



Potential of Natural Plant Extracts in Combating the Bacterial Contaminants from Poultry Water Sample

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Abstract

Background: The poultry industry represents an integral part of worldwide livestock economies; however, it is highly jeopardized by bacterial and parasitic infections. An increased development of antibiotic-resistant bacteria worsens this challenge. This study investigates the potential of plant-based extracts as alternative antibacterial agents in poultry management. Bacterial contaminants were isolated by serial dilution agar plate technique. **Result:** Two bacterial contaminants were isolated namely Poultry Water isolate 1 (PW1) and Poultry Water isolate 2 (PW2). A total of 21 plant extracts of 7 plants (ginger (*Zingiber* Sp.), mulberry (*Morus* Sp.), mango (*Mangifera* Sp.), curry (*Murraya* Sp.), neem (*Azadirachta* Sp.), black pepper (*Piper* Sp.), and ajwain (*Trachyspermum* Sp.) were prepared in different solvents such as distilled water, ethanol and petroleum ether and screened against bacterial contaminants by using agar well diffusion. Extracts from mango and mulberry leaves demonstrated potent action against PW1, whereas curry and mango leaves demonstrated efficacy against PW2. The rhizomes of ginger and neem also produced significant impacts, while Cephalexin showed the strongest antibacterial efficacy overall. The phytochemical examination of the mango leaf extract showed flavonoids, triterpenes, alkaloids, saponins, and tannins. The mango leaf extract's minimum inhibitory concentration (MIC) was 0.15% against PW1 and 0.625% against PW2. **Conclusion:** The findings suggest that plant extracts, especially those derived from mango leaves, present a promising natural substitute for traditional antibiotics in poultry farming, with the potential to lower antibiotic usage and counteract antibiotic resistance. To investigate their modes of action and efficacy in diverse poultry production scenarios, more investigation is required. The purpose of this study was to assess the plant extracts' antibacterial efficacy against bacterial pollutants found in chicken water samples.

Keywords: Antibacterial; Alternative Control; Phytochemical; Poultry Farm

Introduction

Poultry, the most important division of the livestock economy worldwide, contributes to a good balance of protein in the human diet through meat and eggs. However, bacterial and parasite illnesses pose a serious threat to the poultry business, resulting in significant losses to the health of poultry and the general public. Excessive resource use, water pollution, ammonia emissions, antibiotic resistance, and ethical issues are all consequences of intensive traditional agricultural methods (Abiala *et al.* 2016, Gržinić *et al.*, 2023). Since that water makes up over 70% of a chicken's body, water is crucial to the health of poultry. Healthy birds often consume at least twice as much water as they weigh in food.

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Further, water intake increases immensely in natural and induced heat stress. Thus, high-quality water is considered significant for poultry health in different environmental conditions.

Poultry water systems have a significant potential for microbial contamination, *Salmonella*, *Campylobacter* and *Escherichia coli* that harm to the health and growth of birds. While low-quality water tends to decrease feed intake, impede growth, or raise mortality rates, high-quality water enhances feed efficiency, weight gain, and egg production (Morton, 2022). The commercially available antibiotics are used to treat these bacterial infections on birds. The overuse and abuse of antibiotics in poultry farming have led to the emergence of bacteria that are resistant to them. This issue can only be resolved by combining strict hygienic regulations, immunization campaigns, and careful medication use (Samad et al., 2022). Poultry farming is a major contributor to AMR due to excessive antibiotic use for growth promotion and disease prevention. Resistant bacteria and antibiotic residues from poultry enter the food chain, water, and soil, raising public health risks (Singh et al., 2025).

One of the most frequent sources of foodborne diseases is poultry products. These meat products have the potential to get contaminated during processing with a variety of pathogens, including *Campylobacter* and *Salmonella*, which provide a direct or indirect danger to human health. It is important to pay attention to the microbiological safety of poultry meat products since contamination might happen at different stages of production. For instance, contamination may happen during the processing and killing phases (Rouger et al., 2017; Wardhana et al., 2021).

E. coli, especially the APEC strains, is of keen interest in poultry production since it has diseases like colibacillosis that result in high morbidity and mortality. These bacteria could be transferred from infected eggs or by fecal contamination. Amongst the conditions enhancing the problem are high levels of ammonia, bad litter quality, and overcrowding. The preventive measures are majorly directed to the maintenance of good sanitary conditions, dry litter, and sufficient ventilation while the antibiotic treatments prove less effective due to resistance (Eid & Erfan, 2013; Stromberg et al., 2017). Treatment involves the administration of antibiotics and supportive therapies; however, mechanisms are shifting towards prevention given the problem of growing antibiotic resistance (Samad et al., 2022).

Prevention and control can effectively be done through vaccination, strict biosecurity measures, and good managerial practices. With the increased production and consumer demand, the microbiological safety of poultry meat products cannot be ruled out. Contamination may emanate during and after slaughter, which can, later on, develop on account of poor processing and storage. Among these pathogens are *E. coli* that raise alarm for good hygiene measures (European Food Safety Authority, 2017).

Other means in disease control involving medicinal plants are yet to participate to play their vital role in reducing the use of antibiotics in poultry. Generally, plant extracts possessing bioactive compounds such as alkaloids, flavonoids, and essential oils showed strong antibacterial activity, and this forms a suitable alternative to traditional use of antibiotics. For example, *Mangifera* sp. (Mango leaves), *Murraya* sp. (Curry leaves), and *Morus* sp. (Mulberry leaves) have shown remarkable antibacterial activities against different pathogens (Abiala et al., 2016; Katariya & Arjankumar, 2019; Nguyen et al., 2024). Medicinal herbs are increasingly applied in poultry; these herbs are a natural tool in the management of diseases. Herbs like *Echinacea purpurea* and *Artemisia annua* have given positive results in treating parasitic infections, while extracts from plants have proven efficient in enhancing gut health and reducing colonization of bacteria in poultry. In view of the potential of these natural alternatives, more studies are to be done on their efficacy and mechanisms of action in poultry health management (Seidavi et al., 2021; Jamil et al., 2022).

