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Research article

Efficacy of edible plant extracts against multi-drug resistant *Salmonella* serovars in layer flocks

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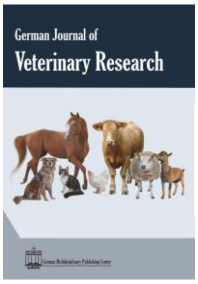

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Abstract

The present study examines the antibacterial effects of edible plants (Neem, Garlic, and Ginger) extracts against *Salmonella* serovars isolated from layer flocks using phenotypic antimicrobial resistance techniques. The incidence of positive isolates was higher in Dinajpur (13.89%), followed by Thakurgoan (11.91%), Nilphamari (10%), and Panchagarh (8.33%). Organoleptic analysis showed a higher prevalence in the liver (17.86%) compared to the heart (7.14%) and lung (10.72%). Ethanolic extractions were performed from the plants, and the antibacterial activity of these extracts was assessed using the disc diffusion method at concentrations of 80, 100, and 120 mg/mL against multidrug-resistant (MDR) bacteria, which were identified from selected layer flocks using conventional laboratory techniques. These bacteria are resistant to at least two antibiotics. The maximum inhibition zones for Neem, Garlic, and Ginger extracts at 120 mg/mL were 14 mm, 10 mm, and 2 mm, respectively, while the minimum inhibition zones at 80 mg/mL were 10 mm, 3 mm, and none, respectively. Neem leaf extract showed the highest effectiveness against MDR bacteria compared to Garlic and Ginger extracts. Although the organisms were sensitive to ciprofloxacin and colistin, they showed intermediate resistance to levofloxacin and chloramphenicol and resistance to neomycin and kanamycin. When comparing the antibacterial efficacy of resistant antibiotics and plant extracts *in vitro*, plant extracts showed larger inhibition zones. Therefore, it was concluded that ethanolic Neem leaf extract could serve as an alternative to conventional antibiotics against field isolates of *Salmonella* spp. in poultry.

Keywords: Layer flocks, *Salmonella* serovars, Antibacterial effect, Plant extract, Multi-drug resistance

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Introduction

Poultry is vital for Bangladesh's national economy and public welfare. Salmonellosis is a common problem in poultry farms in Bangladesh that causes heavy economic loss through mortality and reduced production (Hossain et al., 2021). The disease is of significant importance since the pathogen can be transmitted vertically from parents to offspring, thus further complicating control and prevention efforts. Despite ≥2600 serotypes of *Salmonella* identified, only ~10% of these have been isolated from poultry (Gast, 1997). Chickens host *S.* Pullorum and *S.* Gallinarum (Snoeyenbos, 1991), with pullorum disease affecting in the first 2-3 weeks of age (Calnek, 1997) and fowl typhoid in adult birds. They also cause high mortality in young chicks (Wales & Lawes, 2023). The transmission of pullorum disease and fowl typhoid is closely linked through eggs, posing a risk for human infection with *Salmonella* Enteritidis (Tariq et al., 2022; Lublin and Farnoushi, 2023). Eggs may become contaminated with *Salmonella* in two main ways: (i) *Salmonella* may silently infect the ovaries of apparently healthy hens and contaminate the eggs before the shells are formed. (ii) *Salmonella*-infected bird droppings contain *Salmonella* that can contaminate the outer eggshells and may penetrate when the shell cracks (Galiş et al., 2013).

Several challenges, such as infectious

diseases, poor husbandry, low productivity, and feed shortage, affect the optimal production of this industry (Serbessa et al., 2023). With the great expansion of poultry rearing and farming, pullorum disease and fowl typhoid have become widespread problems in Bangladesh (Munir et al., 2023). Controlling salmonellosis relies mainly on the use of antibiotics, whose overuse has led to increased microbial resistance (Algarni et al., 2022). Age-wise prevalence of avian salmonellosis showed highest infection rates in adult layers (53.25%) in comparison to brooder (14.55%), grower (16.10%), pullet (16.10%) (Rahman et al., 2004) and starter (5.71%), grower (3.33%) and layer (12.86%) at district Dinajpur (Akter et al., 2007). *Salmonella* is known to carry plasmids, which encode for drug resistance (Salehi *et al.,* 2005). As a result, there is a critical need to find alternatives to chemotherapeutic drugs, particularly those of plant origin, which are readily accessible and have fewer side effects (Khulbe and Sati, 2009). The use of plants and their extracts for treating infectious diseases has a long history in many parts of the world (Abdallah, 2011).

