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Research Article

In Vitro-Antibacterial Activity of Five Essential Oils against *Salmonella enterica* Subspecies *enterica* Serovar Typhimurium of Poultry Origin and their Correlation with Antibiotics

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Abstract

Salmonellosis is the major bacterial disease of poultry, causing heavy economic losses. *Salmonella enterica* subspecies *enterica* serovar Typhimurium, (S. Typhimurium) is one of the most common serovars responsible for human and animal salmonellosis around the world. The present study aimed to investigate *in vitro*-antimicrobial activity of five different essential oils (EOs) procured in pure form, against S. Typhimurium (N = 66) and its correlation with MICs of twelve different antibiotics (Abs) which are most commonly used in poultry flocks in India. The broth microdilution method determined the minimum Inhibitory Concentration (MIC) of EOs and Abs. Results of MICs (mg/ml) of EOs in ascending order were cinnamaldehyde EO (2.10 ± 0.11), carvacrol EO (2.35 ± 0.13), thymol EO (3.54 ± 0.20), eugenol EO (3.61 ± 0.28) and citral EO (4.08 ± 0.31). The correlation coefficients between MICs of these five EOs and twelve Abs revealed a 60% (36/60) negative correlation. Cinnamaldehyde EO had the lowest MICs and was found to be the most effective against avian S. Typhimurium. The more negative correlations with the MICs of antibiotics were reported for EOs of carvacrol (10/12 Abs) and thymol (09/12 Abs), indicating greater susceptibility of antibiotic-resistant *Salmonella* toward carvacrol and thymol. Because of the negative correlation between MICs of EOs and Abs, it is concluded that the studied essential oils, particularly cinnamaldehyde, carvacrol, and thymol can be used as effective alternatives to conventional antibiotics for treating non-typhoid infections caused by S. Typhimurium in poultry.

Keywords: Antibiotics; Antimicrobial Activity; Essential Oils; *Salmonella* Typhimurium

Introduction

The *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S*. Typhimurium) belonging to Family Enterobacteriaceae is a rod-shaped, Gram-negative, flagellated, facultative intracellular pathogen that infects wide hosts and has zoonotic potential. This organism is responsible for food-borne gastroenteritis in humans worldwide, mainly caused by contaminated poultry by-products [1]. The spread of *S*. Typhimurium occurs through the consumption of contaminated food or water and contact with infected poultry, animals, or human [2]. Antibiotics were previously used as antibiotic growth promoters (AGP) to prevent pathogenic bacterial growth in the poultry gut and to enhance poultry growth. However, antibiotics are currently prohibited as feed additives in poultry because of the persistence of antibiotic residues in food-producing animals [3]. Also, in the case of poultry, the administration of antibiotics is the primary control strategy for Salmonellosis, but it rarely prevents carrier status in birds [4]. The Gram-negative bacteria like *Salmonella* spp. have a thick lipopolysaccharide (LPS) membrane that limits the susceptibility of microorganisms to various antimicrobials, whereas the Gram-positive bacteria lack this LPS layer, enabling them more susceptible to antibiotics than Gram-negative bacteria [4]. Overuse and misuse of antibiotics in animals and poultry have resulted in the emergence and selection of multi-drug resistant (MDR) strains in the food chain [5]. The biofilm formation ability in *S.* Typhimurium and the emergence and spread of MDR strains have significantly jeopardized current antibacterial therapy since they have widely been recognized as a severe threat to global health and food safety in the 21st century [6,7]. An increase in antibiotic resistance in *S.* Typhimurium was reported recently by various organizations such as the World Health Organization (WHO), the Center for Disease Control and Prevention (CDC), and the Food and Agriculture Organization of the United Nations (FAO) [8,9]. As a result, there is an urgent need to find appropriate alternatives to antibiotics for treating *S.* Typhimurium strains of poultry origin; herbal EO therapy is one among them.