Research has concentrated on developing alternatives to traditional antibiotics, such as antimicrobial peptides, bacteriophages, probiotics, and nanoparticles, as well as the application of alternative therapies, in response to the rise of resistant microbes. According to Rodrigues et al. (2022), these remedies are essential for halting the spread of resistant microbes. Serious illnesses in both humans and animals are caused by the increasing prevalence of multidrug-resistant bacteria (Abreu et al., 2023). In the gigantic challenges posed by bacterial and parasitic infections, alternative strategies like

the use of medicinal plants have become an option in poultry farming. These plants do not only manage diseases, but have some positive effects on the health of the birds thereby assuring a relatively healthy Poultry farm and such methods are, therefore, relevant to sustainable and environmentally friendly poultry farming. Keeping in view the above justification, the natural plant extracts were screened for their antibacterial activity against bacterial contaminants from poultry water sample.

Material & Methods

Sample Collection

Water sample was collected in sterile Petri plates from Gulati poultry farm, Kharar, Punjab, in February 2024. The water sample was transported to the laboratory for isolation of bacterial contaminants by serial dilution agar plate technique. The water sample was sealed, labeled, and stored at a temperature range of 2-8°C to preserve quality after collection.

Isolation of bacterial contaminants by serial dilution agar plate technique

In serial dilution agar plate technique, a total of 8 test tubes of water blanks (9mm) were prepared and autoclaved for serial dilution agar plate technique. One ml sample of the bacterial stock culture was added to the first tube creating a 10^{-1} . 1 ml was shaken and then transferred from this tube to the next test tube (10^{-2} dilution). This process was repeated until a 10^{-8} dilution. One milliliter of each diluted sample was put in a Petri plate, poured molten nutrient agar on the plates and mixed well, and the solidified plates were incubated at 35-37°C for 24-48 hours. The plates supported the growth of bacterial colonies, with a countable number of colonies on the 10^{-5} plate. Observed the colonies on Petri plates and purified bacterial culture by streaking method and maintained at 4°C (Aneja, 2007). The pure culture of *E. coli* as test bacterium was collected from the Department of Biosciences, UIBT, Chandigarh University, Mohali, Punjab, India.

Preparation of Plant Extracts

A total of 7 plants such as Mango (*Mangifera* sp.) leaves, Curry (*Murraya* Sp.) leaves, Mulberry (*Morus* Sp.) leaves, Neem (*Azadirachta* Sp.) leaves, Black pepper (*Piper* Sp.) seeds, Ginger (*Zingiber* Sp.) Rhizome, Ajwain (*Trachyspermum* Sp.) seeds. In the present study, all the plant parts required were purchased from local shops of Kharar, Mohali (Punjab) India. The collected plant parts were brought to the laboratory and washed it properly with tap water. Spread the plant parts on silver foil properly and placed the silver foil paper in the oven and dried in a controlled temperature 60°C for 24 hours. The dried parts were crushed into a fine powder by using a mortar and pestle ensuring homogeneity. 20% (w/v) of the plant extract was prepared in different solvents such as distilled water, ethanol and petroleum ether. The powder was mixed with solvents and kept at room temperature for 24 hrs. The extract was filtered using the Whatman filter paper and the filtered extracts were stored in the refrigerator for the further experiment (Enejyon et al., 2020).

Antibacterial activity of the plant extracts by agar well diffusion method

Antibacterial studies were tested using agar well diffusion method (Sharma & Pundir, 2018). Sterile nutrient agar plates were prepared. 100µl of bacterial suspension (1.5×10^6 Cells per ml) was spread in sterilized NA plates using sterile cotton swabs. 6mm wells were punched on agar plates using sterilized borer. 50-100 microliter of each plant extract was poured in the wells. Plates were incubated at 37°C for 24 hours. Solvents were poured in wells used a negative control. Zone of inhibition was observed and diameters of ZOI were measured using transparent ruler meter rule in mm.

Antibiotic Susceptibility Pattern of test bacteria

Antibiotic susceptibility pattern test bacteria were done by using Kirby-Bauer disc diffusion method. In this method, 100microliter of both the bacterial suspension was spread on sterilized NA plates using sterile cotton swabs. Antibiotic disc (Hi-Media, Mumbai) was placed on the surface of agar plates having bacterial culture suspension. The plates were incubated for 24 hours. After 24 hours, ZOI was observed and diameter of ZOI was recorded in mm (Yao et al., 2021).

Minimum Inhibitory Concentration (MIC) of the mango leaves

The maximum dilution or lowest concentration of an antimicrobial agent that will stop the growth or kill the bacterium is known as the minimum inhibitory concentration, or MIC. Macro dilution agar plate method was used to determine MIC of most potent plant extract, Mango leaf ethanolic Extract (Nrior *et al.*, 2023). Different concentrations (20%-0.07% w/v) of best antibacterial plant extract were prepared by two-fold dilution. Three agar plates were taken and in each of the plate a set of 3 concentrations of the extract were put into the wells. Plate 1 contains set of 20%, 10% and 5%. Plate 2 contains set of 2.5%, 1.25% and 0.625% while Plate 3 contains 0.31%, 0.15% and 0.07%. Then the plates were incubated at (37°C) for 18-24 hours and measured the diameter of the resistance after 24 hours.

Phytochemical analysis of the selected best antibacterial plant extract

A total of 8 Phytochemicals were tested for the best selected plant extract which includes Alkaloids, Carbohydrates, Saponins, Triterpenes, Phenols, Flavonoids, Proteins and Amino Acids.

