa* on *Salmonella* in the literature. However, contrary to the present study's results, weak to moderate antioxidant potential and no significant antimicrobial for *A. setosa* have been reported (34, 35). On the other hand, the chemical composition of the methanolic extract of *Alcea setosa* from Jordan showed 290 compounds, among which flavonoids (flavones) were diversified (34). Phenolic compounds, including flavonoids, exhibit various biological activities and might explain the potent anti-*Salmonella* effect of this species.

5.1. Conclusions

The current study showed that bacterial resistance to conventional antibiotics is expanding even in regions with low antibiotic consumption. Moreover, the tested medicinal plant extracts revealed effective antimicrobial properties against resistant *Salmonella* strains, with *M. sylvestris* and *A. setosa* as the most active bactericide extracts at a concentration of 50 mg/mL. The alcoholic extracts of these two Malvaceae species are remarkably more effective than tetracycline, gentamicin, and cephalosporin and almost as potent as ciprofloxacin against *Salmonella* strains extracted from poultry feces. Due to the growing ineffectiveness of antibiotics against infectious diseases, the introduction of new antibiotics or complementary agents with fewer risks (e.g., drug resistance, allergies, and cancers) is of high necessity. It is recommended that *M. sylvestris* and *A. setosa* extracts containing useful antimicrobial agents be used not only as treatment or preventive supplements in poultry food but also to combat the present health challenge due to the antimicrobial resistance of foodborne pathogens. However, the results obtained in laboratory conditions should be redone and confirmed in vivo to evaluate the possible toxicity, side effects, or adverse reactions with foods or animals.

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Footnotes

Authors' Contribution: S S. contributed to the original idea and acquisition of the data. M D. contributed to the analysis and interpretation of the data and preparation of the final version of the manuscript.

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