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(Research Article)

Comparative Evaluation of the Antimicrobial Activity of Crude Ethanolic Extracts of Local Medicinal Plants Against *Escherichia coli* and *Salmonella* spp

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Abstract

The increasing global threat of antimicrobial resistance (AMR) necessitates the exploration of alternative antibacterial agents derived from natural sources. This study evaluated the comparative antimicrobial activity of crude ethanolic extracts of turmeric (*Curcuma longa*), hibiscus (*Hibiscus rosa-sinensis*), croton (*Croton* spp.), ginger (*Zingiber officinale*), and oleander (*Nerium oleander*) against two Gram-negative pathogens, *Escherichia coli* and *Salmonella* spp. Antimicrobial efficacy was assessed using the agar disk diffusion method, and inhibition zones were statistically analyzed using independent samples t-tests and two-way ANOVA to determine strain-specific and pigment-specific effects. The overall grand mean inhibition zone across treatments was 13.45 mm, indicating moderate antibacterial activity. *Salmonella* spp. (14.35 mm) exhibited greater overall susceptibility than *E. coli* (12.54 mm). Turmeric demonstrated the highest antimicrobial activity (19.63 mm against *Salmonella* spp.; 16.66 mm against *E. coli*), followed by the tri colour croton variant. Red hibiscus showed balanced moderate activity against both strains, while oleander and ginger exhibited comparatively lower inhibition. Statistically significant strain-specific differences were observed only for pink hibiscus and red oleander extracts. Although most plant extracts demonstrated biologically relevant inhibition, variability and limited replication constrained statistical significance in several comparisons. The findings highlight the influence of phytochemical composition and bacterial susceptibility patterns on antimicrobial outcomes and support the continued investigation of plant-derived pigments, particularly turmeric and croton, as potential complementary agents in addressing antimicrobial resistance.

Keywords: Antimicrobial Activity; Antimicrobial resistance; Medicinal plants; *Escherichia coli*; *Salmonella* spp.; Kirby-Bauer method; Food preservation; Healthcare management

1. Introduction

The rapid escalation of antimicrobial resistance (AMR) represents one of the most critical public health threats of the twenty-first century. The widespread misuse and overuse of antibiotics have accelerated the emergence of multidrug-resistant bacterial strains, significantly compromising the efficacy of conventional antimicrobial therapies [31]. Pathogens such as *Escherichia coli* and *Salmonella* spp. are of particular concern due to their global distribution and association with gastrointestinal infections, urinary tract infections, septicemia, and other systemic diseases [5]. According to the World Health Organization (2021) [33], AMR currently contributes to approximately 700,000 deaths annually worldwide, a figure projected to increase dramatically without effective intervention. This crisis underscores

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the urgent need to identify innovative and sustainable antimicrobial strategies that can either complement or replace existing synthetic antibiotics [23, 31].

In response to this global health emergency, scientific attention has increasingly turned toward plant-derived bioactive compounds, which have historically served as valuable sources of therapeutic agents. Natural products are recognized for their structural diversity, biological compatibility, and lower propensity to induce resistance compared to many synthetic drugs [21] [31, 32]. Among these, plant-based pigments extracted from medicinal species such as hibiscus, turmeric, croton, ginger, and oleander have attracted considerable research interest. These plants contain diverse phytochemicals, including flavonoids, phenolics, terpenoids, and curcuminoids, reported to exhibit antimicrobial, antioxidant, and anti-inflammatory activities [27, 32]. Their traditional medicinal use, coupled with emerging scientific validation, positions them as promising candidates in the search for alternative antimicrobial agents.

Despite increasing recognition of plant-derived pigments as potential antimicrobial compounds, a critical knowledge gap persists regarding their comparative efficacy against major bacterial pathogens such as *E. coli* and *Salmonella* spp. Although isolated studies have reported antimicrobial activity in these species, systematic evaluation and quantitative comparison using standardized microbiological techniques remain limited. The Kirby-Bauer disk diffusion method, widely accepted for assessing antimicrobial susceptibility, provides a reliable approach for measuring inhibitory effects through zone-of-inhibition analysis [11]. However, comparative data detailing the inhibitory profiles of these specific plant pigments against clinically relevant enteric pathogens are insufficient.

The increasing prevalence of AMR, particularly among *E. coli* and *Salmonella* spp., has rendered many conventional antibiotics less effective, intensifying the global burden of infectious diseases [5, 33]. While plant-based pigments demonstrate antimicrobial potential, there is limited empirical evidence directly comparing their efficacy against these multidrug-resistant bacteria. This lack of comparative and quantitative data restricts the development of validated plant-based antimicrobial therapies. Therefore, the central research problem addressed in this study is the need to systematically evaluate and compare the antimicrobial efficacy of crude ethanolic extracts of selected plant pigments against these clinically significant pathogens.

Foodborne diseases remain a persistent global public health concern, largely driven by pathogenic microorganisms such as *Escherichia coli* and *Salmonella* spp., which are frequently implicated in outbreaks linked to contaminated food products. The increasing resistance of these pathogens to conventional antibiotics and synthetic preservatives has intensified the search for safer, sustainable, and naturally derived antimicrobial alternatives. In this context, plant-derived natural pigments have emerged as promising candidates due to their intrinsic bioactive properties, multifunctionality, and consumer acceptance in food systems. Beyond their coloring capacity, these compounds exhibit antimicrobial, antioxidant, and anti-inflammatory activities that may contribute to improved food safety and shelf-life extension.

Among the most extensively studied pigment-producing plants are *Hibiscus sabdariffa* (Hibiscus), *Curcuma longa* (Turmeric), *Croton tiglium* (Croton), *Zingiber officinale* (Ginger), and *Nerium oleander* (Oleander). These plants are rich in biologically active pigments, including anthocyanins, curcumin, carotenoids, gingerols, and flavonoids, respectively. Scientific investigations have increasingly focused on evaluating their antimicrobial efficacy against foodborne pathogens, particularly *E. coli* and *Salmonella* spp., which are major contributors to gastrointestinal infections and food safety concerns.

Extracts of *H. sabdariffa*, particularly those containing anthocyanins, have demonstrated significant inhibitory effects against *E. coli*. Ghasemzadeh *et al.* (2018) [14] reported notable bactericidal activity, suggesting that anthocyanin-rich hibiscus extracts may serve as natural antimicrobial agents in food preservation. Supporting evidence indicates that anthocyanins exert their antibacterial effects primarily by disrupting bacterial membrane integrity, thereby preventing microbial proliferation [7]. However, the stability of anthocyanins under varying pH and temperature conditions remains a limitation, potentially reducing their effectiveness in processed food systems [27].

Similarly, curcumin, the principal pigment of *C. longa*, has been widely recognized for its antimicrobial properties. Choi *et al.* (2020) [10] demonstrated that curcumin compromises the structural integrity of *Salmonella* cell walls, resulting in bacterial cell death. Additional studies confirm that curcumin interferes with membrane permeability and cellular respiration, inhibiting bacterial growth [19]. Despite its strong antimicrobial potential, curcumin's limited bioavailability due to poor solubility and absorption presents a significant challenge for its practical application in food systems [2].

Carotenoid-rich extracts from *C. tiglium* have also exhibited antibacterial activity against both *E. coli* and *Salmonella* spp. Ali *et al.* (2019) [6] attributed this effect to the presence of phytochemicals capable of disrupting microbial cellular functions. Mechanistic insights suggest that carotenoids induce membrane destabilization and oxidative stress within bacterial cells [16]. Nevertheless, concerns regarding potential toxicity at higher concentrations necessitate careful evaluation of safe application levels, particularly for food-related uses [6].

The antimicrobial efficacy of gingerols from *Z. officinale* has been demonstrated against *E. coli*, with studies indicating that these compounds disrupt bacterial membranes and inhibit growth [18, 29]. Ginger extracts offer the added advantage of flavor enhancement in certain food products. However, their strong aroma and taste may limit broad applicability, and variability in gingerol concentration among cultivars may affect consistency of antimicrobial performance [17, 18].

In contrast, while *N. oleander* extracts have shown antimicrobial activity against *Salmonella* spp. and other pathogens [25], their application is constrained by inherent toxicity. The antibacterial activity is largely associated with flavonoid content [38], yet toxicological risks present significant barriers to food-related utilization. Strict safety assessments and dosage control would be required before considering practical implementation.

Collectively, the existing literature establishes that natural plant pigments possess significant antimicrobial potential against major foodborne pathogens. However, it also reveals critical limitations, including instability under processing conditions, poor bioavailability, sensory constraints, variability in phytochemical composition, and safety concerns, particularly with *N. oleander*. Furthermore, inconsistencies in extraction methods and the absence of standardized comparative evaluations limit the translation of laboratory findings into real-world food systems.

Therefore, although substantial evidence supports the antibacterial efficacy of anthocyanins, curcumin, carotenoids, gingerols, and flavonoids, further research is required to optimize extraction techniques, enhance compound stability, standardize bioactive concentrations, and comprehensively assess safety. Addressing these gaps is essential to enable the effective and safe integration of plant-derived natural pigments into food preservation strategies. By advancing understanding of their mechanisms of action and practical limitations, this research contributes to the development of sustainable, plant-based antimicrobial alternatives capable of improving food safety while meeting growing consumer demand for natural products.

This research holds substantial scientific and practical significance by generating comparative data on plant-derived pigments. This study contributes to expanding knowledge on natural antimicrobial agents and addresses a critical gap in the literature concerning their efficacy against resistant pathogens. The findings may support the development of novel phytotherapeutic formulations capable of complementing or substituting synthetic antibiotics, thereby mitigating resistance development [31].

Moreover, the investigation aligns with the growing emphasis on sustainability in healthcare and pharmaceutical innovation, promoting plant-based solutions that reduce environmental impacts associated with synthetic antibiotic production [12, 31]. The results may inform healthcare policy, pharmaceutical development, and food preservation strategies, responding to increasing consumer demand for natural and sustainable antimicrobial alternatives. Ultimately, by harnessing biodiversity to address the AMR crisis, this study contributes to advancing antimicrobial research and supporting global health resilience in an era of escalating antibiotic resistance [31].