Alkaloids Test

Picric Acid Test - Three to four drops of a 2% picric acid solution were added to a few millilitres of the filtrate. Alkaloids are indicated by the colour orange.

Detection of Carbohydrates

Fehling's Test- Boil 1 mL of the spice extract in a water bath with 1 mL of Fehling's solutions A and B. Carbohydrates are present when a crimson red precipitate form (Mohammed, 2023).

Test for Saponins

Foam Test- Shake well after combining 0.5 g of the plant extract with 2 mL of water. Saponins are present when the foam lasts for ten minutes (Olasehinde *et al.*, 2018).

Test for Triterpenes

Salkowski's Test- Mix 2-3 drops of the sample with 2 mL of chloroform, then carefully add concentrated sulfuric acid. A red or reddish-brown color in the chloroform layer indicates the presence of cholesterol (Tripathy & Sahoo, 2024).

Test for Phenols

Ferric Chloride test- Add 1-2 drops of 5% ferric chloride solution to 1-2 mL of the sample. A color change to purple, blue, green, or red indicates the presence of phenols or certain other compounds (Tripathy & Sahoo, 2024).

Test for Flavonoids

Alkaline Reagent Test- Two millilitres of 2% NaOH solution are combined with one millilitre of the extract, and a few drops of diluted HCl are added. The presence of flavonoids is indicated by a bright yellow tint that goes colourless when the acid was added (Somkuwar *et al.*, 2013).

Test for Proteins

Xanthoproteic Test- Adding a few drops of concentrated nitric acid to 1-2 mL of the sample, producing a yellow color. Adding a strong base like NaOH deepens the color to orange, confirming the presence of these amino acids (Somkuwar *et al.*, 2013).

Test for Amino acids

Ninhydrin Test - adding a few drops of 0.2% ninhydrin solution to 1-2 mL of the sample and heating it. A blue or purple color indicates the presence of free amino acids or amines (Nwafor *et al.*, 2022).

Results

Isolation of bacterial contaminants

Bacterial contaminants were isolated by serial dilution agar plat technique. Two bacterial contaminants were isolated namely Poultry Water isolate 1 (PW1) and Poultry Water isolate 2 (PW2). The pure culture of the bacterial isolates as shown in the Fig. 1. The pure culture of *E. coli* test bacterium was collected from the Department of Biosciences, UIBT, Chandigarh University, Mohali, Punjab, India.

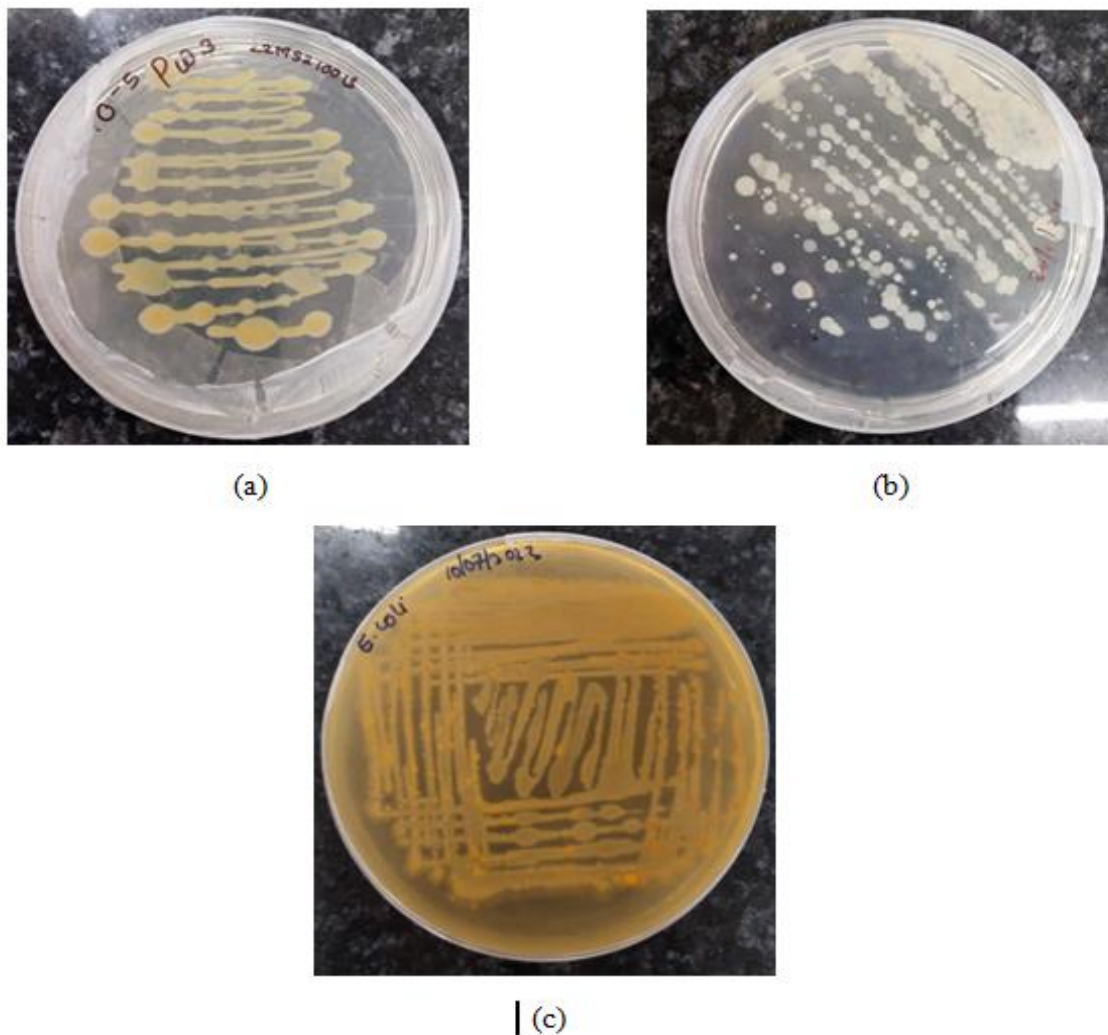


Figure 1: The Pure Culture of the Test Bacteria (a) Poultry Water Isolate 1 (PW1) (b) Poultry Water Isolate 2 (PW2) and (c) *E. coli*