Plant materials continue to be an important source for combating infectious diseases, and many of the plants have been investigated for novel drugs or templates for the development of new therapeutic agents (Sharma et al., 2023; Abdallah et al., 2023). Bangladesh is rich in natural flora, among them medicinal plants as a traditional system of therapy, have long been used to cure diseases in humans and animals (Maisha et al., 2024). Bangladesh, Pakistan, and India are bound with plants that have medicinal effects (Rahman et al., 2023). Therefore, several research works have been conducted to evaluate the antibacterial effects of Garlic and Ginger extracts against *Salmonella* serovars in many countries of the world (Murungan et al., 2015; Odo, 2023).

In Bangladesh, however, the information on the antibacterial effects of Neem (*Azadirachta indica*), Garlic (*Allium officinale*), and Ginger (*Zingiber officinale*) extracts against *Salmonella* serovars isolated from layer chickens is scarce, with few exceptions e.g., Hernández-Ledesma et al., (2023) who investigated food samples from humans and Mahbuba et al., (2012), who worked on *Salmonella* serovars isolated from poultry. These studies used Arjuna (*Terminalia arjuna*) extracts against identified isolates. However, the antibacterial effects of Neem, Garlic, and Ginger extracts have not yet been researched in the country. To address this gap, the present study was conducted to evaluate the antibacterial effects of extracts from Neem, Garlic, and Ginger against *Salmonella* serovars isolated from selected layer flocks by using cultural, morphological, biochemical, and antibiogram studies. This research aimed to be a novel contribution to the field of veterinary microbiology in Bangladesh by comparing the *in vitro* efficacy of commercial antibiotics and plant extracts against field isolates.

Materials and methods

Study area and research design

The samples were collected from the selected commercial layer flocks at different areas (Dinajpur, Thakurgoan, Panchagahr, Nilphamari District) of Rangpur division and then transferred to the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU) for laboratory analysis. Completely Randomized Design (CRD) and descriptive cross-sectional survey were used to isolate and identify the *Salmonella* serovars from selected layer flocks. The design was chosen because the study was concerned with the identification of *Salmonella* serovars from selected layer flocks by using morphological, cultural, and biochemical techniques. The structured questionnaire was used for the data collection by using face-to-face farmer interviews.

Sample size determination

A total of 168 field samples (liver, heart, and lung) of the layer birds were aseptically collected just after postmortem examination from the study area based on three groups, categorized on the basis of their age as Group A**:** 0-8 weeks, Group B**:** 9-20 weeks and Group C**:** above 20 weeks. The sample size was determined by using a prevalence rate of 12.86% from previous studies (Akter et al., 2007) at a 5% level of significance, and the following formula was employed (Addis et al., 2011).

$$N = \frac{Z^2pq}{d^2}$$

Where N= is the desired sample size; Z = is the standard normal deviation that provides a 95% confidence interval (1.96); P = is the prevalence rate of *Salmonella* in layers from previous studies (Akter et al., 2007). Also, q = 1-p and d = is the absolute precision (error bound 0.05)

$$N = \frac{(1.96)^2 \times (.13) \times 0.87}{(0.05)^2} = \frac{3.84 \times 0.87}{(0.05)^2}$$
$$= \frac{0.4224}{0.0025} = 168.96 = 169 \approx 168$$

Collection, preparation of edible plant extracts, and disc preparation

The Neem leaves were collected from the HSTU campus, and fresh garlic and ginger were collected from the local market of Dinajpur town. Garlic and ginger were sliced with a sterile sharp knife. The sliced pieces and Neem leaves were cleaned and washed using sterile distilled water. For the preparation of powder, the collected samples were sun-dried for a week and followed by oven at 55-60°C for two days. The dried samples were pulverized in an electric grinder to get extractable powder. The powder was preserved in an airtight plastic container until they were directly used for screening and preparation of ethanol extract. Ten Grams of each powder were added to 80 mL of ethanol and shaken overnight at room temperature; then, the suspensions were filtered. After that, filtrates were concentrated using a Rotary-evaporating machine to get the viscous substance. These were transferred to a beaker and taken on a water bath for further drying at room temperature. Finally, a solid mass was obtained and stored at 4°C until use. This solid mass was considered the 100% concentration of the extract. The concentrations of 80%, 100%, and 120% were made by diluting the concentrated extract with appropriate volumes of 100% ethanol. Discs having different concentrations [80% (80 mg/mL), 100% (100 mg/mL), and 120% (120 mg/mL)] of ethanolic extract of Neem, Garlic, and Ginger were prepared using filter paper, and it was allowed to air dry for 30 minutes. The extraction process of Neem is shown in Figure 1. Similarly, Garlic and Ginger were extracted by the same process.

Isolation and identification of *Salmonella* spp.