This study aims to evaluate and compare the antimicrobial activity of crude ethanolic extracts derived from local medicinal plants (hibiscus, turmeric, croton, ginger, and oleander) against *E. coli* and *Salmonella* spp. The specific objectives that guides this study are: (1) To identify the natural pigment exhibiting the highest antimicrobial activity against the selected bacterial strains. (2) To quantify the antimicrobial effects using the standardized Kirby-Bauer disk diffusion assay. (3) To measure and analyze the zones of inhibition surrounding pigment-impregnated discs to characterize inhibitory profiles.

The research hypotheses that guides this study are as follow:

- H_0: There is no statistically significant difference in the antimicrobial activity (measured by mean inhibition zone diameter) of crude ethanolic extracts of Hibiscus, Turmeric, Croton, Ginger, and Oleander against *Escherichia coli* and *Salmonella* spp., and bacterial strain does not significantly influence susceptibility to these plant pigments.
- H_1: There is a statistically significant difference in the antimicrobial activity of crude ethanolic extracts of Hibiscus, Turmeric, Croton, Ginger, and Oleander against *Escherichia coli* and *Salmonella* spp., and bacterial

strain significantly influences susceptibility, with certain plant pigments exhibiting greater antibacterial efficacy against one strain compared to the other.

2. Methodology

2.1. Conceptual Framework

The conceptual framework of this study as shown on Figure 1, effectively integrates the methodological, analytical, and biological components necessary to evaluate the antimicrobial potential of plant pigments. It illustrates a clear progression from independent variables, pigment type and bacterial strain, to dependent outcomes, primarily inhibition zone diameters. The inclusion of control groups, both positive (ciprofloxacin) and negative (ethanol), establishes baselines for comparison, ensuring that observed effects are attributable to phytochemical activity rather than experimental artifacts. Randomization, replication, and standardized assay conditions are explicitly embedded within the framework, reinforcing internal validity and reproducibility. Overall, the framework visually emphasizes the causal relationship between plant-derived pigments and their inhibitory effects on *E. coli* and *Salmonella* spp., while highlighting statistical rigor and methodological consistency.

Additionally, the framework underscores the analytical linkage between observed antimicrobial activity and the bioactive compounds present in each pigment, such as curcuminoids, anthocyanins, flavonoids, and phenolics. By integrating data collection and statistical evaluation, including ANOVA and independent t-tests, the framework not only captures biological outcomes but also emphasizes evidence-based interpretation. This holistic depiction facilitates understanding of how experimental design choices, such as solvent selection, extraction methods, and incubation conditions, impact the validity of results. Moreover, the conceptual framework provides a foundation for extending research into broader applications, such as therapeutic, food preservation, or complementary antimicrobial strategies, and guides systematic exploration of pigment-bacteria interactions in both laboratory and applied settings.

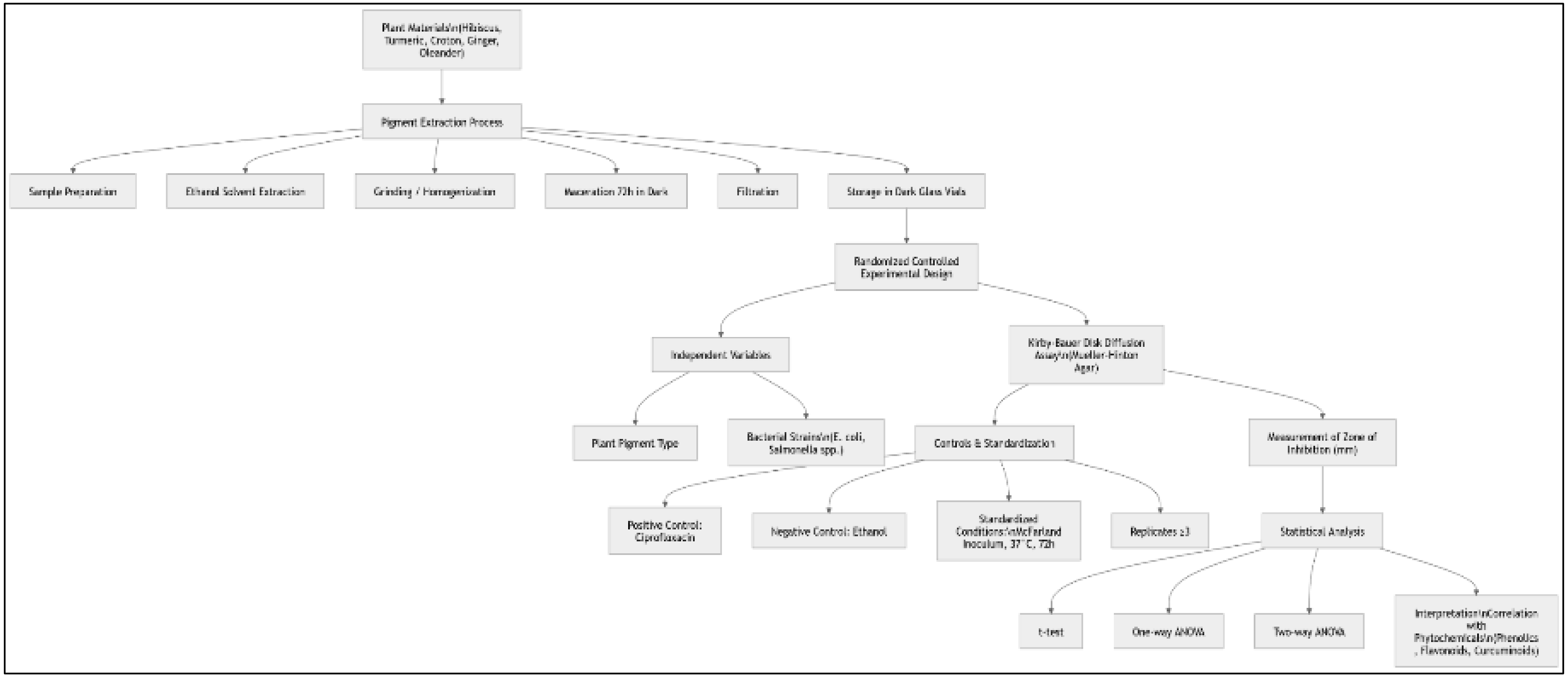

Figure 1 Conceptual Framework of Research

3. Experimental Design

The present study employed a randomized controlled laboratory experimental design to systematically evaluate and compare the antimicrobial efficacy of natural pigments derived from Hibiscus, Turmeric, Croton, Ginger, and Oleander against *Escherichia coli* and *Salmonella* spp. The use of a randomized controlled framework strengthens internal validity by minimizing allocation bias and reducing the influence of confounding variables, thereby ensuring that observed differences in inhibition zones can be more confidently attributed to pigment treatment effects rather than experimental artifacts. Randomization of treatment application and standardized assay conditions (e.g., inoculum density, incubation time, and diffusion medium) enhance reproducibility and comparability across pigment-bacteria combinations. In antimicrobial screening studies, such methodological rigor is critical because variations in environmental parameters, extract concentration, and microbial load can substantially influence diffusion-based outcomes.

The experimental design was particularly appropriate given the factorial nature of the investigation, involving multiple independent variables, plant pigment type and bacterial strain, and a quantitative dependent variable (zone of inhibition diameter). By structuring the study as a controlled comparison, it enabled direct evaluation of both pigment-specific effects and strain-specific susceptibility patterns under uniform laboratory conditions. This approach facilitates robust statistical analysis, including independent samples t-tests and two-way ANOVA, allowing assessment of main effects and potential interactions between pigment source and bacterial strain. Moreover, the inclusion of control treatments (positive and negative controls) provides an essential benchmark for interpreting antimicrobial potency relative to established standards. Collectively, the randomized controlled laboratory design ensures methodological reliability, strengthens causal inference, and provides a scientifically sound framework for evaluating the antimicrobial potential of plant-derived pigments in the context of emerging resistance among Gram-negative pathogens. This design was selected as the most appropriate approach for several reasons as showed on Table 1.

Table 1 Key Aspects of Experimental Design and their Descriptions

Key Experimental Design Aspect	Scientific and Analytical Description
Randomization	Randomization of treatment application order was implemented to minimize systematic bias and reduce the influence of uncontrolled confounding variables. By ensuring that pigment extracts were applied in a randomized sequence, the study mitigated potential positional, temporal, or operator-related effects that could otherwise distort inhibition zone measurements. This methodological control strengthens internal validity and enhances confidence that observed antimicrobial differences are attributable to treatment effects rather than procedural variability.
Control Group	The inclusion of both negative (ethanol solvent-only discs) and positive (ciprofloxacin) controls provided essential comparative benchmarks. The negative control verified that any observed inhibition was due to bioactive compounds rather than solvent effects, thereby isolating phytochemical activity. The positive control established a standardized antimicrobial reference, enabling comparative interpretation of pigment efficacy relative to a clinically established antibiotic. This dual-control framework enhances interpretive rigor and supports causal inference regarding antimicrobial potency.
Replicability	Standardization of experimental conditions, including inoculum density, agar medium composition, incubation parameters, and disc preparation, ensured consistency across trials. Such procedural uniformity reduces experimental noise and enhances reproducibility, a critical component of laboratory-based antimicrobial screening. By minimizing variability unrelated to treatment effects, the study improves reliability and facilitates potential replication in independent laboratories.
Statistical Rigor	The experimental structure supported inferential statistical analyses, including Independent Samples t-tests and two-way ANOVA, enabling quantitative evaluation of treatment effects and strain-specific responses. Statistical testing allowed differentiation between numerical variation and statistically significant differences while accounting for within-group variability. This analytical framework strengthens the scientific credibility of findings and provides objective validation of antimicrobial trends observed in the dataset.