Antibacterial activity of Plant extracts by Agar well diffusion method

Antibacterial activity of Plant extracts against *E. coli*

Curry leaf and Ginger rhizome (Fig. 2 a & b) ethanolic extracts showed maximum inhibition activity against *E. coli* with zone of inhibition (24mm) and (20mm) followed by Neem leaves (Fig 2. b) and black pepper ethanolic extracts showed (13mm) and (10mm) zone of inhibition respectively. All the other test plants mango leaves, mulberry leaves and ajwain extracts petroleum ether and aqueous solvent did not showed activity as shown in Table 1.

Antibacterial activity of Plant extracts against Poultry water- 1 (PW1)

Mango leaves & Mulberry leaves ethanolic extracts showed maximum inhibition activity against PW1 with zone of inhibition 44mm & 24mm respectively (Fig 3. a & b). All the other test plants curry leaves,

neem leaves, ginger rhizome, black pepper and ajwain did not showed activity as mentioned in Table 1.

Antibacterial activity of Plant extracts against Poultry water- 2 (PW2)

Mango leaves and Curry leaves ethanolic extract showed maximum inhibition activity against PW2 with zone of inhibition 45mm & 35mm (Fig 4a&, b) followed by Neem& Ginger rhizome ethanolic extract showed 25mm & 22mm zone of inhibition respectively (Fig. 4. c). Curry leaves petroleum ether extract showed 14mm zone inhibition (Fig 4. b). All the other plants mulberry leaves, black pepper, ajwain extracts aqueous did not showed activity as mentioned in table 1.

Water, petroleum ether, and ethanol were used as solvents to test the extracts' efficacy. Interestingly, ethanol extracts were more successful generally; ginger and curry leaf were efficient against several strains, whereas mango demonstrated suppression against PW1 and PW2. Petroleum ether usually displayed lower activity, with certain zones of inhibition visible for black pepper and curry leaf. There was no discernible antibacterial action in the water extracts.

Table 1: Antimicrobial Activity of Plant Extracts Against E. Coli, PW1 And PW2 By Agar Well Diffusion

Sl. No.	Plant Extract	Zone of Inhibition								
		Ethanol			Petroleum ether			Water		
		E. coli	PW1	PW2	E. coli	PW1	PW2	E. coli	PW1	PW2
1	Mango (<i>Mangifera</i> sp.) leaves	NA	44mm	45mm	NA	NA	NA	NA	NA	NA
2	Curry (<i>Murraya</i> sp.) leaves	24mm	NA	35mm	NA	NA	14mm	NA	NA	NA
3	Mulberry (<i>Morus</i> sp.) leaves	NA	24mm	NA	NA	NA	NA	NA	NA	NA
4	Neem (<i>Azadirachta</i> sp.) leaves	13mm	NA	25mm	NA	NA	NA	NA	NA	NA
5	Ginger (<i>Zingiber</i> sp.) rhizome	20mm	NA	22mm	NA	NA	NA	NA	NA	NA
6	Black pepper (<i>Piper</i> sp.) seeds	11mm	NA	NA	NA	NA	NA	NA	NA	NA
7	Ajwain (<i>Trachyspermum</i> sp.) seeds	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA: No activity

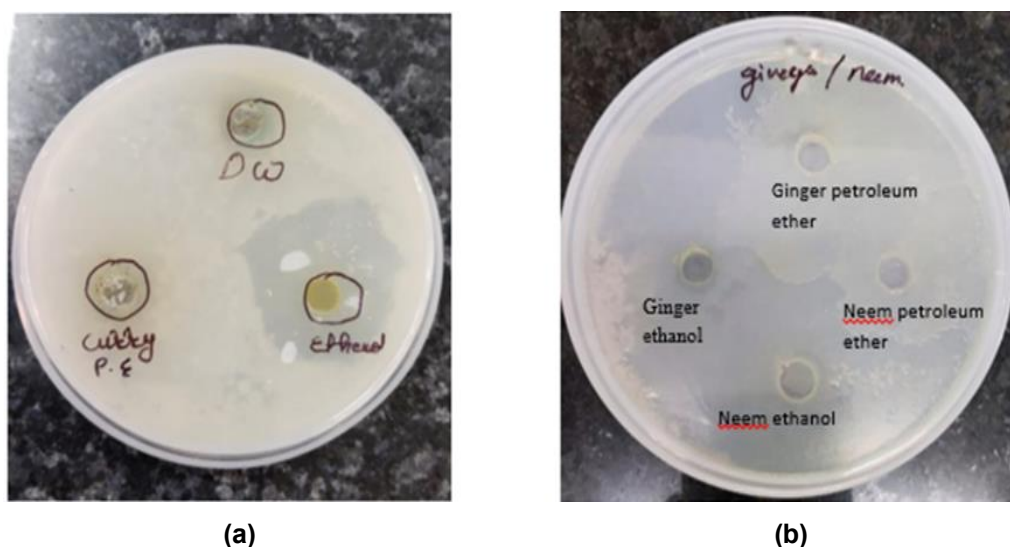
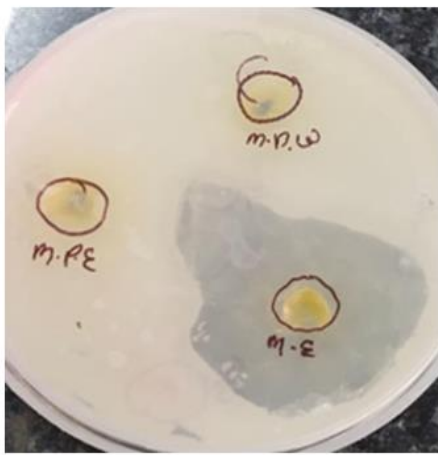
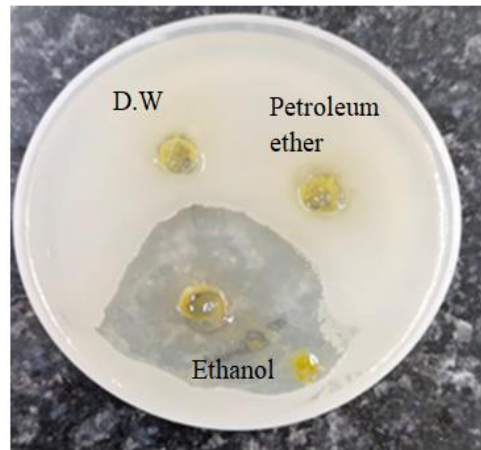