Essential oils (EOs) obtained from various herbs are effective against MDR strains of bacteria [10]. Various methods such as steam distillation, hydro-distillation, hydro-diffusion, and solvent extraction can be used to extract EOs from herbal plants [10]. The EOs predominantly disintegrate the outer membrane of Gram-negative bacteria [11]. Many EOs has multiple antimicrobial mechanism properties and has been correlated with their components. But the exact mechanisms underlying the antimicrobial properties of EOs are unknown. A variety of mechanisms have been proposed, including protein denaturation and cell membrane destruction [12]; inhibition of the production of essential enzymes and degradation of the bacterial cell wall [13]; inhibition of the synthesis of microbial toxins, prevention of the development of biofilms, reduce the fimbriae production [10]; releasing LPS and increasing the permeability of the cytoplasmic membrane [11]; leakage of cellular contents, inactivation of extracellular enzymes, reduction of intracellular ATP, cytoplasmic coagulation and interruption of electron flow [14]. Furthermore, EOs have shown immunostimulatory effects in poultry without significant biotoxicity [15]. Significant reductions in the levels of pro-inflammatory cytokines and induction of anti-inflammatory cytokines were reported after treating with EOs [15]. Moreover, EOs protects the birds from the possible adverse effects of commercial antibiotics and the consumers from the harmful impact of antibiotic residues [16]. Further, the U.S. Food and Drug Administration (FDA) classifies the majority of spice essential oils as 'generally recognized as safe' [17].

Most of the available scientific literature on MICs of EOs is based on the extract of herbal plants, which contains a mixture of phytoconstituents. Also, till now no correlation of MICs of EOs and MICs of Abs against *S.* Typhimurium was carried out. Thus, there is virtually no literature revealing MICs of pure EOs containing single phytoconstituents against *S.* Typhimurium and its correlation with MICs of different antibiotics. Hence, the main objective of this study is to evaluate the *in vitro* activity (MICs) of five purified EOs (cinnamaldehyde EO, carvacrol EO, thymol EO, eugenol EO, and citral EO) derived from various herbal plants against *S.* Typhimurium and further correlate their MICs with 12 different antibiotics. To the best of our knowledge, this appears to be the first of its kind in reporting the activity of EOs containing single phytoconstituent bearing EOs and antibiotics against *S.* Typhimurium and their correlation. Thus, the study is novel.

Materials and Methods

Essential Oils (EOs) and antibiotics

The five essential oils (EO) i.e cinnamaldehyde EO (extracted from *Cinnamomum ceylanicum*), carvacrol EO (extracted from *Origanum vulgare*), thymol EO (extracted from *Thymus citriodorus*), eugenol EO (extracted from *Syzygium aromaticum*), and citral EO (extracted from *Cymbopogon citratus*) used in the present study were procured in pure form (> 99%) from IgY Immunologix India Pvt. Ltd., Hyderabad, India. The twelve antibiotics used in this study were ciprofloxacin, neomycin, doxycycline, enrofloxacin, chloramphenicol, amikacin, tetracycline, gentamicin, cefalexin, oxytetracycline, trimethoprim, and bacitracin. The antibiotics were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

Bacterial cultures

Salmonella Typhimurium infection continues to be one of the biggest issues for the poultry sector in India, not only because of the drastic drop in production and significant mortality but also its zoonotic importance [18]. Because of its economical and zoonotic significance, *S.* Typhimurium was selected for the present study. The *S.* Typhimurium isolates (n = 66) of poultry origin available at the Department of Veterinary Pathology, Nagpur Veterinary College, Nagpur, Maharashtra, were used in the present study to evaluate the *in vitro*-antimicrobial activity. The bacterial cultures mentioned in this study were confirmed using conventional microbiological assays and polymerase chain reaction (PCR) employing *invA* and *Spy* genes [19,20]. The primers used in the present study are depicted in table 1.

Gene	Forward primer	Reverse primer	Amplicon Size (bp)	Reference
*Inv*A	5'TCGTGACTCGCG-TAAATGGCGATA 3'	5'GCAGGCG-CACGCCATAAT-CAATAA 3'	423	[20]
Spy	5'TTGTTCACTTTT-TACCCCTGAA 3'	5'CCCTGACAGC-CG TTAGATATT3'	401	[20]

Table 1: Details of Primer sequences used for amplification of *inv*A and *Spy* genes.