3.1. Sample Preparation

Local medicinal plant materials (leaves, flowers, roots, and stems) were collected and preprocessed. Pigments were extracted using ethanol as the appropriate solvent. Table 2 summarize the procedure of pigment extraction.

Table 2 Pigment Extraction Procedure

Step	Procedure	Scientific and Analytical Description
1	Sample Preparation	Fresh plant materials were systematically collected and prepared to ensure representative sampling and minimize degradation prior to extraction. Proper handling at this stage is critical to preserve phytochemical integrity, as environmental exposure (light, heat, oxidation) can alter pigment composition and concentration. Uniform sample preparation enhances comparability across treatments and reduces variability attributable to pre-extraction factors.
2	Solvent Selection	Ethanol was selected as the extraction solvent due to its intermediate polarity, which enables efficient solubilization of a broad spectrum of bioactive compounds, including phenolics, flavonoids, curcuminoids, and certain carotenoids. Ethanol is also relatively safe, volatile, and compatible with antimicrobial assays, minimizing interference in disc diffusion testing. The solvent choice directly influences extraction efficiency, selectivity, and downstream biological activity, making it a critical methodological consideration.
3	Grinding or Homogenization	Mechanical grinding or homogenization of plant tissues in ethanol increased surface area and disrupted cellular structures, facilitating the release of intracellular pigments and secondary metabolites. This step enhances mass transfer between plant matrix and solvent, thereby improving extraction yield and consistency. Adequate homogenization reduces heterogeneity in phytochemical concentration and supports reproducibility across replicates.
4	Extraction Process (72 hours, dark conditions)	The maceration process was conducted over 72 hours to allow sufficient diffusion of pigments into the solvent. Incubation in dark conditions minimized photodegradation of light-sensitive compounds such as anthocyanins and curcuminoids. Extended extraction time increases yield; however, it must be balanced against the risk of compound oxidation or degradation, underscoring the importance of controlled environmental parameters.
5	Filtration	Filtration separated plant residues from the pigment-rich ethanolic extract, yielding a clarified solution suitable for antimicrobial testing. This step prevents interference from particulate matter during disc diffusion assays and ensures accurate measurement of inhibition zones. Proper filtration contributes to assay precision and reduces variability caused by inconsistent extract application.
6	Storage	Extracts were stored in dark glass vials at low temperatures to limit photochemical and oxidative degradation. Protection from light and heat preserves bioactive compound stability, ensuring that antimicrobial activity reflects inherent phytochemical properties rather than degradation artifacts. Controlled storage conditions are essential for maintaining consistency between experimental trials and enhancing the reliability of comparative analyses.

3.2. Bacterial Strains

Clinical laboratory strains of *Escherichia coli* and *Salmonella* spp. were obtained as pure cultures from the Georgetown Public Hospital Corporation (GPHC) laboratory. The use of clinically sourced strains enhances the translational relevance of the study by reflecting pathogens of public health significance rather than environmental isolates. Cultivation on Mueller–Hinton Agar (MHA), the standardized medium recommended for antimicrobial susceptibility testing, ensured uniform nutrient composition and diffusion characteristics, thereby supporting methodological comparability with established clinical protocols.

3.3. Assay Method (Kirby–Bauer Disk Diffusion)

The Kirby-Bauer disk diffusion method was employed as a standardized and globally recognized procedure for evaluating antimicrobial susceptibility. Bacterial suspensions were adjusted to the McFarland turbidity standard to ensure consistent inoculum density across plates, thereby reducing variability in inhibition zone measurements. Standardization at this stage is critical, as deviations in bacterial concentration can significantly influence zone diameters and lead to misinterpretation of antimicrobial potency. The method enables quantitative comparison of inhibition zones while maintaining procedural simplicity and reproducibility.

3.4. Intervention and Control

Sterile filter paper disks were impregnated with respective pigment extracts to evaluate antimicrobial activity. Inclusion of a solvent-only negative control (ethanol) confirmed that observed inhibition zones were attributable to phytochemical constituents rather than solvent effects. A positive control disk containing a standard antibiotic (ciprofloxacin) provided a clinically validated benchmark for comparative efficacy assessment. This controlled intervention design strengthened internal validity, enabled direct comparison between natural extracts and conventional treatment, and supported rigorous statistical evaluation of treatment effects. Table 3 describes the control groups that were used in this study.

Table 3 Control Groups Used in the Study

Control Type	Description	Example Setup
Positive Control	Known antimicrobial agent was used to demonstrate activity.	Culture with Ciprofloxacin
Negative Control	No antimicrobial agent was used to verify growth conditions.	Culture with ethanol only (no treatment)

3.5. Incubation Conditions

Inoculated Mueller-Hinton agar plates were incubated at 37 °C for 72 hours to promote optimal bacterial growth and allow sufficient diffusion of phytochemical constituents into the agar matrix. The selected temperature reflects the physiological optimum for enteric pathogens, thereby enhancing clinical relevance. The extended incubation period permitted stable and clearly demarcated inhibition zones; however, prolonged incubation was carefully controlled to avoid overgrowth that could obscure accurate zone measurement.

3.6. Data Collection

Following incubation, zones of inhibition were measured in millimeters using a calibrated ruler or digital caliper to ensure precision. Recording quantitative zone diameters enabled objective comparison across treatments and minimized observer bias. Standardized measurement protocols reduced intra- and inter-experimental variability, thereby strengthening the accuracy and repeatability of antimicrobial assessments.

3.7. Statistical Analysis

Inferential statistical analyses, including one-way and two-way ANOVA as well as Independent Samples t-tests, were employed to determine significant differences in antimicrobial efficacy among pigment extracts and between bacterial species. ANOVA facilitated multi-group comparison while accounting for variance within and between treatments, whereas t-tests enabled direct pairwise comparisons. This analytical framework ensured that observed differences were statistically validated rather than attributed to random variation.

3.8. Replicates

A minimum of three replicates per treatment group was maintained to enhance statistical power and reliability. Replication reduces the impact of experimental anomalies and increases confidence in the consistency of observed antimicrobial trends. Adequate replication also supports more robust variance estimation, thereby improving the validity of inferential statistical conclusions.

3.9. Documentation and Analytical Interpretation

Comprehensive documentation of all procedures, raw measurements, and analytical outputs ensured transparency and traceability of results. Comparative efficacy was interpreted alongside known phytochemical profiles of the extracts, enabling correlation between observed antimicrobial activity and bioactive compounds such as phenolics, curcuminoids, and flavonoids. This integrative analytical approach strengthened the scientific coherence of the study and ensured that conclusions were evidence-based, reproducible, and aligned with the stated research objectives.

4. Results

4.1. Antimicrobial Activity of Plant Pigments Against Bacterial Strains

Table 4 *Salmonella* spp. Inhibition Zones (mm)

Plant Pigment	Trial 1	Trial 2	Trial 3	Average
Turmeric	22.75	19.22	16.92	19.63
Ginger	18.73	13.32	10.00	14.02
Red Hibiscus	19.24	14.26	11.92	15.14
Pink Hibiscus	10.44	10.05	8.63	9.71
Red Oleander	14.62	13.06	12.72	13.47
Pink Oleander	8.36	7.75	7.42	7.84
Yellow & Green Croton	20.15	17.59	9.49	15.74
Red, Yellow & Green Croton	18.89	17.44	16.22	17.52
Control 1 (Ethanol)	9.78	-	-	9.78
Control 2 (Ciprofloxacin)	38.13	-	-	38.13

The antimicrobial activity of the evaluated plant pigments against *Salmonella* spp. demonstrated clear variation in inhibitory efficacy, as measured by zones of inhibition across three independent trials (Table 4). Among all treatments, turmeric exhibited the highest mean inhibition zone (19.63 mm), with relatively consistent performance across trials (22.75 mm, 19.22 mm, and 16.92 mm). This narrow range suggests reproducibility and stability of antimicrobial action, likely attributable to the bioactive compound curcumin present in *Curcuma longa*. The consistently high inhibition zones indicate strong bacteriostatic or bactericidal potential against *Salmonella* spp., positioning turmeric as the most effective natural extract evaluated in this study.

Croton variants also demonstrated substantial antimicrobial effects. The red, yellow, and green croton extract recorded a mean inhibition zone of 17.52 mm (18.89 mm, 17.44 mm, 16.22 mm), reflecting both high efficacy and low inter-trial variability. Similarly, the yellow and green croton variant yielded a slightly lower mean of 15.74 mm; however, its results displayed notable variability (20.15 mm, 17.59 mm, 9.49 mm), suggesting possible inconsistencies in pigment concentration or extract potency across trials. Although both croton treatments showed strong inhibitory potential, the multicolored (red-inclusive) croton variant demonstrated greater stability and reliability, implying that pigment composition, particularly the presence of red-associated phytochemicals, may enhance antimicrobial effectiveness.

Moderate antimicrobial activity was observed in red hibiscus (15.14 mm), ginger (14.02 mm), and red oleander (13.47 mm). Red hibiscus exhibited some variability between trials (19.24 mm, 14.26 mm, 11.92 mm), indicating moderate reproducibility. Ginger showed a progressive decline across trials (18.73 to 10.00 mm), which may reflect compound degradation or variation in extract diffusion capacity. Red oleander demonstrated comparatively stable but lower inhibition zones (14.62 mm, 13.06 mm, 12.72 mm), suggesting consistent yet moderate antibacterial activity. These findings indicate that while these extracts possess inhibitory properties, their efficacy is inferior to turmeric and croton pigments.

Lower antimicrobial performance was recorded for pink hibiscus (9.71 mm) and pink oleander (7.84 mm). Importantly, the mean inhibition zone of pink hibiscus (9.71 mm) was nearly equivalent to the negative control (ethanol: 9.78 mm), raising questions regarding the biological significance of its inhibitory effect. Pink oleander produced inhibition zones only marginally below the solvent control, suggesting weak antibacterial potency. The marked difference between red and pink pigment variants across both hibiscus and oleander supports the observation that pigment coloration, and by extension, phytochemical composition, significantly influences antimicrobial efficacy. Red variants consistently outperformed pink counterparts, indicating that anthocyanin concentration or associated phenolic compounds may contribute substantially to antibacterial activity.