Figure 2: Antibacterial Activity of (A) Curry Leaves (B) Neem and Ginger Against E. Coli

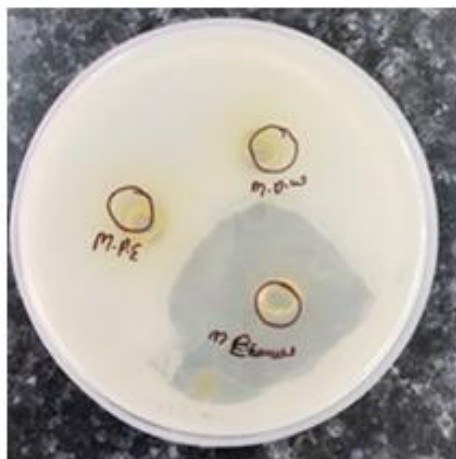


(a)

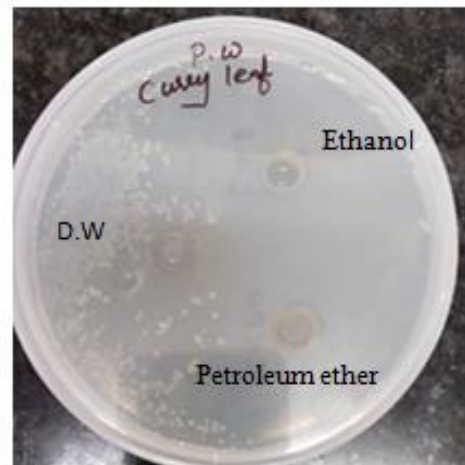


(b)

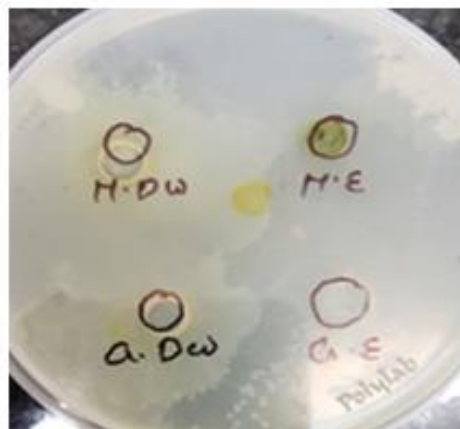
Figure 3: Antibacterial Activity of (A) Mango Leaves (B) Mulberry Leaves Ethanolic Extract Against PW1



(a)



(b)



(c)

Figure 4: Antibacterial Activity of Ethanolic Extract of (A) Mango Leaves (B) Curry Leaves (C) Neem & Ginger on PW2

Antibiotic susceptibility pattern of test bacteria

Cephalexin exhibited the highest antibacterial activity, as indicated by the zone of inhibition (measured in mm), against PW1 (30mm) and PW2 (35mm). This was followed by Gentamicin showed antibacterial activity against *E. coli* (24mm), PW1 (30mm) and PW2 (25mm) and Erythromycin showed antibacterial activity against *E. coli* (12mm), PW1 (28mm) and PW2 (25mm). Ampicillin showed activity against PW1 (25mm) and PW2 (30mm) followed by Vancomycin (30mm) & (11mm) against PW1 & PW2 respectively. While as indicated by the zone of inhibition Oxacillin (30mm), Chloramphenicol (25mm) and Clindamycin (27mm) showed the antibacterial activity against PW1. While Oxacillin (30mm) and Clindamycin (15mm) showed activity against PW2. While Ampicillin, Vancomycin, Clindamycin and Oxacillin did not show any inhibition against *E. coli*. The results of Antibiotic susceptibility test conducted against test bacteria is shown in table 2.

Table 2: Zone of Inhibition Exhibited by Antibiotics Against *E. Coli*, PW1 And PW2

Zone of Inhibition in mm								
Antibiotics Test Bacteria	AMP	VA	OX	GEN	E	CD	C	CEP
Escherichia coli	NA	NA	NA	24mm	12mm	NA	20mm	NA
PW1	25mm	30mm	30mm	30mm	28mm	27mm	25mm	30mm
PW2	30mm	11mm	NA	25mm	20mm	15mm	NA	35mm

NA: No Activity; AMP: Ampicillin; VA: Vancomycin; OX: Oxacillin; GEN: Gentamicin; E: Erythromycin; CD: Clindamycin; C: Chloramphenicol and CEP: Cephalexin