Salmonella serovars were isolated and identified from samples taken from layer birds, specifically from the liver, heart, and lungs. Initially, the samples were cultured in Selenite Broth Base (Oxoid, UK), which promotes the growth of *Salmonella* spp. while inhibiting other enteric organisms. Subsequently, they were cultured on various media, including Nutrient Agar (NA; Hi-Media, India), MacConkey agar (MC; Hi-Media, India), Brilliant Green Agar (BGA; Hi-Media, India), and *Salmonella-Shigella* agar (SS; Hi-Media, India) to observe morphological and cultural characteristics. Biochemical tests: Methyle Red (MR), Simmons citrate utilization (SC), Triple Sugar Iron (TSI), Voges-Proskauer (VP), Indole, and Motility Indole Urea (MIU), following the methods outlined by Buxton and Fraser (1977), Merchant and Packer (1967), and OIE (2004), were then conducted to confirm the identification of *Salmonella* spp.

Antibiotic sensitivity tests

All bacterial isolates were subjected to an antibiotic sensitivity test by the Kirby-Bauer Disc diffusion method according to the guidelines of The European Committee on Antimicrobial Susceptibility Testing standard (EUCAST). The antimicrobial discs (6 antibiotic discs; ciprofloxacin 5 µg, colistin 10 µg, levofloxacin 5 µg, chloramphenicol 30 µg, neomycin 30 µg, and kanamycin 30 µg) were applied to the plates. After incubation, the diameter of the zones of complete inhibition (including the diameter of the disc) was measured and recorded in millimeters. Antimicrobial testing results were recorded as sensitive, intermediate, and resistant according to zone diameter interpretative standards provided by EUCAST.

Antibacterial activity of medicinal plant extracts

Susceptibility and resistance to different antibiotics were measured *in vitro* by employing the modified Kirby-Bauer (Bauer et al., 1966) method. This method allowed for the rapid determination of a drug's efficacy by measuring the diameter of the zone of inhibition that resulted from the agent's diffusion into the medium surrounding the disc. The concentrations of the plant extracts (Neem, Garlic, and Ginger) were 80%, 100%, and 120%, respectively, with the disc concentrations measured in mg/mL.

Disk diffusion method

Filter paper discs of 6 mm in diameter were prepared and sterilized. The cultures were enriched in sterile nutrient broth for 6-8 hours at 37 °C. Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile nutrient agar plates; the different concentrations of ethanolic extract of Neem, Garlic, and Ginger discs were aseptically placed over the seeded agar