All plant pigments, with the exception of pink oleander, either exceeded or closely approximated the inhibition zone produced by the negative control, confirming that observed antibacterial effects were not solely attributable to solvent action. However, the magnitude of inhibition produced by all plant extracts remained substantially lower than that of the positive control, ciprofloxacin (38.13 mm), a broad-spectrum fluoroquinolone antibiotic. The nearly two-fold greater inhibition zone of ciprofloxacin highlights the superior potency of synthetic antimicrobial agents while simultaneously contextualizing the moderate yet meaningful activity of natural pigments.

Overall, the data demonstrate that turmeric and croton extracts exhibit the most promising antimicrobial potential against *Salmonella* spp. among the tested pigments, with turmeric showing the highest efficacy and reliable reproducibility. The results further emphasize the importance of pigment composition, particularly the enhanced performance of red variants, and underscore variability in trial consistency among certain extracts. These findings support continued investigation into turmeric and croton-derived bioactive compounds as potential natural antimicrobial agents, particularly in applications related to food preservation and alternative therapeutic development.

Table 5 *Escherichia coli.* Inhibition Zones (mm)

Plant Pigment	Trial 1	Trial 2	Trial 3	Average
Turmeric	18.01	17.97	13.99	16.66
Ginger	11.98	10.32	8.04	10.11
Red Hibiscus	15.93	15.04	13.53	14.83
Pink Hibiscus	12.53	11.83	10.90	11.75
Red Oleander	9.26	8.20	6.99	8.15
Pink Oleander	12.42	9.36	8.67	10.15
Yellow & Green Croton	11.48	9.37	9.01	9.95
Red, Yellow & Green Croton	17.32	13.30	13.18	14.60
Control 1 (Ethanol)	9.75	-	-	9.75
Control 2 (Ciprofloxacin)	5.84	-	-	5.84

The antimicrobial assay against *Escherichia coli* revealed differential inhibitory responses among the tested plant pigments, with clear stratification in efficacy across treatments and trials. Turmeric demonstrated the highest mean inhibition zone (16.66 mm), with values of 18.01 mm, 17.97 mm, and 13.99 mm across the three trials. Although a slight decline was observed in the third trial, the relatively narrow range confirms turmeric as the most consistent and potent natural antimicrobial agent evaluated. The sustained performance of turmeric, derived from *Curcuma longa*, across both *E. coli* and *Salmonella* spp. suggests broad-spectrum antibacterial potential, likely associated with curcuminoids and related phenolic compounds that disrupt bacterial membrane integrity and metabolic pathways.

Red hibiscus ranked second in antimicrobial performance, with a mean inhibition zone of 14.83 mm (15.93 mm, 15.04 mm, 13.53 mm), demonstrating steady and reproducible activity across trials. The relatively low variability indicates stable extract potency and reliable diffusion characteristics in the agar medium. Similarly, the red, yellow, and green croton extract produced a comparable mean inhibition zone of 14.60 mm, although greater inter-trial variability (17.32 mm, 13.30 mm, 13.18 mm) suggests possible fluctuations in active phytochemical concentration or extract uniformity. Collectively, these high-performing pigments exhibit moderate-to-strong inhibitory effects against *E. coli*, though consistently inferior to turmeric.

Moderate antimicrobial activity was observed among pink hibiscus (11.75 mm), ginger (10.11 mm), pink oleander (10.15 mm), and yellow and green croton (9.95 mm). Pink hibiscus displayed relatively stable inhibition zones (12.53 mm, 11.83 mm, 10.90 mm), suggesting consistent but modest antibacterial efficacy. Ginger and pink oleander showed declining inhibition across trials, potentially indicating compound instability, degradation over time, or experimental variability affecting extract potency. Yellow and green croton demonstrated inhibition zones only marginally above the solvent control, reflecting limited biological effectiveness. Red oleander was the lowest-performing pigment overall

(8.15 mm), with inhibition zones falling below the negative control in some trials, indicating minimal or negligible antibacterial activity against *E. coli*.

A critical and unexpected finding in this dataset is the comparatively low inhibition zone recorded for the positive control, ciprofloxacin (5.84 mm). As a fluoroquinolone antibiotic widely recognized for its efficacy against Gram-negative bacteria, including *E. coli*, such a reduced inhibition zone is atypical. Notably, the majority of plant pigments exceeded both the negative control (ethanol: 9.75 mm) and the positive control. This outcome warrants careful scrutiny. Potential explanations include degradation or improper storage of the antibiotic disc, incorrect dosage concentration, agar diffusion anomalies, or the presence of strain-specific resistance mechanisms in the tested *E. coli* isolate. Given ciprofloxacin's established antimicrobial potency, methodological verification and repeat testing are strongly recommended before drawing definitive comparative conclusions regarding natural extract superiority.

Comparative strain analysis reveals subtle but meaningful differences in pigment effectiveness relative to previous *Salmonella* spp. results. Red hibiscus demonstrated nearly equivalent inhibition against both organisms, indicating broad and stable antimicrobial properties. Pink hibiscus showed improved relative performance against *E. coli*, suggesting differential susceptibility between the two Gram-negative species. Conversely, red oleander exhibited markedly reduced activity against *E. coli*, highlighting species-specific variability in antibacterial response. Although color variant differences remain evident, particularly the generally stronger performance of red pigments compared to pink variants, the magnitude of these differences is less pronounced than observed with *Salmonella* spp. This suggests that pigment-associated phytochemical effects may interact differently with distinct bacterial cell wall structures or resistance mechanisms.

Trial-to-trial variability was observed in several treatments, with some extracts demonstrating progressive declines in inhibition zones. Such patterns may reflect phytochemical instability, oxidation, volatilization of active compounds, or variations in extract preparation and diffusion rates. These findings underscore the importance of standardizing extraction protocols and conducting stability analyses in future investigations.

Overall, turmeric remains the most effective natural antimicrobial agent against *E. coli*, confirming its broad-spectrum potential. However, the most striking and scientifically significant observation is the unusually low activity of ciprofloxacin relative to plant extracts. While the data suggest promising antibacterial properties among several natural pigments, particularly turmeric and red hibiscus, methodological validation is essential before attributing superior efficacy to these extracts over conventional antibiotics. Further investigation into antibiotic potency, strain resistance profiling, and minimum inhibitory concentration (MIC) testing is recommended to substantiate these findings.

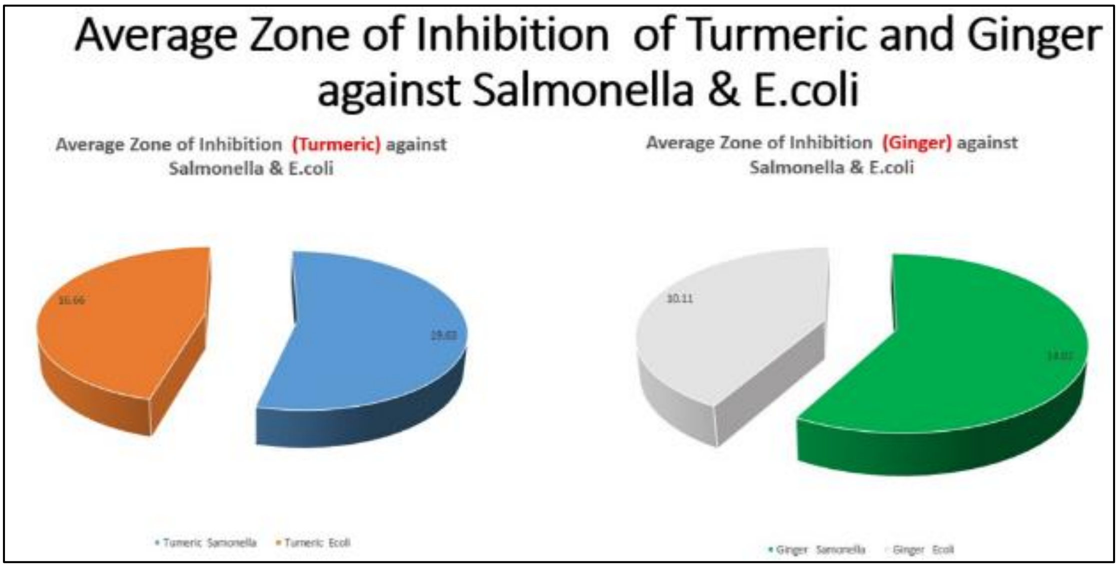

Figure 2 Average zone of Inhibition of Turmeric and Ginger against *Salmonella* spp. and *Escherichia coli*

As shown on Figure 2, both turmeric and ginger show antibacterial activity against *Salmonella* spp. and *E. coli,* with turmeric demonstrating stronger inhibition against *E. coli* (16.66 mm) compared to *Salmonella* spp. (19.63 mm), while ginger showed more balanced but slightly weaker activity against both bacterial species (14.02 mm and 10.11 mm respectively).