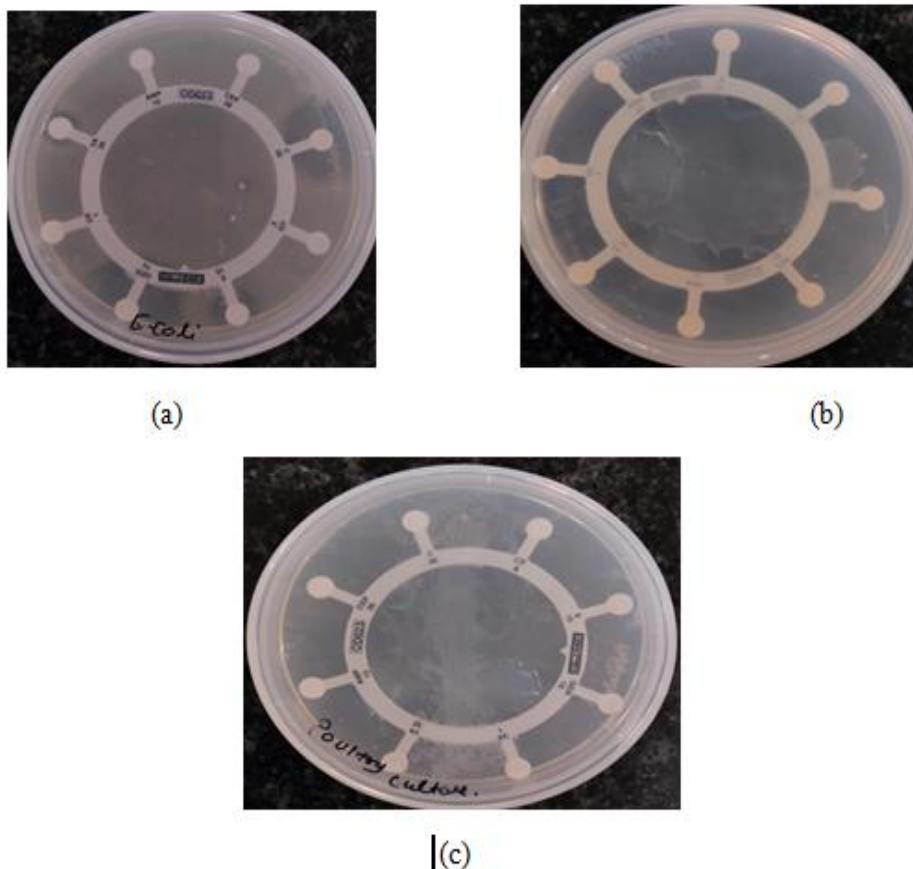


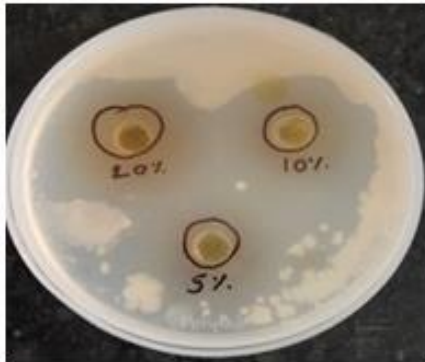
Figure 5: Antibiotic Susceptibility Pattern (a) *Escherichia Coli* (b) PW1 (c) PW2

Minimum Inhibitory Concentration of Mango leaves ethanolic extract

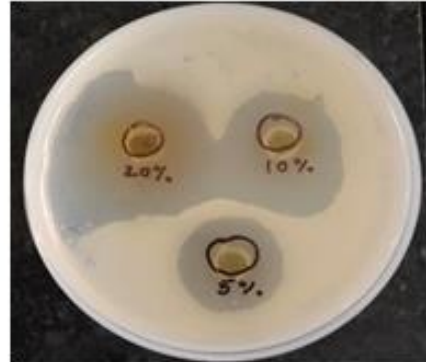
The ethanolic extract of Mango leaves demonstrated a minimum inhibitory concentration (MIC) of 0.15% against the bacteria PW1 (producing a 10mm inhibition zone) and 0.625% against PW2 (producing a 17mm inhibition zone). The detailed results are presented in the following table 3.

Table 3: Minimum Inhibitory Concentration of Mango Leaves Ethanolic Extract Against PW1 & PW2

Sl. No.	Mango Extract Conc. (in %)	Zone of Inhibition	
		PW1	PW2
1	20%	42mm	40mm
2	10%	40mm	35mm
3	5%	38mm	26mm
4	2.5%	25mm	24mm
5	1.25%	18mm	19mm
6	0.625%	16mm	17mm
7	0.31%	11mm	NA
8	0.15%	10mm	NA
9	0.07%	NA	NA



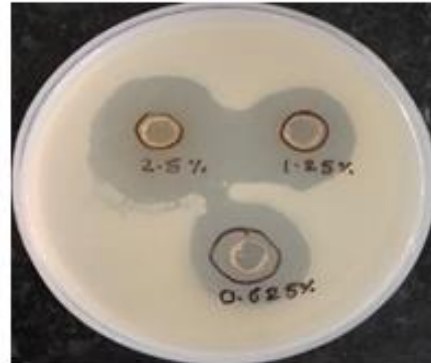
(a)



(d)



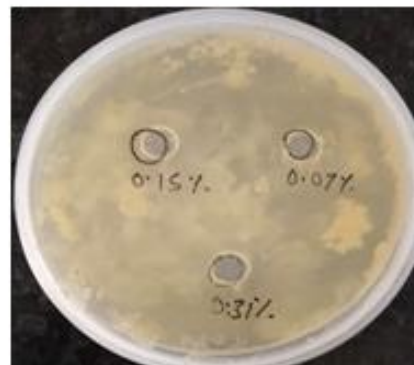
(b)



(e)



(c)



(f)

Figure 6: Images (A), (B) And (C) Showing Different Concentrations of Mango Leaves Ethanolic Extract Against Poultry Farm-1 (PW1). Images (D), (E) and (F) Showing Different Concentrations of Mango Leaves Ethanolic Extract Against Poultry Farm-2 (PW2).