Determination of MIC of EOs and antibiotics

The MIC values of these five different EOs and twelve antibiotics against *S.* Typhimurium (N = 66; 1×10^6 CFU/ml) were determined by broth microdilution method using 96 well microtitre plates as per the standard guidelines specified by Clinical and Laboratory Standard Institute [21,22]. Briefly, a hundred µl of these EO/Ab was added to the first well. Fifty µl of cation-adjusted Mueller-Hinton broth was added from the 2nd to the 12th well. Serial dilution of EO/Ab was made from the 2nd to the 10th well. Fifty µl of bacterial cells (1×10^6 CFU/ml) were added from the 1st to 11th well. The culture plate was incubated for 18 h at 37^{0}C. After 18hours of incubation, 15 µl of 0.015% resazurin was added to each of the wells. After adding resazurin, the plates were incubated again at 37^{0}C for 1.5h and the color change from purple to pink was recorded as an indicator of the presence of bacteria. MIC was read as the lowest concentration of these EOs and antibiotics that inhibited the growth of indicator organisms [23].

Correlation between MICs

The linear associations between MICs of these studied five EOs were estimated by the Pearson Correlation Coefficient. Also, the MICs of these EOs were correlated with the MICs of 12 antibiotics by the same method [24].

Data analysis

All the MIC assays of selected five EOs and 12 Abs against 66 isolates of *S.* Typhimurium were carried out in triplicates, as 3 independent experiments. From that mean MICs were calculated and results were expressed as Mean ± standard error (SE). Data were statistically analyzed by one-way ANOVA by using Web Agri Stat Package (WASP) statistics software (www.icargoa.res.in). The confidential intervals at 95% of MICs of EOs and Abs were calculated simultaneously. Similarly, Web Agri Stat Package (WASP) statistics software also determined the correlation (Pearson Correlation Coefficient) between MICs of all the studied EOs and Abs.

Sr No	EO	Mean MIC (mg/ml for EOs, and ug/ml for Abs)	CI (95%)
1	Cn	2.10 ± 0.11^{b}	1.88 - 2.33
2	Ca	2.35 ± 0.13^{b}	2.09 - 2.61
3	Th	3.54 ± 0.20^{a}	3.14 - 3.94
4	Eu	3.61 ± 0.28^{a}	3.04 - 4.18
5	Ct	4.08 ± 0.31^{a}	3.46 - 4.70
6	CIP	30.41 ± 0.49^{f}	20.8 - 40.1
7	N	37.98 ± 0.87^{f}	20.9 - 55.1
8	DO	52.07 ± 0.37^{f}	44.8 - 59.3
9	Ex	77.88 ± 1.21^{ef}	54.1 - 102.0
10	C	80.25 ± 1.39^{ef}	53.2 - 107.2
11	Ak	85.69 ± 2.73^{ef}	31.0 -140.3
12	TE	125.82 ± 1.66^{de}	93.1 - 159.3
13	GEN	169.26 ± 3.85^{cd}	93.8 - 245.1
14	CN	188.32 ± 3.09^{c}	128.1 - 249.3
15	O	190.51 ± 1.91^{c}	153.3 - 228.2
16	TR	381.86 ± 3.00^{b}	323.4 - 441.5
17	B	503.78 ± 2.10^{a}	461.7 - 545.8

Table 2: The mean MIC of different EOs and Abs against *Salmonella enterica* subspecies *enterica* serovar Typhimurium of poultry origin (N = 66).

Mean ± SE with common alphabets as superscripts do not differ significantly, Significance level ≤ 0.01.

(EOs: Essential Oils; Abs: Antibiotics; Cn: Cinnamaldehyde; Ca: Carvacrol; Th: Thymol; Eu: Eugenol; Ct: Citral; CIP: Ciprofloxacin; N: Neomycin; DO: Doxycycline; Ex: Enrofloxacin; C: Chloramphenicol; Ak: Amikacin, TE: Tetracycline; GEN: Gentamicin; CN: Cefalexin; O: Oxytetracycline; TR: Trimethoprim; B: Bacitracin)

Results

MICs of EOs and Abs

The mean MICs of EOs ranged from 2.10 to 4.08 mg/ml while the mean MICs of Abs were in the range of 30.41 to 503.78 µg/ml. The statistically significant differences were reported between the mean MICs of EOs as well as antibiotics against *S.* Typhimurium of poultry origin, which are depicted in table 2.