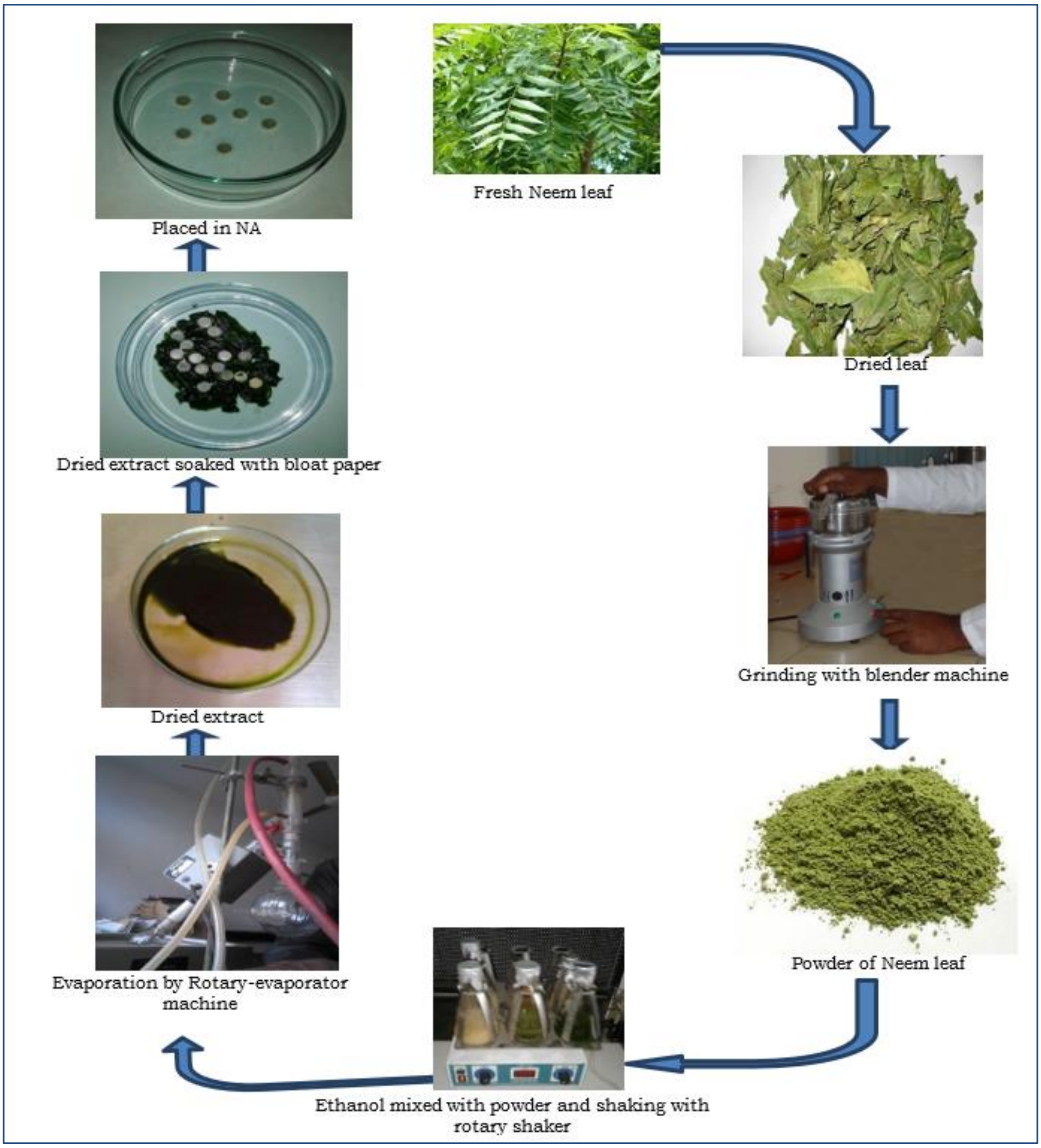

Figure 1: A diagram showing the preparation of Neem extract and Neem disc.

plates sufficiently separated from each other to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zone was measured in mm (Chand, 2013).

Data analysis

The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (*IBM SPSS Statistics* V21.0, USA). Initially, all the data was arranged in Microsoft Excel (Version-2013, USA) for further analysis in SPSS. The Chi-square test was employed to assess the association between variables. The level of significance was set at $p<0.05$. Prevalence rates were calculated using SPSS software based on the observed data.

Results

Isolation and identification of *Salmonella* spp.

Salmonella serovars were isolated and identified from the layer bird samples (liver, heart, and lung) after cultivation on NA, MC agar, BGA, and SS agar medium. They produced circular, smooth, opaque, translucent colonies on NA; Pale, colorless, smooth, transparent raised colonies on MC; opaque, translucent, colorless, smooth, round colonies with black center appeared on SS; pale, pink color colonies against a pinkish background on BGA for all samples from liver, lungs, and heart. Organoleptic characterization of *Salmonella* serovars by biochemical tests showed positive for MR (Methyle Red), SC (Simmons citrate utilization), TSI (Triple Sugar Iron), and Negative for VP (Voges-Proskauer), Indole and MIU (Motility Indole Urea) test. Out of 168 tested samples 20 samples were confirmed as positive. Among the positive samples, five farms were from Dinajpur, six farms were from Thakurgoan, four farms were from Panchagarh, and five farms were from Nilphamari. The percentages of positive isolates from different samples (liver, heart, and lung) of the areas mentioned above were 13.89%, 11.91%, 8.33%, and 10%, respectively, and the average prevalence of *Salmonella* serovars in the study area was 11.9% (Table 1). In the case of internal organs (liver; 10, heart; 4, lung; 6), the prevalence of *Salmonella* spp. was also 17.86%, 7.14%, and 10.72%, respectively (Table 2). Results of biochemical tests of *Salmonella* serovars are shown in Table 3.

Table 1: Prevalence of *Salmonella* serovars based on spatial (study area) differences.

Name of District	Types of samples	No. of samples tested	No. of positive isolates	Prevalence of isolates (%)	χ^2 value	Level of significance
Dinajpur	Liver	24	4	13.89	0.666	0.881 (n.s.)
	Heart	24	3			
	Lung	24	3			
Thakurgoan	Liver	14	2	11.91		
	Heart	14	1			
	Lung	14	2			
Panchagarh	Liver	8	2	8.33		
	Heart	8	-			
	Lung	8	-			
Nilphamari	Liver	10	2	10		
	Heart	10	-			
	Lung	10	1			
Total		**168**	**20**	**11.9**		

Table 2: Organoleptic prevalence of *Salmonella* infection in dead birds.

Sample	No. of samples tested	No. of positive isolates	Prevalence of isolates (%)	χ^2 value	Level of significance
Liver	56	10	17.86	3.18	0.20 (NS)
Heart	56	4	7.14		
Lung	56	6	10.72		
Total	168	20	11.9		

Table 3: Organoleptic characterization of *Salmonella* serovars by biochemical tests.