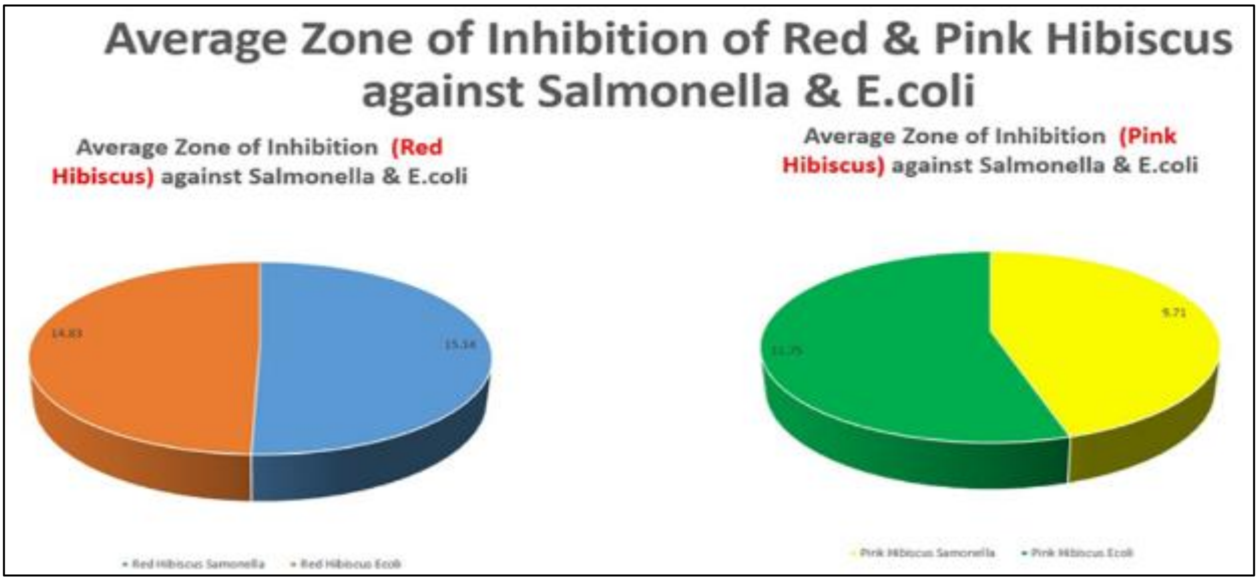

Figure 3 Average zone of Inhibition of Red Hibiscus and Pink Hibiscus against *Salmonella* spp. and *Escherichia coli*

Figure 3 shows that red hibiscus has similar antibacterial activity against both *Salmonella* spp. (15.14 mm) and *E. coli* (14.83 mm), while pink hibiscus demonstrates stronger inhibition against *E. coli* (11.75 mm) compared to *Salmonella* spp. (9.71 mm), with red hibiscus showing greater antibacterial effectiveness than pink hibiscus.

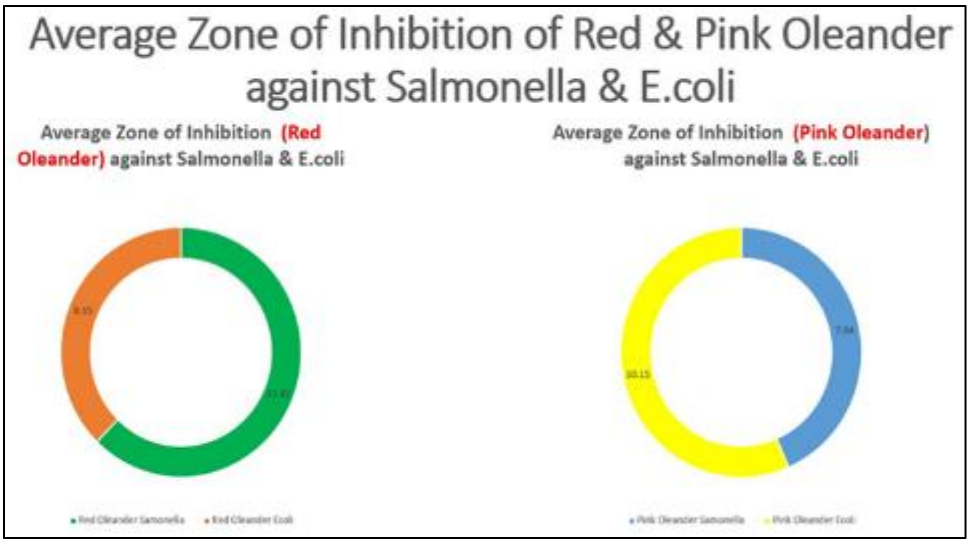

Figure 4 Average zone of Inhibition of Red Oleander and Pink Oleander against *Salmonella* spp. and *Escherichia coli*

As displayed on Figure 4, red oleander stronger antibacterial activity against *Salmonella* spp. (13.02 mm) than *E. coli* (8.15 mm), while pink oleander demonstrates the opposite patterns with greater effectiveness against *E. coli* (10.15 mm) compared to *Salmonella* spp. (7.84 mm), with red oleander overall showing superior antibacterial performance.

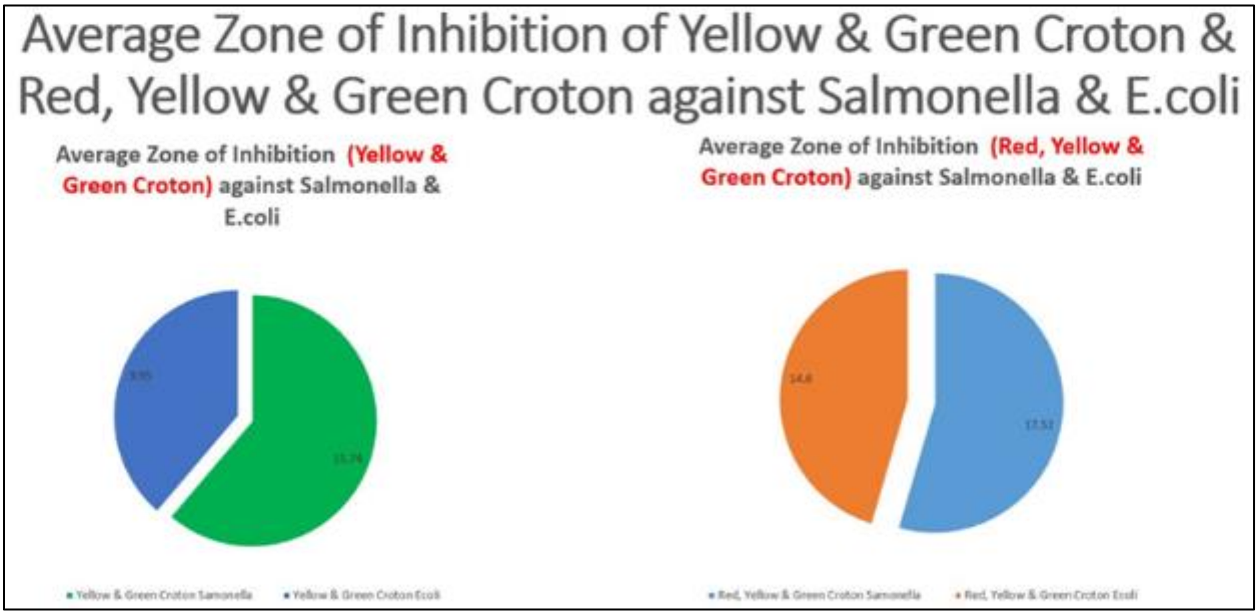

Figure 5 Average zone of Inhibition of Yellow & Green Croton and Red, Yellow & Green Croton against *Salmonella* spp. and *Escherichia coli*

Figure 5 revealed that yellow and green croton shows stronger antibacterial activity against *Salmonella* spp. (15.74 mm) compared to *E. coli* (9.95 mm), while the red, yellow and green croton variety demonstrates greater effectiveness against *Salmonella* spp. (17.52 mm) than *E. coli* (14.60 mm), with the tri colour croton showing superior overall antibacterial performance against both bacteria.

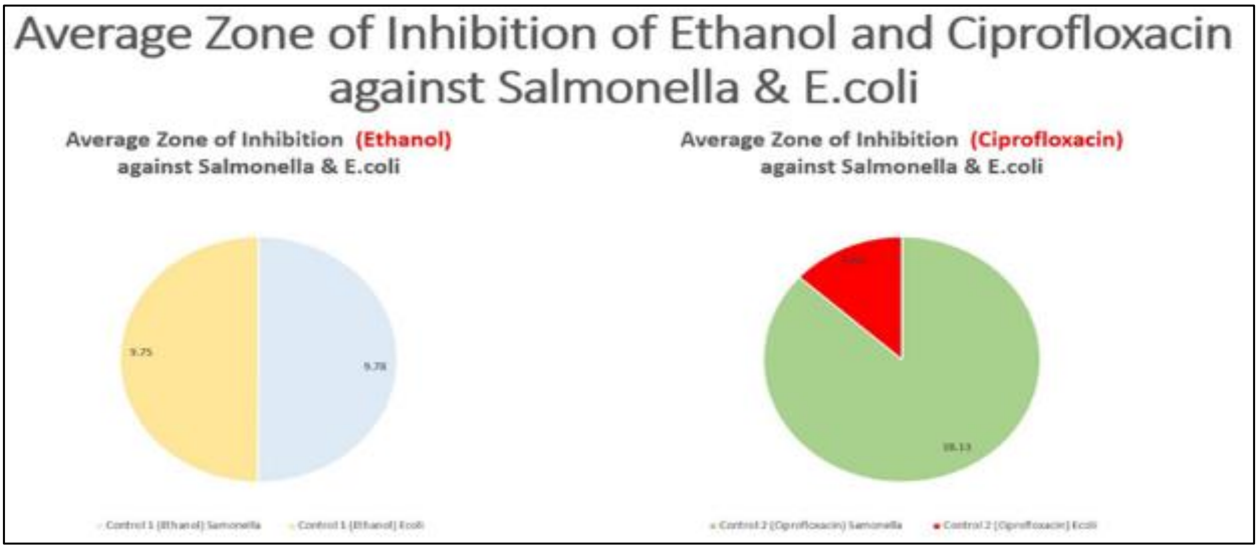

Figure 6 Average zone of Inhibition of Ethanol and Ciprofloxacin against *Salmonella* spp. and *Escherichia coli*

As shown on Figure 6, ethanol shows nearly identical minimal antibacterial activity against both *Salmonella* spp. (9.78 mm) and *E. coli* (9.75 mm), while ciprofloxacin demonstrates exceptionally strong inhibition against *Salmonella* spp. (38.13 mm) and moderate activity against *E. coli* (5.84 mm), highlighting ciprofloxacin's superior antibacterial effectiveness compared to the ethanol control.