Correlation between MIC of EOs

The linear correlation between the MICs of different EOs against *S.* Typhimurium is depicted in table 3. We reported positive correlations between all tested pairs of EOs among the pairwise (10 pairs) possible combinations that we had studied.

EO	Cn	Ca	Th	Eu	Ct
Cn	------	-------	-----	-------	-----
Ca	0.27187	------	------	------	------
Th	0.34965	0.47901	------	------	------
Eu	0.20541	0.5007	0.34764	------	------
Ct	0.33933	0.36535	0.16989	0.62989	------

Table 3: Pearson Correlation Coefficient between MICs of EOs.

(EOs: Essential Oils; Cn: Cinnamaldehyde; Ca: Carvacrol; Th: Thymol; Eu: Eugenol; Ct: Citral)

Correlation between MIC of EOs and antibiotics

The linear correlation between the MICs of EOs and Abs against *S.* Typhimurium is depicted in Table 4. We have studied all possible 60 combinations of EOs and antibiotics; among those, 40% (24/60) were positively correlated with the MICs of antibiotics. For the remaining 60% (36/60), the MICs of EOs were negatively correlated with the MICs of antibiotics. The antibiotics (Abs) chloramphenicol, gentamycin, amikacin, bacitracin, and trimethoprim revealed a negative correlation with all the tested EOs, while doxycycline and enrofloxacin revealed a positive correlation with all the tested EOs. The maximum negative correlations with antibiotics were observed for carvacrol and thymol. Carvacrol showed a negative correlation with 10 out of 12 tested antibiotics, while thymol revealed a negative correlation with 9 out of 12 tested antibiotics.

Discussion

Non-typhoid infections in poultry are caused by a non-typhoidal group of *Salmonella* like *S*. Typhimurium and *S.* Enteritidis. *Salmonella* Typhimurium is prevalent in poultry and linked to salmonellosis in humans [13]. To control non-typhoid infections in poultry, mostly antibiotics are used. Inappropriate use of antibiotics in food-producing animals and poultry resulted in bacterial resis-

EOs/Abs	CIP	N	DO	Ex	C	Ak	TE	GEN	CN	O	TR	B
Cn	0.36	-0.07	0.51	0.13	-0.15	-0.04	0.19	-0.16	0.03	0.18	-0.27	-0.20
Ca	-0.03	-0.06	0.14	0.19	-0.36	-0.31	-0.10	-0.49	-0.29	-0.08	-0.13	-0.26
Th	0.11	-0.04	0.05	0.29	-0.21	-0.11	-0.03	-0.32	-0.15	-0.02	-0.16	-0.09
Eu	0.03	0.10	0.01	0.17	-0.08	-0.12	-0.08	-0.23	0.04	0.17	-0.08	-0.08
Ct	0.09	0.11	0.13	0.14	-0.14	-0.11	0.04	-0.25	0.06	0.14	-0.23	-0.25

Table 4: Pearson Correlation Coefficient between MICs of EOs and Abs.

(EOs: Essential Oils; Abs: Antibiotics; Cn: Cinnamaldehyde; Ca: Carvacrol; Th: Thymol; Eu: Eugenol; Ct: Citral; CIP: Ciprofloxacin; N: Neomycin; DO: Doxycycline; Ex: Enrofloxacin; C: Chloramphenicol; Ak: Amikacin, TE: Tetracycline; GEN: Gentamicin; CN: Cefalexin; O: Oxytetracycline; TR: Trimethoprim; B: Bacitracin) tance, which can then be transmitted to humans in three different ways: firstly, through contact with animals; secondly, through the preparation and consumption of contaminated food; and thirdly due to the excretion of resistant bacteria and unmetabolized antibiotics by animals and poultry [25]. Hence, a search for a new antimicrobial against Salmonellosis is crucial to overcome these drug resistance patterns towards antibiotics. EOs obtained from various herbal plants are used to control and treat various ailments [16]. Therefore, in the present experiment, various EOs in pure form were studied for their efficacy against Salmonellosis as an alternative to antibiotics. All tested EOs in the present study revealed variable action against *S.* Typhimurium.