Isolates	Indo	MR	VP	SC	MIU	TSI Butt	TSI Slant	TSI H_2S
Liver	_	+	_	+	_	Y	R	+
Heart	_	+	_	+	_	Y	R	+
Lung	_	+	_	+	_	Y	R	+

Indo = Indole; MR = Methyle Red; VP = Voges-Proskauer; SC = Simmons citrate utilization; MIU = Motility Indole Urea; TSI = Triple Sugar Iron; Y= Yellow; R= Red

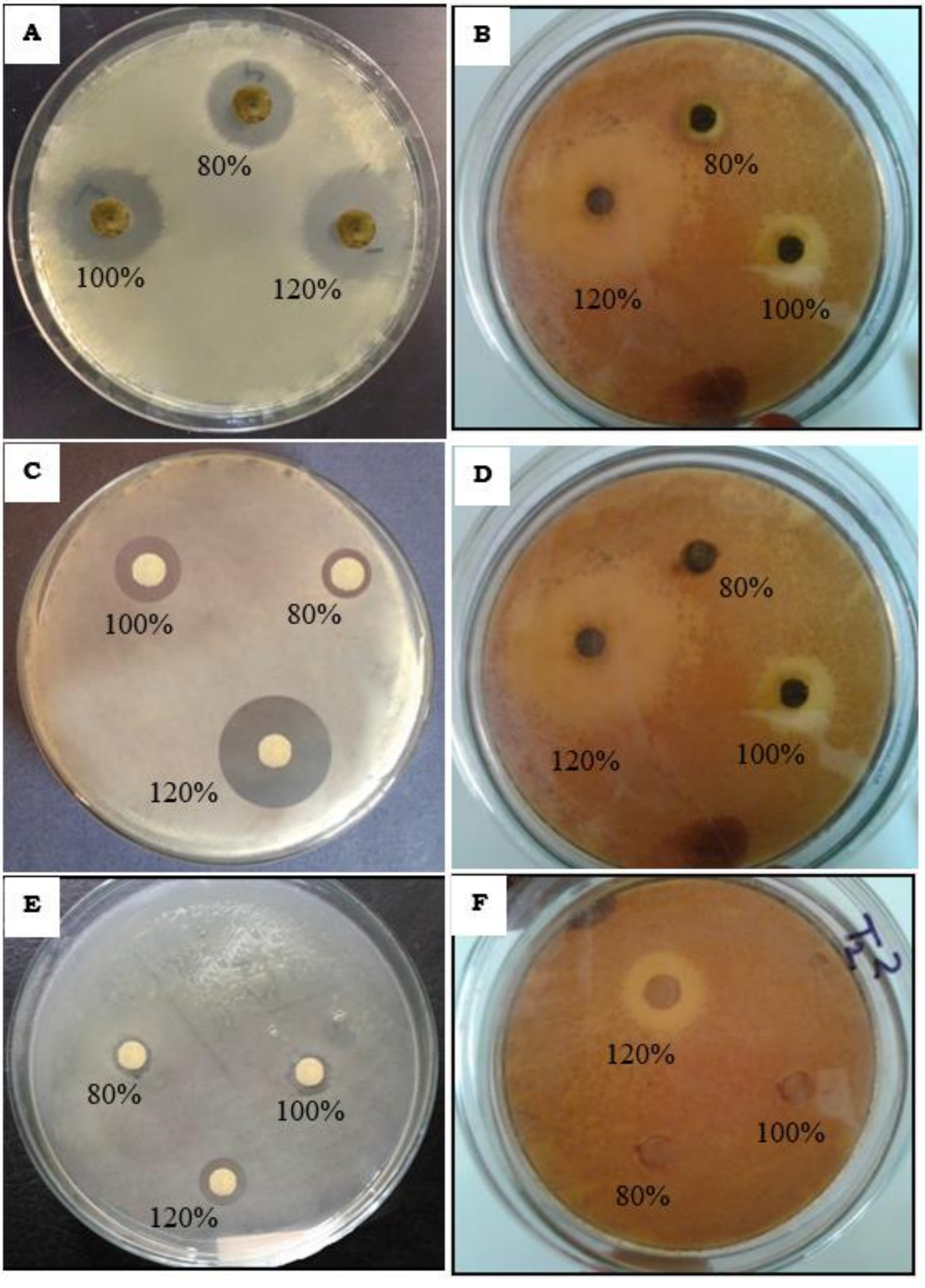

Figure 2: Antimicrobial sensitivity test of Neem extract on nutrient agar (A) and SS agar (B); Garlic extract on nutrient agar (C) and SS agar (D), and Ginger extract on nutrient agar (E) and SS agar (F). 80%=80 mg/mL, 100%?100 mg/mL, and 120% =120 mg/mL.