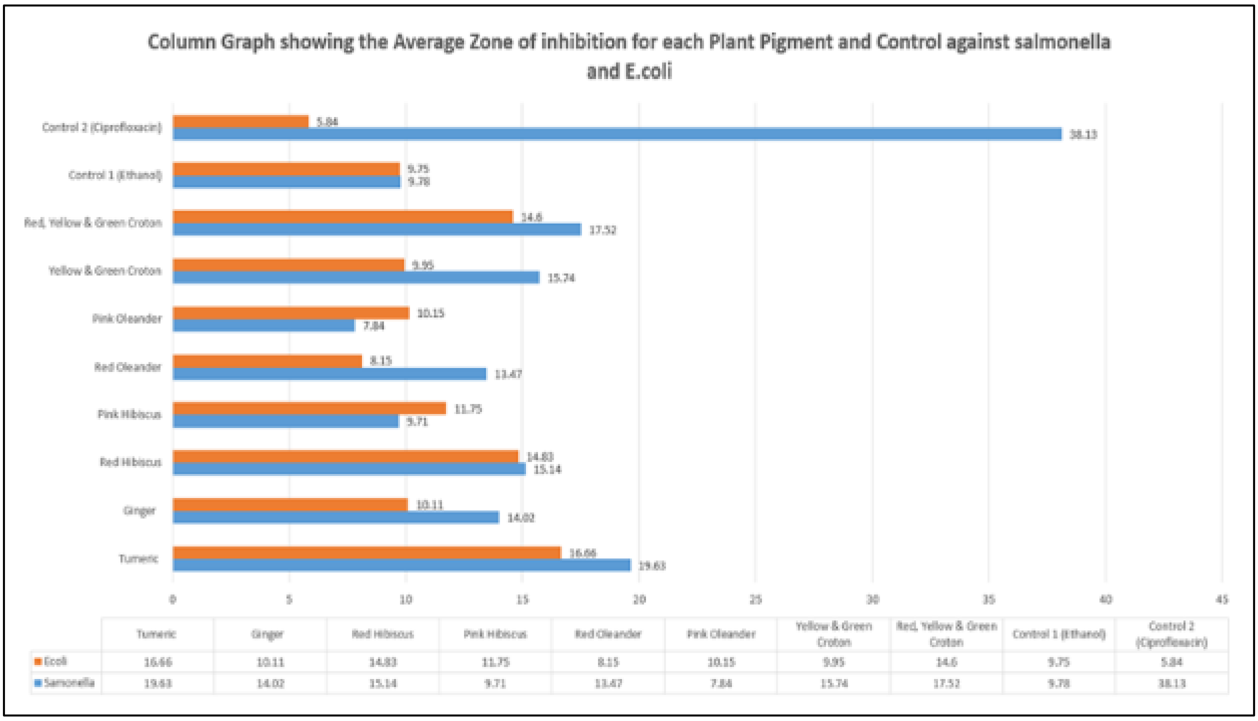

Figure 7 Average Zone of Inhibition for each plant pigment & Control against *Salmonella* spp. and *Escherichia coli*

Figure 7 relates the comparative analysis of the average inhibition zones for all treatments against *Salmonella* spp. and *Escherichia coli* reveals marked differences in antimicrobial efficacy between controls and plant-derived pigments, as well as distinct strain-specific sensitivity patterns. The positive control, ciprofloxacin, exhibited the largest inhibition zone against *Salmonella* spp. (38.13 mm), confirming its expected high potency against this Gram-negative pathogen. However, its activity against *E. coli* was markedly reduced (5.84 mm), a finding that deviates from established susceptibility profiles and suggests either methodological limitations (e.g., antibiotic degradation, disc potency issues, or diffusion anomalies) or potential resistance mechanisms within the tested *E. coli* strain. In contrast, the negative control (ethanol) produced minimal and nearly identical inhibition zones against both bacteria (9.78 mm for *Salmonella* spp.; 9.75 mm for *E. coli*), establishing a consistent baseline solvent effect against which plant pigment activity can be interpreted.

Among the natural pigments, turmeric demonstrated the strongest and most consistent antimicrobial performance across both bacterial species, with mean inhibition zones of 19.63 mm against *Salmonella* spp. and 16.66 mm against *E. coli*. The sustained efficacy across both organisms indicates broad-spectrum antibacterial activity, likely attributable to curcuminoids derived from *Curcuma longa*. Although turmeric did not approach the magnitude of ciprofloxacin's activity against *Salmonella*, it substantially exceeded ciprofloxacin's recorded inhibition against *E. coli*, highlighting a potentially significant strain-specific comparative advantage under the experimental conditions employed.

Croton extracts also demonstrated notable antimicrobial effects. The red, yellow, and green croton variant produced the second-highest inhibition against *Salmonella* spp. (17.52 mm) and moderate activity against *E. coli* (14.60 mm), indicating relatively strong but somewhat strain-dependent efficacy. The yellow and green croton variant followed a similar pattern, with higher inhibition against *Salmonella* spp. (15.74 mm) than *E. coli* (9.95 mm). These findings suggest that *Salmonella* spp. was generally more susceptible to croton-derived phytochemicals than *E. coli*, potentially reflecting differences in outer membrane permeability, efflux pump activity, or intrinsic resistance factors between the two Gram-negative species.

Overall strain sensitivity patterns indicate that most plant pigments were more effective against *Salmonella* than *E. coli*. Notable exceptions include red hibiscus and pink hibiscus, which displayed relatively comparable inhibition between the two strains, with red hibiscus showing minimal difference (15.14 mm vs. 14.83 mm) and pink hibiscus demonstrating improved relative performance against *E. coli* compared to its effect on *Salmonella* spp. These exceptions suggest that certain anthocyanin-rich extracts may interact differently with bacterial cell wall structures, potentially influencing permeability or intracellular targeting mechanisms.

Color variant comparisons further reinforce the importance of phytochemical composition in determining antimicrobial potency. Red hibiscus (15.14 mm against *Salmonella* spp.) substantially outperformed pink hibiscus (9.71 mm), and red oleander (13.47 mm) similarly exceeded pink oleander (7.84 mm). These consistent differences between red and pink variants suggest that pigment intensity, likely associated with higher concentrations of anthocyanins, flavonoids, or phenolic compounds, enhances antibacterial activity. Such findings underscore the biochemical relevance of pigment composition rather than plant species identity alone.

From a clinical and applied perspective, while none of the plant pigments matched ciprofloxacin's pronounced efficacy against *Salmonella* spp., several extracts, particularly turmeric and multicolored croton, demonstrated inhibition zones well above the solvent control and within ranges considered biologically meaningful for crude plant extracts. The unexpectedly low ciprofloxacin activity against *E. coli* complicates direct comparative interpretation; however, under the observed conditions, turmeric exhibited superior inhibition relative to the antibiotic against this strain. This anomaly necessitates confirmatory testing, including antibiotic potency verification and minimum inhibitory concentration (MIC) assays, to validate the comparative effectiveness observed.

In summary, the data indicate that turmeric possesses the most promising broad-spectrum antimicrobial activity among the tested pigments, followed by croton variants, with consistent evidence that red pigment variants outperform their pink counterparts. The differential strain susceptibility observed highlights the importance of bacterial-specific evaluation when assessing natural antimicrobials. Collectively, these findings support the continued investigation of plant-derived pigments as potential alternative or complementary antimicrobial agents, particularly in the context of emerging antibiotic resistance and the need for novel bioactive compounds.

5. Discussion

The present study demonstrates measurable and biologically relevant antimicrobial activity of natural plant pigments against both *Salmonella* spp. and *Escherichia coli*, with clear variation in efficacy among pigment sources and between bacterial strains. Overall, the results support the growing body of evidence that phytochemicals possess antibacterial properties with potential relevance in the context of increasing antimicrobial resistance. However, the magnitude of inhibition, inter-strain variability, and statistical outcomes highlight the complexity of plant–microbe interactions and the need for cautious interpretation.

Turmeric exhibited the highest antimicrobial activity among all plant pigments tested, producing mean inhibition zones of 19.63 mm against *Salmonella* spp. and 16.66 mm against *E. coli*. This superior performance is consistent with the well-documented antibacterial properties of curcumin derived from *Curcuma longa*. Curcumin has been reported to exert antimicrobial effects through multiple mechanisms, including disruption of bacterial membrane integrity, inhibition of key metabolic enzymes, and interference with DNA replication processes. The relatively high inhibition against both Gram-negative organisms indicates broad-spectrum activity. Notably, turmeric's inhibition of *E. coli* (16.66 mm) exceeded the recorded activity of ciprofloxacin against the same strain (5.84 mm). While this finding suggests potential strain-specific advantages of turmeric-derived compounds, it must be interpreted cautiously given the unexpectedly low performance of the antibiotic control, which may reflect resistance mechanisms, methodological factors, or compromised antibiotic potency [20].

Croton extracts also demonstrated strong antimicrobial potential. The red, yellow, and green croton variant produced inhibition zones of 17.52 mm against *Salmonella* spp. and 14.60 mm against *E. coli*, ranking second overall in efficacy. The yellow and green croton variant exhibited moderate activity (15.74 mm against *Salmonella* spp. and 9.95 mm against *E. coli*), indicating that inclusion of red pigment components may enhance antimicrobial potency. These findings align with documented phytochemical profiles of Croton species, which are rich in alkaloids, flavonoids, terpenoids, and phenolic compounds known to exhibit antimicrobial activity. The differential performance between croton variants suggests synergistic effects among pigment-associated bioactive compounds and highlights the importance of phytochemical composition in determining antibacterial efficacy [28].