Phytochemical Analysis of Mango Plant Extract

The presence of alkaloids, saponins, phytosterols, triterpenes, phenols, flavonoids, and tannins were discovered through phytochemical analysis of mango plant extract in ethanol solvent. The results are shown in detail in table 4 below.

Table 4: Phytochemical Analysis of Mango Plant Extract

Sl. No.	Compounds	Test	Result
1	Alkaloids	Picric Acid Test:	+
2	Carbohydrates	Benedict's Test	-
3	Saponins	Froth Test	+
5	Triterpenes	Salkowski's Test	-
6	Phenols	Ferric Chloride Test	+
7	Flavonoids	Lead Acetate Test	+
8	Proteins	Xanthoproteic Test	+
9	Amino Acids	Ninhydrin	+

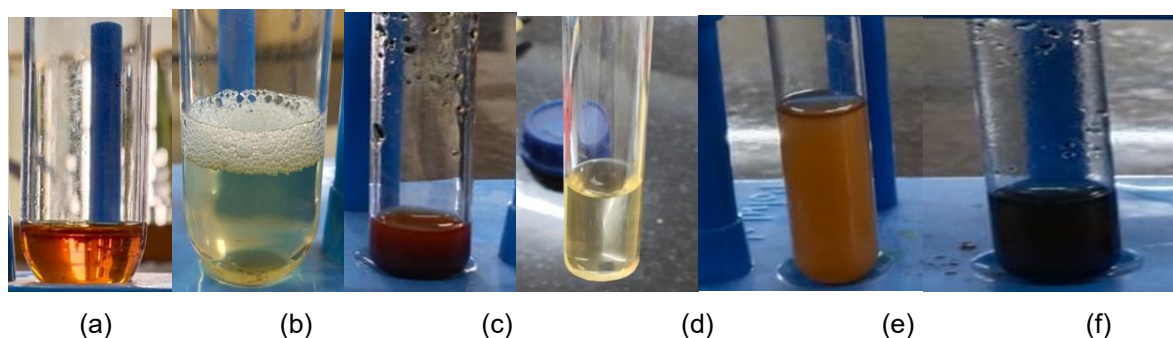


Figure 7: Phytochemical Analysis of Mango Leaves Ethanolic Extract (a) Alkaloids (b) Saponins (c) Phenols (d) Flavonoids (e) Proteins (f) Amino Acids are Present

Discussion

In the present study, bacterial contaminants were isolated by serial dilution agar plat technique. Two bacterial contaminants were isolated namely Poultry Water isolate 1 (PW1) and Poultry Water isolate 2 (PW2). According to (Okafor *et al.*, 2022), using agar plating and serial dilution, it is possible to isolate bacterial contaminants like PW1 and PW2 from poultry farm water while adhering to accepted microbiological techniques for identifying waterborne pathogens in agricultural environments. Food security is a big concern due to the constantly growing human population, hence it is important to make sure that food production systems can accommodate this growth [UN DESA]. Meat and other animal products are essential components of the human diet. Demand for this food is rising, and during the past 50 years, meat consumption has more than 4 folds (Richie *et al.*, 2019). While has shown how successful these methods are at separating various bacterial strains from poultry farm environments, highlighting the significance of water quality monitoring in halting the spread of illness. Furthermore (Hooda *et al.*, 2000). Emphasized the importance of determining certain bacterial pollutants in order to enhance biosecurity protocols in poultry farms.

Consistent with previous study, the agar well diffusion method results demonstrate the strong antibacterial activity of several plant extracts against *E. coli* and poultry farm bacteria. Abiala *et al.* (2016) stated that mango leaf ethanolic extracts showed potent antibacterial activity, especially against gram-negative bacteria, as demonstrated by a 44mm inhibitory zone against PW1. Mulberry leaves are also effective in preventing bacterial growth, as (Ustundag & Ozdogan, 2015) showed, confirming the 24mm inhibition zones against PW1. Furthermore, (Ofongo *et al.*, 2021) discovered noteworthy antibacterial characteristics in the extracts of neem and ginger, which correspond with their mild inhibition zones in this study. According to Anizoba *et al.* (2024) neem leaf and ginger rhizome contain numerous chemical components that are biologically active and are widely utilized in medications to treat various illnesses. Anizoba *et al.* (2024) studied to assess the effect of aqueous neem leaf and

ginger rhizome extracts on the growth performance and hematological parameters in the three breeds of chicken.

The results of earlier study are consistent with the antibacterial activity of cephalexin, gentamicin, and other antibiotics against isolates from poultry farms (PW1 and PW2) and *E. coli*. Cephalexin's greater efficacy against gram-positive bacteria was reported by (Khanal *et al.*, 2017) this was demonstrated in this research by the 30mm and 35mm inhibition zones against PW1 and PW2, respectively. Aslam *et al.*, (2024) examined the efficacy of various generations of cephalosporins against biofilms developed by pathogenic *S. aureus* and *E. coli*. In microplate and petri-plate tests, both bacterial strains showed well-formed biofilms. Phase contrast microscopy verified that *S. aureus* was more susceptible to the effects of all cephalosporin generations than *E. coli*.

Shaikh *et al.* (2015) reported that gentamicin has a well-documented 24mm zone of inhibition against *E. coli*, as well as broad-spectrum activity. The compound is also highly effective against strains that have developed resistance. The moderate effect of erythromycin, especially its 12 mm inhibition against *E. coli*, is in line with the findings of (Akonda *et al.*, 2009), who reported that responses varied according to the strain of bacteria. The poor efficacy of ampicillin and vancomycin against *Escherichia coli*, however, their stronger activity against PW1 and PW2 is indicative of established patterns of susceptibility and resistance as documented by (Woodford & Livermore, 2009). In line with findings by (Agyare, *et al.* 2018) the notable effectiveness efficacy of clindamycin, chloramphenicol, and oxacillin, particularly against PW1, highlights their potential utility in the fight against certain bacterial illnesses linked to poultry. Mukhtar *et al.* (2023) evaluated the potency of selected antibiotic medications dispensed in community pharmacies in Ngeria. According to the MIC and zone of inhibition, the ethanol extract of mango leaves showed variable levels of antibacterial activity against PW1 and PW2, which is in line with other research that looked at the antimicrobial qualities of plant extracts. Mango leaf extracts exhibit strong antibacterial properties, especially at higher concentrations, according to (Alzoreky & Nakahara, 2003).