Antimicrobial sensitivity of plant extracts using the disc diffusion method

The results of the antibacterial activity of plant extracts using the disc diffusion method are presented in Table 4. It indicates that *Salmonella* serovars showed different levels of sensitivities in the tested samples of Neem, Garlic, and Ginger extracts. The zone of inhibition of the Neem and Garlic extracts was higher than that of the Ginger extract for the corresponding concentrations (80% (80 mg/mL), 100% (100 mg/mL), and 120% (120 mg/mL). In Neem extract, the zone of inhibition varied from 9 mm to 14 mm in diameter. The greater zone of inhibition was observed at 120 mg/mL of concentration (14 mm), and the lower zone of inhibition at 80 mg/mL of concentration (9 mm) [Figure 2 (A, B), Table 5]. In Garlic extract, the zone of inhibition varied from 2 mm to 10 mm in diameter. The greater zone of inhibition was observed at 120 mg/mL of concentration (10 mm), and the lower zone of inhibition at 80 mg/mL of concentration (2 mm) [Figure 2 (C, D), Table 5]. In Ginger extract, the zone of inhibition varied from 2 mm to 3 mm in diameter. The greater zone of inhibition was observed at 120 mg/mL of concentration (3 mm), and the lower zone of inhibition at 100 mg/mL of concentration (2 mm) [Figure 2 (E, F), Table 5].

Antimicrobial sensitivity pattern of commonly used antibiotics

Antibiotic sensitivity was performed against six commonly used antibiotics belonging to different groups (Table 6). In this study, the *Salmonella* serovars showed sensitivity to ciprofloxacin (21 mm) and colistin (11 mm), intermediate to levofloxacin (15 mm) and chloramphenicol (16 mm), and resistance to neomycin (13 mm) and kanamycin (13 mm). Comparing plant extracts with resistant antibiotics, this work revealed that Neem extract showed the highest zone of inhibition against *Salmonella* serovars, which was 21 mm, while garlic presented an 11 mm zone, except for the ginger, which was about to be resistant. Neomycin and kanamycin showed around a 7 mm zone even though they were initially resistant (Figure 3).

Discussion

This study investigates the antibacterial effectiveness of Neem, Garlic, and Ginger extracts against MDR *Salmonella* serovars in layer flocks. It aims to address salmonellosis and its economic impact on layer farming by conducting a comparative analysis with commercial antibiotics. Among the 168 samples examined, 20 tested positive for *Salmonella* serovars based on cultural, morphological, and biochemical characteristics. Our findings indicate variations in the prevalence of *Salmonella* serovars, with higher rates reported in Dinajpur (13.89%) compared to Thakurgoan (11.91%), Panchagarh (8.33%), and Nilphamari (10%). The organoleptic prevalence of *Salmonella* infection in dead birds was highest in the liver (17.86%) and lowest in the heart (7.14%). Mahmud et al. (2011) reported an overall prevalence of 21.1%, with 31.2% *Salmonella* infection found in dead birds, which was higher than our findings.

Conversely, the findings of Rani et al. (2022) were lower than ours. The observed variation in *Salmonella* infection prevalence could be attributed to various factors, including geoclimatic conditions, immune status, infecting dose, concurrent infections, stress, management practices, biosecurity measures, and study area locations. Poultry farming is rapidly expanding in Bangladesh due to the high demand for meat and eggs (Amin et al., 2023). However, the indiscriminate use of antibiotics in poultry production, both for treatment and as feed supplements, has been reported (Akter et al., 2023). The residual effects of antibiotics in meat and eggs can pose public health risks, such as the development of drug-resistant bacteria in humans (Diarra and Malouin, 2014). Poultry veterinarians face challenges in treating MDR bacteria, leading to increased production costs and mortality rates, as most antibiotics are ineffective against pathogenic MDR bacteria (Abreu et al., 2023). In light of these issues, our study aims to assess the effectiveness of plant extracts against field isolates of *Salmonella* spp.

Table 4: Antimicrobial sensitivity pattern of different plant extracts against identified field isolates.