In the present study, statistically significant differences (as shown in Table 2) were reported between the mean MICs of EOs as well as antibiotics against *S.* Typhimurium of poultry origin. Our results revealed that the MIC of cinnamaldehyde EO was significantly lower than that of all other studied EOs. Further, the MIC of citral EO, reported in the present study was significantly higher than that of cinnamaldehyde EO. The higher value of MICs of citral EO reported in the present study might be attributed to the susceptibility of citral to oxidative degradation, which resulted in the loss of antimicrobial activity of citral under normal storage conditions [12]. Our findings were also supported by previous researchers who observed lower MICs and better antibacterial activities of cinnamaldehyde-containing EOs than that of other EOs [16,26,27].

Our results are in accordance with an earlier study [24] in which minimum inhibitory concentrations of commercial essential oils against common chicken pathogenic bacteria were studied. In that study, the cinnamaldehyde-containing essential oil (cinnamon) was reported to be most effective against chicken pathogenic bacteria than that of other EOs [24]. As we recorded, their study also revealed that the MIC values for most EOs are higher than those of conventional antibiotics. But, because of less toxicity of EOs than Abs, they are excellent alternatives to Abs [24]. Similar to antibiotic growth promoters (AGP), the productive performance of the poultry was enhanced after supplementation of cinnamaldehyde EO [28], carvacrol EO, and thymol EO [29]. Hence, apart from the therapeutic purpose they can be used as a supplement in poultry for better production.

The lower MICs of eugenol EO and higher MICs of citral EO than that of the present study were reported earlier [13]. Likewise, a higher MIC of *Cinnamomum zeylanicum* than that of our present study was also reported previously [30]. The differences observed are correlated with the bioactivity of EOs which is influenced by the oil's entire composition, including minor components and potential synergistic, additive, and antagonistic effects. Different bioactivity profiles within the same EO have been reported due to variations in the oil's chemical composition [31]. Also, the slight differences in the activity of essential oils can be attributed to the susceptibility of EOs to degradation by various external factors such as light, temperature, oxidation, or hydrolysis [4].

Mechanisms underlying the antimicrobial properties of the EOs were explained earlier [10,12,13,32]. Cinnamaldehyde is a naturally occurring aromatic aldehyde found in the bark and leaves of cinnamon trees and other species of the genus *Cinnamomum.* Cinnamaldehyde, first reacts with lipid and hydroxyl radicals, converting them into stable products via their hydrogen-donating ability. And later, these components can inhibit the production of essential enzymes by bacteria due to the presence of a carbonyl group that binds to and inactivates them and/or damages the bacteria's cell wall [13]. Carvacrol, oxygenated monoterpenoid phenol, is the major phytoconstituent of *Origanum vulgare* of the Mediterranean region, exhibits antibacterial activity by permeabilizing and depolarizing the cytoplasmic membrane, inhibits the synthesis of microbial toxins, prevents the development of biofilms, reduce the fimbriae production and anti-inflammatory actions [10]. Thymol is the natural oxygenated monoterpenoid phenol derivative of p-Cymene obtained from *Thymus citriodorus.* Thymol and carvacrol disintegrate the outer membrane of *S.* Typhimurium [32]. Eugenol is included in the phenolic group having allyl chain-substituted guaiacol, a member of the allylbenzene class of chemical compounds, which is the major phytoconstituent Syzygium *aromaticum*, can inhibit the production of essential enzymes by bacteria due to the presence of a carbonyl group that binds to and inactivates them and/or damages the bacteria's cell wall [13]. Citral is an acyclic monoterpene aldehyde, the major phytoconstituent of *Cymbopogon citrates.* Citral can disrupt and penetrate the lipid structure of bacterial cell walls. Further, citral induces protein denaturation and cell membrane destruction, followed by cytoplasmic leakage, cell lysis, and death of the bacterial cell [12].

The correlation between MICs of different pairs EOs were analyzed in the present study and is shown in table 3. If the resistance mechanism to EOs and other EOs/antibiotics/other antimicrobials is intertwined, we could expect a positive correlation between the two substances. We reported all positive correlations between all the pairs of EOs. It indicates that an increase in the MIC of one EOs is associated with increased tolerance to other EOs and vice-versa. The positive correlation between studied essential oils also suggests that they can be used in combination form for better anti-*Salmonella* activity. Combining the various major phytoconstituents together in the EOs can enhance the antibacterial properties and lower the therapeutic dose. The enhanced antimicrobial activity of the EO mixture is due to synergistic effects and is mainly attributed to aldehyde groups [3]. Thus, the combinations of EOs help to alleviate issues related to flavor, palatability, and cost when used in poultry as feed additives.