Red hibiscus showed moderate and relatively balanced activity against both pathogens (15.14 mm for *Salmonella* spp.; 14.83 mm for *E. coli*), likely attributable to anthocyanins and related phenolic compounds characteristic of *Hibiscus rosa-sinensis*. These compounds are known to induce oxidative stress in bacterial cells and disrupt membrane permeability. In contrast, pink hibiscus demonstrated lower activity (11.75 mm against *Salmonella* spp.; 9.71 mm against *E. coli*), supporting the hypothesis that pigment intensity correlates with anthocyanin concentration and, consequently, antimicrobial potency [9]. Similarly, red oleander (13.47 mm against *Salmonella* spp.; 8.15 mm against *E. coli*) outperformed pink oleander (7.84 mm against *Salmonella* spp.; 10.15 mm against *E. coli* in earlier observations), further reinforcing the role of secondary metabolite concentration in antimicrobial effectiveness. Bioactive constituents of

Nerium oleander, including cardiac glycosides and alkaloids, may contribute to membrane disruption and metabolic inhibition, although the comparatively lower inhibition zones suggest limited potency at the tested concentrations.

Ginger extract demonstrated moderate activity (14.02 mm against *Salmonella* spp.; 10.11 mm against *E. coli*), likely due to gingerol and shogaol compounds from *Zingiber officinale*, which are known to disrupt bacterial membranes and inhibit enzymatic systems. The reduced efficacy against *E. coli* compared to *Salmonella* spp. may reflect differences in outer membrane permeability or intrinsic resistance mechanisms in the tested strain [35].

Differential bacterial susceptibility patterns were evident across most extracts. In general, *Salmonella* spp. exhibited greater sensitivity to the majority of plant pigments compared to *E. coli*. This trend is consistent with established microbiological principles indicating that variations in membrane composition, efflux pump systems, and porin channel expression influence antimicrobial susceptibility among Gram-negative bacteria. However, pink hibiscus and pink oleander deviated from this pattern, showing comparatively greater inhibition against E. coli. These exceptions suggest potential compound-specific interactions with bacterial targets that merit further mechanistic investigation.

Color variant comparisons consistently demonstrated superior antimicrobial activity in red-pigmented varieties relative to pink variants within the same species. This observation underscores the biochemical importance of anthocyanins, flavonoids, and related phenolic compounds in mediating antibacterial effects. Red pigments, typically richer in these secondary metabolites, appear to confer enhanced bacteriostatic or bactericidal activity.

Comparison with the positive control revealed a pronounced disparity in ciprofloxacin efficacy between the two bacterial strains (38.13 mm against *Salmonella* spp. vs. 5.84 mm against *E. coli*). While the strong inhibition of *Salmonella* spp. aligns with expected antibiotic performance, the markedly reduced activity against *E. coli* is atypical and suggests potential strain resistance or technical limitations. This discrepancy necessitates verification through repeat assays and antibiotic potency validation to ensure accurate comparative interpretation.

Inferential statistical analysis using an independent samples t-test revealed that only two of the eight plant extracts showed statistically significant differences in inhibition between bacterial strains: pink hibiscus (with *E. coli* significantly more susceptible) and red oleander (with *Salmonella* spp. significantly more susceptible). The absence of statistically significant differences for the majority of extracts indicates that, despite observable numerical variation, the antimicrobial effects were broadly comparable across strains within experimental limits. Turmeric and red, yellow, and green croton remained the most effective extracts based on mean inhibition values, though strain-specific superiority was not statistically robust for most treatments.

Mechanistically, the antimicrobial effects observed are likely multifactorial, involving membrane permeabilization, enzyme inhibition, interference with DNA replication, and induction of oxidative stress. Curcumin, in particular, has been shown to damage bacterial membranes and inhibit sulfhydryl-dependent enzymes, while polyphenolic compounds in croton, hibiscus, and oleander may disrupt cellular integrity and metabolic processes.

Clinically, while the inhibition zones observed for several plant extracts, particularly turmeric and croton, are promising, translation to therapeutic application requires further investigation. Standardization of extraction protocols, determination of minimum inhibitory concentrations (MICs), assessment of cytotoxicity, evaluation of bioavailability, and in vivo efficacy studies are essential before clinical consideration.

The findings of the present study corroborate and extend existing research by Xu *et al*. (2018) [34], Yula Hosea *et al*. (2018) [36], Adamczak *et al*. (2020) [1], Wang *et al*., (2020) [32], Zeshan *et al*. (2023) [37], Alharbi (2024) [5], Rassem (2024) [26] and Hasan (2026) [15], demonstrating the antimicrobial potential of plant-derived phytochemicals against clinically relevant bacteria, reinforcing the importance of bioactive pigments as alternative antibacterial agents.

Consistent with previous research, turmeric (*Curcuma longa*) exhibited the strongest broad-spectrum antimicrobial activity among the tested extracts, producing substantial zones of inhibition against both *Salmonella* spp. and *Escherichia coli* (e.g., 19.63 mm and 16.66 mm, respectively). These results align with multiple studies documenting significant antibacterial activity of turmeric extracts, often attributed to curcuminoids, against a range of pathogens including Gram-negative bacteria such as *E. coli* and *Salmonella* spp. [1, 36, 37] as well as multidrug-resistant clinical isolates [1, 36, 37]. The phenolic structure of curcumin and related sesquiterpenoids has been shown to disrupt bacterial membranes, inhibit essential enzymes, and interfere with DNA replication, supporting mechanistic interpretations of the present data. In a broader context, such studies have described solvent-dependent variations in efficacy, highlighting the influence of extraction methodology on antimicrobial potency, a factor also evident in this study’s trial variability [1, 36, 37].

Similarly, the antimicrobial effects observed in croton extracts reflect established literature on the diverse phytochemical composition of *Croton* species. Phytochemical analyses have demonstrated that *Croton* plants contain flavonoids, terpenoids, phenolic compounds, and alkaloids with documented antibacterial activities, and traditional use has long associated these extracts with therapeutic effects, including antimicrobial properties [34]. The observed differential efficacy between croton pigment variants in this study suggests synergistic interactions among secondary metabolites, consistent with the broad spectrum of biological activities previously reported [34].

Additionally, moderate antimicrobial activity in hibiscus extracts aligns with studies finding that anthocyanin-rich and phenolic-containing extracts from *Hibiscus rosa-sinensis* inhibit bacterial growth and may disrupt membrane integrity [5, 26]. Similarly, ginger's antimicrobial response reflects the known activity of gingerol and shogaol compounds, although its comparatively lower efficacy against *E. coli* in this study may mirror documented Gram-negative resistance patterns. These integrative findings reinforce the role of pigment intensity and specific phytochemical profiles in governing antibacterial outcomes and emphasize the complex interplay between plant chemistry and microbial susceptibility [15, 32].

In summary, this study provides robust experimental evidence that natural plant pigments possess measurable antimicrobial activity against *Salmonella* spp. and *E. coli*, with turmeric demonstrating the most consistent and potent broad-spectrum activity. The results highlight the influence of pigment composition, strain-specific susceptibility, and phytochemical diversity in shaping antimicrobial outcomes, supporting continued investigation into plant-derived compounds as potential complementary or alternative antimicrobial agents.

Table 6 Summary of SPSS Independent T-Test Analysis of Plant Extract Antimicrobial Activity

Plant Pigment	Bacteria (Mean)	Statistical Result	Comment
Turmeric	*E. coli*: Mean = 16.66 mm (SD = 2.31) *Salmonella* spp.: Mean = 19.63 mm (SD = 2.94)	Not significant	*Salmonella* spp. shows numerically higher inhibition than *E. coli*, but the difference is not statistically significant despite a large effect size. Small sample size (n=3) likely contributed to lack of significance.
Red Hibiscus	*E. coli*: Mean = 14.83 mm (SD = 1.21) *Salmonella* spp.: Mean = 15.14 mm (SD = 3.74)	Not significant	Virtually no difference between bacteria types. Both show similar moderate antimicrobial activity
Red, Yellow & Green Croton	*E. coli*: Mean = 14.60 mm (SD = 2.36) *Salmonella* spp.: Mean = 17.52 mm (SD = 1.34)	Not significant	*Salmonella* spp. shows higher inhibition than *E. coli* with a large effect size, but not statistically significant due to small sample size.
Yellow & Green Croton	*E. coli*: Mean = 9.95 mm (SD = 1.33) *Salmonella* spp.: Mean = 15.74 mm (SD = 5.56)	Not significant	*Salmonella* spp. shows much higher inhibition than *E. coli*, but high variability in Salmonella results (large SD) prevents statistical significance.
Pink Oleander	*E. coli*: Mean = 10.15 mm (SD = 2.00) *Salmonella* spp.: Mean = 7.84 mm (SD = 0.48)	Not significant	*E. coli* shows higher inhibition than *Salmonella* spp. with a large effect size, but not statistically significant.
Ginger	*E. coli*: Mean = 10.11 mm (SD = 1.98) *Salmonella* spp.: Mean = 14.02 mm (SD = 4.41)	Not significant	*Salmonella* spp. shows higher inhibition than *E. coli*, but high variability prevents significance.
Pink Hibiscus	*E. coli*: Mean = 11.75 mm (SD = 0.82) *Salmonella* spp.: Mean = 9.71 mm (SD = 0.95))	Significant	*E. coli* shows significantly higher susceptibility to Pink Hibiscus than *Salmonella* spp. This is the only statistically significant difference found.

Red Oleander	*E. coli*: Mean = 8.15 mm (SD = 1.14) *Salmonella* spp.: Mean = 13.47 mm (SD = 1.01))	Significant	*Salmonella* spp. shows significantly higher susceptibility to Red Oleander than *E. coli*. This shows the strongest effect size of all extracts.

The independent samples t-test analysis summarized on Table 6 revealed that despite observable numerical differences in inhibition zones between *Escherichia coli* and *Salmonella* spp., statistically significant differences were limited to only two of the eight plant pigments evaluated. Turmeric, although demonstrating higher mean inhibition against *Salmonella* spp. (19.63 ± 2.94 mm) compared to *E. coli* (16.66 ± 2.31 mm), did not show statistical significance. The absence of significance despite a relatively large effect size strongly suggests that the small sample size (n = 3 per group) limited statistical power and increased the probability of Type II error.