Name of Farm	Age of birds (Weeks)			Sample no. (Positive)	Zone of inhibition (mm)								
					Neem			Garlic			Ginger		
	0-9	10-20	Above 20		80%	100%	120%	80%	100%	120%	80%	100%	120%
Robi, Nil	1000	-	-	-	-	-	-	-	-	-	-	-	-
Mos, Nil	-	1000	-	-	-	-	-	-	-	-	-	-	-
Rej, Nil	1000	-	-	-	-	-	-	-	-	-	-	-	-
Sel, Nil	-	-	1500	18(2)	10	12	14	3	7	10	-	-	3
Moj, Nil	-	-	1000	12(1)	10	11	13	3	6	10	-	-	3
Ash, Panch	-	1000	-		-	-	-	-	-	-	-	-	-
Kris, Panch	1500	-	-	-	-	-	-	-	-	-	-	-	-
Kud, Panch	-	2500	-	15(2)	10	11	14	3	7	9	-	-	2
Shah, Panch	-	-	3000	9(0)	-	-	-	-	-	-	-	-	-
Haz, Thaku	-	-	1000	12(0)	-	-	-	-	-	-	-	-	-
Mot, Thaku	8000	-	-	18(3)	9	12	14	3	6	10	-	2	3
Mas, Thaku	1200	-	-	12(2)	8	10	13	2	6	10	-	-	3
Sad, Thaku	-	2500	-		-	-	-	-	-	-	-	-	-
Joy, Thaku	-	-	3000		-	-	-	-	-	-	-	-	-
Ani, Thaku	-	-	6000		-	-	-	-	-	-	-	-	-
Has, Dinj	-	-	8000		-	-	-	-	-	-	-	-	-
SPP, Dinj	-	10,000	-	18(2)	9	11	14	3	6	10	-	2	3
Naz, Dinj	-	-	3000		-	-	-	-	-	-	-	-	-
Mot, Dinj	-	1500	-		-	-	-	-	-	-	-	-	-
Sor, Dinj	-	-	2000		-	-	-	-	-	-	-	-	-
Abd, Dinj	500	-	-		-	-	-	-	-	-	-	-	-
Arif, Dinj	-	1000	-		-	-	-	-	-	-	-	-	-
Mah, Dinj	-	1000	-		-	-	-	-	-	-	-	-	-
Agro, Dinj	-	-	4000	12(0)	-	-	-	-	-	-	-	-	-
Mono, Dinj	-	-	2800		-	-	-	-	-	-	-	-	-
Vai, Dinj	-	-	9200		-	-	-	-	-	-	-	-	-
Rai, Dinj	1000	-	-	12(3)	9	12	14	3	6	10	-	-	3
Ras, Dinj	1000	-	-		-	-	-	-	-	-	-	-	-
SR, Dinj	-	-	10000	18(3)	9	11	14	3	5	10	-	-	2
Lot, Dinj	-	-	1500	12(2)	10	12	14	3	6	10	-	2	3
Total				168(20)									
Sig					0.00								
Average±SD					0.00	11.48±1.95	0.00	6.30±2.95	0.00	0.00	0.00	1.03±1.2	0.00
mean±SD				168(20)	9.33±0.70	11.33±0.70	13.77±0.44	2.89±0.44	6.11±0.6	9.9±0.33		0.75±1.03	2.75±0.46

Legends (District Name): Nil = Nilphamari, Panch = Panchagarh, Thaku = Thakurgaon, Dinj = Dinajpur. All are significant at 0.1% level. In this present study, Table 4 shows the antibacterial sensitivity pattern of different plant extracts against identified field isolates. It was observed that there were significant (*p*<0.01) differences among the three plant extracts.

Table 5: Antimicrobial activity of Neem, Garlic, and Ginger extract on *Salmonella* serovars.

Tested sample	Concentration (%)	Zone of inhibition (mm)			Average
		T1	T2	T3	
Neem	80	10	10	9	9.67
	100	12	12	11	11.67
	120	14	13	13	13.34
Garlic	80	3	2	3	2.67
	100	7	6	7	6.67
	120	9	10	10	9.67
Ginger	80	_	_	_	_
	100	_	2	2	1.33
	120	2	3	2	2.33