The correlation between MIC of different EOs and antibiotics reported in the present study is shown in table 4. In the present study, we reported a positive correlation between enrofloxacin and doxycycline with all studied EOs. This indicates tolerance against enrofloxacin and doxycycline of bacteria is associated with tolerance to all the studied EOs. In our study, antibiotics such as chloramphenicol, gentamicin, amikacin, bacitracin, and trimethoprim revealed a negative correlation with all five tested EOs indicating increased resistance to these antibiotics against *S.* Typhimurium is associated with increased susceptibility to the EOs. We reported significantly lower MIC for ciprofloxacin than other antibiotics. This higher sensitivity of *S.* Typhimurium to ciprofloxacin could be attributed to its limited use due to more price than other antibiotics. Further, significantly higher MICs of trimethoprim and bacitracin were reported by us. Less sensitivity of *S.* Typhimurium to trimethoprim and bacitracin is attributed to their indiscriminate use at the field level. Drug-resistant forms of Salmonellosis are evolving due to the indiscriminate use of antibiotics [33]. Antibiotics are double-edged swords, its non-rational uses in food-producing animals and poultry resulted in pressure selection of antimicrobial-resistant *S.* Typhimurium, endangering both the animal and public health [18]. Compared to other species such as bovines or porcine, the chicken showed higher antibiotic-resistance diversity to *S.* Typhimurium strains [34]. The emergence of antibiotic-resistant *S.* Typhimurium strains in humans and poultry is concerning. The two antibiotic-resistant patterns; Penta-resistant pattern ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) and tetra-resistant pattern ASSuT (ampicillin, streptomycin, sulfonamides, and tetracycline) in *S.* Typhimurium were reported earlier [34]. Antibacterial resistance in *S.* Typhimurium is mainly mediated by two mechanisms; the first one is the drug inactivation and the second mechanism is the reduction in the membrane permeability. In the first mechanism, Abs are inactivated through chemical modification using enzymes that catalyze different reactions such as acetylation, phosphorylation, and adenylation. For example, enzymes such as penicillinase and chloramphenicol acetyltransferase can acetylate the ß-lactam ring of penicillin and some cephalosporins and the two hydroxyl groups of chloramphenicol, respectively. Similarly, aminoglycoside-modifying acetyltransferase AAC(6')-Ib-cr harbors two amino acid substitutions at Trp102Arg and Asp179Tyr, which confers the ability to acetylate the unsubstituted nitrogen of the C7 piperazine ring present in several quinolones [35]. Various natural or acquired mechanisms including horizontal gene transfer, gene deletions, and mutations are responsible for the development of antimicrobial multi-resistance [36].

Contradictory to our findings, a more positive correlation between MICs of EOs containing multiple phytoconstituents and antibiotics was reported previously against poultry pathogens other than *Salmonella* spp. [24], As reported in the present study, the previous research [24] also described a more positive correlation of EOs with doxycycline than other antibiotics, indicating that increased bacterial tolerance against doxycycline is associated with increased tolerance to all the studied Eos.

As shown in table 4, we reported a negative correlation of carvacrol (10 out of 12 antibiotics) and thymol (9 out of 12 antibiotics) with antibiotics, indicating greater susceptibility of antibiotic-resistant *Salmonella* toward carvacrol and thymol. Similarly, carvacrol and thymol were reported to be most effective against antibiotic-resistant Gram-negative bacteria in the previous study [17,37]. Pronounced antimicrobial activity and anti-biofilm activity against *S. typhimurium* were shown by those essential oils having a higher content of oxygenated monoterpenoid phenol compounds [37]. This might be the reason behind the excellent anti-*Salmonella* activity reported for the carvacrol EO and thymol EO.

We reported more negative correlation (60%) between the MICs of Abs and MICs of EOs, indicating the effectiveness of EOs over antibiotic-resistant *Salmonella*. Our results supported a previous study [38] that revealed the efficacy of EOs over conventional antibiotics by studying the zone of inhibitions against MDR *S.* Typhimurium. The zone of inhibitions observed for the various Abs such as penicillin, clindamycin, amoxicillin, amphotericin, oxacillin, and trimethoprim, etc. were much lower compared to the zone of inhibition displayed by the studied EOs against MDR *S.* Typhimurium. This highlights the superiority of EOs over traditional antibiotics.