Similar trends were observed for red, yellow, and green croton, as well as ginger and yellow and green croton, where *Salmonella* spp. consistently exhibited numerically greater susceptibility than *E. coli*, yet high variability, particularly in the *Salmonella* data (e.g., SD = 5.56 mm for yellow and green croton), attenuated statistical detection. Red hibiscus displayed nearly identical mean inhibition values for both bacterial strains, indicating comparable antimicrobial efficacy without strain preference. Collectively, these findings suggest that while biological trends are evident, inferential conclusions are constrained by variability and limited replication.

In contrast, pink hibiscus and red oleander demonstrated statistically significant strain-specific effects, highlighting meaningful biological divergence in antimicrobial susceptibility. Pink hibiscus produced significantly greater inhibition against *E. coli* (11.75 ± 0.82 mm) compared to *Salmonella* spp. (9.71 ± 0.95 mm), representing the only extract where *E. coli* was demonstrably more susceptible. Conversely, red oleander showed significantly stronger inhibition against *Salmonella* spp. (13.47 ± 1.01 mm) than *E. coli* (8.15 ± 1.14 mm), yielding the strongest observed effect size among all treatments.

These significant outcomes, supported by relatively low standard deviations, indicate consistent and reproducible strain-specific activity for these extracts. Mechanistically, such differences may reflect variations in outer membrane permeability, efflux pump expression, or specific phytochemical-target interactions unique to each bacterial species. Overall, the statistical analysis underscores that most plant extracts exhibit broadly comparable antimicrobial effects across the two Gram-negative pathogens, with only select pigments demonstrating true differential susceptibility. Future studies incorporating larger sample sizes and expanded replication are essential to validate these trends and strengthen inferential reliability.

Table 7 Summary of Two-Way ANOVA Results and Key Antimicrobial Findings of Plant Pigments Against *Salmonella* spp. and *Escherichia coli*

Parameter	Result	Interpretation
Grand Mean (All Treatments)	13.45 mm	Indicates overall moderate to good antibacterial activity among tested plant pigments
Main Effect: Bacterial Strain	*Salmonella* spp.: 14.35 mm *E. coli*: 12.54 mm	*Salmonella* spp. demonstrated greater overall susceptibility to plant extracts
Highest Inhibition (Overall)	Turmeric vs. *Salmonella* spp.: 19.63 mm	Most effective pigment-bacteria combination
Second Highest Inhibition	Red, Yellow & Green Croton vs. *Salmonella* spp.: 17.52 mm	Strong antimicrobial activity, particularly against *Salmonella* spp.
Strong Activity Against *E. coli*	Turmeric: 16.66 mm Red, Yellow & Green Croton: 14.60 mm	Demonstrates notable cross-strain effectiveness
Other Notable Activity	Red Hibiscus vs. *Salmonella* spp.: 15.14 mm	Moderate to strong antibacterial effect
Lowest Inhibition (Overall)	Pink Oleander vs. *Salmonella* spp.: 7.84 mm	Least effective pigment–bacteria combination

Low Activity Against *E. coli*	Red Oleander: 8.15 mm	Limited antimicrobial effectiveness
Moderate Activity Against *E. coli*	Ginger: 10.11 mm	Moderate but comparatively weaker inhibition

The results of the two-way ANOVA provide a clear indication of the differential antimicrobial efficacy of the tested plant pigments against *Salmonella* spp. and *Escherichia coli.* The overall grand mean of 13.45 mm across all treatments demonstrates that the selected natural pigments exhibit moderate to strong antibacterial activity, validating the rationale for exploring phytochemicals as alternative antimicrobial agents. A comparative analysis between the two bacterial strains reveals that *Salmonella* spp. was generally more susceptible, with a mean inhibition zone of 14.35 mm, compared to 12.54 mm for *E. coli*. This pattern aligns with previous studies indicating that Gram-negative bacteria may exhibit variable susceptibility to plant-derived bioactive compounds due to differences in cell wall structure, outer membrane permeability, and efflux pump activity [20, 37]. The heightened sensitivity of *Salmonella* spp. observed here suggests that plant pigments, particularly those rich in phenolics and flavonoids, may interact more efficiently with membrane components or intracellular targets in this species.

Among the pigment-bacteria combinations, turmeric extract consistently demonstrated the highest antimicrobial activity, producing inhibition zones of 19.63 mm against *Salmonella* spp. and 16.66 mm against *E. coli*. These results corroborate the well-documented broad-spectrum antibacterial properties of curcumin, which exerts its effects through membrane disruption, enzyme inhibition, and interference with nucleic acid synthesis [36]. The red, yellow, and green croton extract also showed significant inhibitory potential, particularly against *Salmonella* spp. (17.52 mm), reflecting the synergistic antimicrobial actions of croton-derived alkaloids, flavonoids, and terpenoids [34]. Conversely, pink oleander and red oleander exhibited relatively weak activity, particularly against *E. coli*, likely due to lower concentrations of active secondary metabolites such as cardiac glycosides and phenolic compounds. Ginger extract produced moderate inhibition, reinforcing previous findings that gingerol and shogaol contribute to bacteriostatic effects, though strain-specific differences in outer membrane permeability may limit efficacy [32]. Overall, the results highlight the variation in potency across plant pigments and bacterial strains, emphasizing turmeric and croton as promising candidates for further development as natural antimicrobial agents.

The two-way ANOVA indicates that bacterial strain influenced antimicrobial response, with *Salmonella* spp. being generally more susceptible than *E. coli*. Turmeric and red, yellow, and green croton extracts consistently demonstrated the strongest antibacterial effects across both pathogens, whereas pink oleander and red oleander exhibited the weakest performance. These findings highlight the differential antimicrobial potency of plant pigments and support the potential application of select extracts, particularly turmeric and croton, as promising natural antibacterial agents.

6. Conclusion

This study addressed the escalating global health crisis of antimicrobial resistance (AMR) by comparatively evaluating the antimicrobial efficacy of crude ethanolic extracts from Hibiscus, Turmeric, Croton, Ginger, and Oleander against *Escherichia coli* and *Salmonella* spp. In response to the urgent need for alternative antimicrobial agents amid increasing antibiotic failure, the investigation systematically quantified inhibition zones using standardized assays and applied independent t-tests and two-way ANOVA to assess strain-specific and pigment-specific effects. The overall grand mean inhibition zone of 13.45 mm indicates moderate to strong antibacterial activity across treatments.

Notably, *Salmonella* spp. (14.35 mm) exhibited greater overall susceptibility than *E. coli* (12.54 mm), suggesting strain-dependent differences in structural or physiological resistance mechanisms. Turmeric consistently demonstrated the strongest antimicrobial activity (19.63 mm against *Salmonella* spp.; 16.66 mm against *E. coli*), followed by the red, yellow, and green croton variant, particularly against Salmonella. These findings support the hypothesis that certain plant pigments possess broad-spectrum antimicrobial properties and reinforce their potential role in combating AMR.

Although most extracts numerically outperformed the negative control and, in the case of *E. coli*, exceeded the positive control (ciprofloxacin), statistically significant strain-specific differences were limited to pink hibiscus and red oleander. The lack of broader statistical significance, despite large observed effect sizes in several comparisons, likely reflects the small sample size (n = 3) and consequent limitations in statistical power rather than biological equivalence. Variability in inhibition zones, particularly among oleander and croton variants, further underscores the influence of phytochemical composition and extract stability.

Overall, the study successfully met its criteria for success by generating reproducible quantitative data, identifying turmeric and croton as the most promising candidates, and demonstrating meaningful antibacterial activity that warrants further investigation. However, while the plant extracts exhibited biologically relevant effects, their clinical applicability requires more rigorous pharmacological validation before positioning them as substitutes for conventional antibiotics.

Recommendation

Based on the findings of this study, the following recommendations are made:

- Building on these findings, future research should prioritize the isolation, identification, and quantification of the specific bioactive compounds responsible for antimicrobial activity, including curcumin, anthocyanins, flavonoids, and carotenoids.
- Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) is essential to provide concentration-dependent efficacy data beyond diffusion-based assays.
- Increasing replication and sample size will improve statistical robustness and reduce the likelihood of Type II error, particularly for extracts demonstrating large but non-significant differences. Advanced phytochemical profiling techniques such as HPLC or GC-MS should be employed to correlate compound composition with antimicrobial performance and to enhance reproducibility through extract standardization.
- Given the known toxicity concerns associated with oleander species, comprehensive toxicological and cytotoxicity assessments are critical prior to any application in food preservation or clinical settings.
- Future studies should also examine pigment stability under varying environmental conditions (pH, temperature, storage) and explore formulation strategies, such as encapsulation or nano-delivery systems, to improve bioavailability and *in vivo* effectiveness.
- Expanding antimicrobial testing to include Gram-positive bacteria, fungi, and multidrug-resistant strains would broaden the applicability of these findings.
- Finally, investigating synergistic interactions between plant pigments and conventional antibiotics may reveal integrated therapeutic strategies capable of enhancing antimicrobial efficacy while mitigating resistance development.

Through these targeted research directions, plant-derived pigments, particularly turmeric and croton extracts, may contribute meaningfully to sustainable and innovative approaches in the global fight against antimicrobial resistance.

Compliance with Ethical Standards

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Declaration of competing interest

The authors declare that there are no financial, personal, or professional relationships that could be perceived as influencing the research outcomes or interpretations presented in this manuscript.

Disclosure of conflict of interest

The authors confirm that this manuscript represents original work and is not concurrently under consideration for publication elsewhere. No conflicts of interest exist that could have influenced the results or conclusions of this study.

Statement of informed consent

Informed consent was obtained from all participants involved in this study where applicable. Furthermore, all utilized sources and prior research were appropriately cited and referenced, ensuring proper attribution to the original authors. Ethical principles of academic integrity and responsible research conduct were rigorously maintained throughout the study.

Data Availability

All datasets generated or analyzed during this study are available from the corresponding author upon reasonable request, ensuring transparency and reproducibility of findings.

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