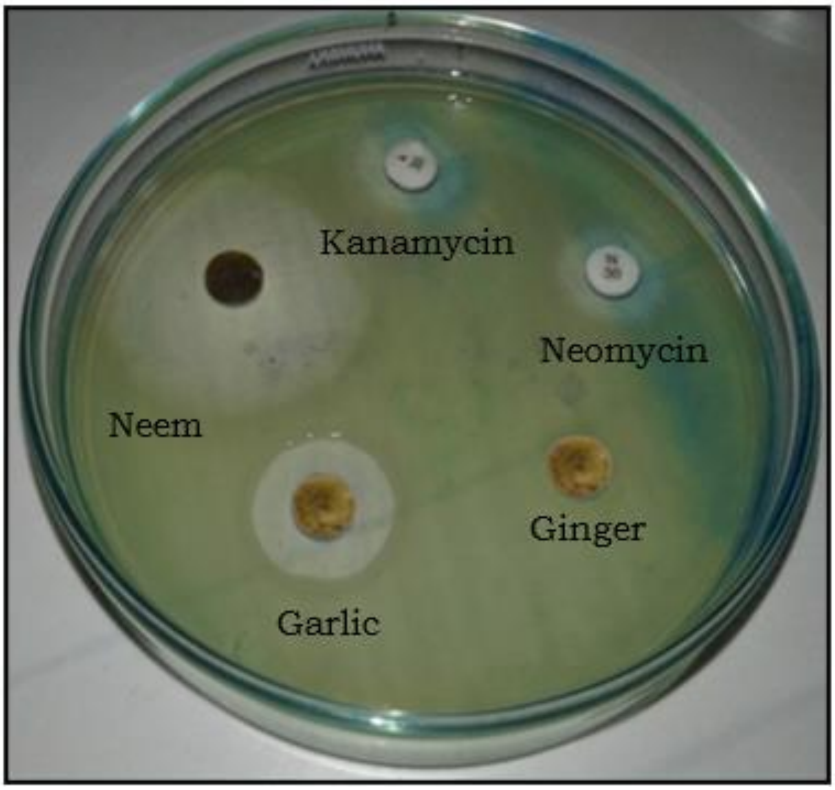

Figure 3: Comparison of plant extracts with resistant antibiotics against *Salmonella* serovars.

Table 6: Zone size interpretative chart (EUCAST standard).

Antibiotics	Symbol	Disc content	Zone of inhibition (mm)		
			Sensitive	Intermediate	Resistance
Ciprofloxacin	CIP	5	21	16-20	15
Colistin	CL	10	11	-	10
Levofloxacin	LE	5	17	14-16	13
Chloramphenicol	C	30	18	13-17	12
Neomycin	N	30	19	14-18	13
Kanamycin	K	30	18	14-17	13

In the present study, the antibacterial sensitivity of different plant extracts revealed that all of the field isolates showed some antimicrobial activity against all tested *Salmonella* isolates at varying concentration levels ranging from 80% to 120%. All the tested isolates were found to be highly sensitive to Neem extracts than Garlic and Ginger extracts, suggesting that these plant extracts could be a good alternative to synthetic antibiotics. These results are similar to the findings of Al Noman et al. (2023) and Akihisa et al. (2011). Mousa et al. (2021) also support these findings. Tarekegn and Balkachew (2023) showed 15 mm and 10.5 mm zones for Garlic and Ginger against *Salmonella typhi*, which are quite higher than our findings but lower than the zone of Neem extract. Variations in the antibacterial sensitivity of plant extracts showed the necessity of an *in vitro* antibacterial sensitivity test prior to the treatment. It also emphasizes a reasonable selection of antibacterial agents for effective treatment.

The present study aimed to compare the efficacy of commercial antibiotics with plant extracts against field isolates. The study revealed a notable resistance among most isolates to synthetic antibiotics, with only ciprofloxacin and colistin demonstrating sensitivity. Additionally, the study compared the resistance of antibiotics with that of plant extracts, finding that the latter exhibited high sensitivity in kanamycin in comparison to neomycin and kanamycin to field isolates. Results from the plant extracts indicated their effectiveness and reliability in treating *Salmonella* infections. However, the recommendation for the practical application of these plant extracts remains tentative. Further research is necessary to clarify serotypes or serovars using molecular techniques and to conduct more extensive field trials. This additional research will provide a more comprehensive understanding of the extracts' efficacy and safety on a broader scale.

Conclusion

The study demonstrated that plant extracts from Neem, Garlic, and Ginger had significant antibacterial activity against *Salmonella* serovars isolated from layer flocks. Neem extract showed the highest efficacy. The antibacterial activity of these extracts was found to be concentration dependent, revealed by the higher concentrations (120%) of the extracts resulting in larger zones of inhibition. The response to the plant extracts varied across different farms, suggesting that environmental factors, differences in bacterial strains, or other local conditions could influence the effectiveness of the plant extracts. Farmers and veterinarians should consider a dose-dependent approach when using plant extracts as antimicrobial agents. Understanding local conditions and specific bacterial strains was needed in the selective use of plant extracts.

Further research into the mechanisms of action, safety, and field applications of these extracts was required. Neem extract, in particular, could be a valuable addition to current treatment protocols. Finally, the study opened perspectives for further research and application in the field, contributing to sustainable and responsible disease management strategies in the poultry industry.

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Author contributions. Concept: M.R.A and M.M.R; research, Data arrangement, and analysis: M.M, M.R.A, and MS; initial draft: M.S.; critical review and writing the final version of the manuscript: M.S. All authors approved the final version of the manuscript.

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