Thus, as compared to antibiotics, the studied EOs are of great significance against *S.* T*yphimurium* mainly due to two reasons. Firstly, the serious side effects of Abs on humans in long term due to antibiotic-residual effects, and secondly the development of MDR strains due to the non-rational use of antibiotics. But, ultimately it highlights the importance of determining the optimum dose and possible synergistic and additive effects of EOs in poultry to avoid the resistance of various poultry pathogens to EOs. Also, it is critical to track the effectiveness of various EOs over time.

Conclusions

It is concluded that the studied essential oils, particularly cinnamaldehyde, carvacrol, and thymol can be used against avian *S.* Typhimurium and can be commercialized as an efficient, ecological alternative to Abs for treating antibiotic resistance non-typhoid infections in poultry. Further *in vivo* studies in poultry are necessary to determine the optimum dose and toxicity of these EOs. Also, further investigation is essential to explore the synergistic effects of different EOs to enhance anti-*Salmonella* activity and reduce the dosage.

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Disclosure Statement

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Bibliography

1. Spickler AR and Leedom Larson KR. "Salmonellosis" (2013).
2. Rukambile E., *et al.* "Infection, colonization and shedding of Campylobacter and Salmonella in animals and their contribution to human disease: A review". *Zoonoses Public Health* 66 (2019): 562-578.
3. Huang Z., *et al.* "Synergistic effects of cinnamaldehyde and cinnamic acid in cinnamon essential oil against *S. pullorum*". *Industrial Crops and Products* 162 (2021): 113296.
4. Nair A., *et al.* "Nanoparticles-Attractive carriers of antimicrobial essential oils". *Antibiotics (Basel, Switzerland)* 11.1 (2022): 108.
5. Solarte AL., *et al.* "Susceptibility distribution to essential oils of *Salmonella enterica* strains involved in animal and public health and comparison of the Typhimurium and Enteritidis Serotypes". *Journal of Medicinal Food* 21.9 (2018): 946-950.
6. Crump JA and Heyderman RS. "A perspective on invasive Salmonella disease in Africa". *Clinical Infectious Diseases* 61 (2015): S235-S240.
7. Martelli G and Giacomini D. "Antibacterial and antioxidant activities for natural and synthetic dual active compounds". *European Journal of Medicinal Chemistry* 158 (2018): 91-105.
8. CDC "Antibiotic resistance threats in the United States". *Centers for Disease Control and Prevention* (2019): 114.
9. World Health Organization. "Global antimicrobial resistance and use surveillance system (GLASS) report: 2021" (2021).

10. Man A., *et al.* "Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study". *Pathogens* 8.1 (2019): 15.

11. Wendy TL., *et al.* "Synergy between essential oil components and antibiotics: A review". *Critical Reviews in Microbiology* 40.1 (2014): 76-94.

12. Wen-Chien L., *et al.* "Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil". *Journal of Food and Drug Analysis* 26.1 (2018): 82-89.

13. Ebani VV., *et al.* "*In vitro*-antimicrobial activity of essential oils against *Salmonella enterica* **serotypes Enteritidis and Typhimurium strain isolated from poultry**". *Molecules (Basel, Switzerland)* 24.5 (2019): 900.

14. Burt S. "Essential oils: Their antibacterial properties and potential applications in foods-A review". *International Journal of Food Microbiology* 94.3 (2004): 223-253.

15. Sandner G., *et al.* "Immunomodulatory activities of selected essential oils". *Biomolecules* 10.8 (2020): 1139.

16. Yasmin S., *et al.* "Phytochemical analysis and *in vitro*-activity of essential oils of selected plants against *Salmonella enteritidis* and *Salmonella gallinarum* of poultry origin". *Pakistan Veterinary Journal* 40.2 (2020): 139-144.

17. Preuss HG., *et al.* "Minimum inhibitory concentrations of herbal essential oils and monolaurin for Gram-positive and Gram-negative bacteria". *Molecular and Cellular Biochemistry* 272 (2005): 29-34.

18. Ruvalcaba-Gómez JM., *et al.* "Non-antibiotics strategies to control *Salmonella* **infection in poultry**". *Animals: An open access journal from MDPI* 12.1 (2022): 102.

19. Wilson K., *et al.* "Preparation of genomic DNA from bacteria". *In Current Protocol in Molecular Biology.* Wiley publishers, New York (1987): 2.4.1-2.4.2.

20. Nair A., *et al.* "Isolation and identification of *Salmonella* from diarrheagenic infants and young animals, sewage waste and fresh vegetables". *Veterinary World* 8.5 (2015): 669-673.

21. CLSI. "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.11th edition". *CLSI standard M07*.Clinical and Laboratory Standards Institute, Wayne, PA (2018).

22. CLSI. "Performance standards for antimicrobial susceptibility testing. 32nd edition". *CLSI Supplement M 100*. Clinical and Laboratory Standard Institute, Wayne PA, (2022).

23. Manandhar S., *et al.* "*In vitro*-antimicrobial activity of some medicinal plants against human pathogenic bacteria". *Journal of Tropical Medicine* 5 (2019): 1-5.

24. Van N., *et al.* "Minimum inhibitory concentrations of commercial essential oils against common chicken pathogenic bacteria and their relationship with antibiotic resistance". *Journal of Applied Microbiology* 132.2 (2022): 1025-1035.

25. Rau RB., *et al.* "Antimicrobial resistance of *Salmonella* **from poultry meat in Brazil: results of a nationwide survey**". *Epidemiology and Infection* 149 (2021): e228.

26. Mayaud L., *et al.* "Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics". *Letters in Applied Microbiology* 47.3 (2008): 167-173.

27. Naveed R., *et al.* **"Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against** *Salmonella* **and other multi-drug resistant bacteria**". *BMC Complementary and Alternative Medicine* 13 (2013): 265.

28. Saied AM., *et al.* "Effect of cinnamon oil supplementation into broiler chicken diets on growth, carcass traits, haemato-biochemical parameters, immune function, antioxidant status, and caecal microbial count". *Journal of Animal and Feed Sciences* 31.1 (2022): 21-33.

29. Gholami-Ahangaran M., *et al.* "Thymol and carvacrol supplementation in poultry health and performance". *Veterinary Medicine and Science* 8.1 (2022): 267-288.

30. Ebani VV., *et al.* "Chemical composition and *in vitro*-antimicrobial efficacy of sixteen essential oils against *Escherichia coli* **and** *Aspergillus fumigatus* **isolated from poultry**". *Veterinary Sciences* 5.3 (2018): 62.

31. Zhang L., *et al.* "Variation in essential oil and bioactive compounds of Curcuma kwangsiensis **collected from natural habitats**". *Chemistry and Biodiversity* 14.7 (2017): 189.

32. Lambert R., *et al.* "A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol, and carvacrol". *Journal of Applied Microbiology* 91.3 (2001): 453-462.

33. Kumar P., *et al.* "Salmonellosis in commercial layer poultry birds in certain parts of India: Prevalence and antibiotic resistance patterns". *Indian Journal of Animal Health* 54 (2015): 139.

34. Wang X., *et al.* "Antibiotic resistance in *Salmonella* **Typhimurium isolates recovered from the food chain through national antimicrobial resistance monitoring system between 1996 and 2016**". *Frontiers in Microbiology* 10 (2019): 985.

35. Castro-Vargas R. E., *et al.* "Antibiotic resistance in *Salmonella* **spp. isolated from poultry: A global overview**". *Veterinary World* 13.10 (2020): 2070-2084.

36. Sharma VK., *et al.* "A review of the influence of treatment strategies on antibiotic-resistant bacteria and antibiotic resistance genes". *Chemosphere* 150. (2016): 702-714.

37. Guillín Y., *et al.* "Effect of essential oils on the inhibition of biofilm and quorum sensing in *Salmonella* Enteritidis 13076 and *Salmonella* Typhimurium 14028". *Antibiotics (Basel, Switzerland)* 10.10 (2021): 1191.

38. Helal IM., *et al.* "Antimicrobial efficiency of essential oils from traditional medicinal plants of Asir region, Saudi Arabia, over drug-resistant isolates". *BioMed Research International* (2019): 8928306.