Using a few clinical isolates as test organisms, Ogbona *et al.* (2018) examined the antibacterial qualities and effectiveness of mango (*Mangifera indica*) leaf extracts. On the NCCLS scale, extracts of mature mango leaves in all solvents and doses did not cause any susceptibility response in the test bacterial species. It was discovered that the range of minimum inhibitory and bactericidal doses was 25–50 mg/ml. Furthermore, it was found that organisms' sensitivity to mango extracts increased with concentration.

These findings are comparable to the 42 mm and 40 mm inhibitory zones against PW1 and PW2 that observed at 20% concentration. Plant-based antimicrobials are concentration-dependent, as seen by the slow decline of inhibition at lower doses (e.g., the 0.15% extract exhibiting 10mm and 17mm zones). The MIC values of mango extracts differed significantly amongst different bacterial strains, according to (Abiala *et al.*, 2016) which is consistent with the variations in MICs against PW1 and PW2 in your data. Furthermore, the absence of inhibition at very low concentrations (0.07%) is consistent with research by (Ofongo *et al.*, 2021), which showed that the extract's efficacy decreases with its concentration, especially when it comes to strains of bacteria that are more resistant.

Nguyen *et al.* (2024) studied on effects of maturation on antibacterial properties of Vietnamese Mango (*Mangifera indica*) leaves and the result showed that extracts from mango leaves at young ages had better antibacterial properties than those from old leaves, as evidenced by the lower minimum inhibitory concentrations and larger inhibitory zones.

The presence of alkaloids, saponins, phytosterols, triterpenes, phenols, flavonoids, and tannins in the phytochemical analysis of the mango plant extract is in line with other studies that have highlighted the diverse phytochemical profile of mango leaves. The strong antioxidant and antibacterial activities of mango extracts are attributed to their considerable levels of phenols and flavonoids, as reported by (Akinmoladun *et al.*, 2007). These findings corroborate the favorable results in the ferric chloride conducted in study. The results of the Picric Acid and Froth tests, which revealed the presence of alkaloids and saponins, respectively, are consistent with the findings of (Ajila *et al.*, 2007) who

characterized these chemicals as important bioactive components in mango leaves that contribute to their therapeutic benefits. The identification of proteins and amino acids by the use of Xanthoproteic and Ninhydrin tests provides additional support for the findings of (Masibo & He, 2008), who noted that the protein content of mango leaves contributes to their nutritional and therapeutic value. The Fehling test result that is negative, which rules out carbohydrates, is consistent with the findings of (Abdullah & Mazlan, 2020) who reported that the phytochemicals largely found in mango leaves are non-carbohydrate.

The study's findings demonstrated that, of all the plant extracts examined, the ethanolic extract of mango leaves had the strongest antibacterial action, particularly when it came to poultry culture PW2, where it inhibited a zone measuring roughly 45mm. This result was consistent with other studies on the plant, which showed that mango leaves have strong antibacterial properties. As stated by According to (Nguyen *et al.*, 2024) the antibacterial potency of Vietnamese mango leaves against test bacterial strains was influenced by the maturity stage of the leaves. This could be attributed to bioactive compounds in mango leaves, such as alkaloids, flavonoids, and tannins, playing a leading role in inhibiting the growth of bacteria.

Conversely, against the studied bacterial strains, the mulberry and curry leaf extracts exhibited moderate to poor antibacterial activity. Despite being widely recognized as an herbal remedy, mulberry did not show any appreciable zones of inhibition in this study. According to earlier research that revealed inconsistent outcomes based on the phytochemical composition and concentration in the extract, this suggests that the antibacterial efficacy of mulberry would rely on the extraction technique and solvent used. Curry leaf extract, on the other hand, showed modest action, particularly against PW1, and the results were consistent with those of (Katariya & Arjunker, 2019) who found that curry leaves have strong antibacterial activity against specific bacterial strains.

These results thus demonstrate that plant extracts, particularly those from mango leaves, are a viable substitute for antibiotics in the farming of poultry. Antibiotic resistance, the usage of antibiotics, and the management of bacterial illnesses can be substantially aided by such natural alternatives. Nevertheless, more investigation into the mechanisms of action of plant extracts is needed to assess their effectiveness in a range of poultry husbandry scenarios. This would apply to both the evaluation of the *in vitro* and *in vivo* activity of such plants as well as the search for alternative medicinal plants containing similar bioactive chemicals. In the long run, this could contribute to the development of an all-encompassing program on bird disease control that guarantees animal and public health safety.

Conclusion

Two bacterial contaminants were isolated namely Poultry Water isolate 1 (PW1) and Poultry Water isolate 2 (PW2) by serial dilution agar plate technique. The agar well diffusion method was used to screen the antibacterial activity of 21 plant extracts of 7 plants. Curry leaf and Ginger rhizome ethanolic extracts showed maximum inhibition activity against *E. coli* followed by Neem leaves and Black pepper seeds ethanolic extracts. Mango leaves and Mulberry leaves ethanolic extracts showed maximum inhibition activity against PW1. Mango leaves and Curry leaves ethanolic extract showed maximum inhibition activity against PW2. The most potent commercially available antibacterial agent was cephalexin against PW1 and PW2. Erythromycin and Gentamicin also showed considerable inhibition against *E. coli*, PW1, and PW2. Strong antibacterial activity was shown by vancomycin and ampicillin against PW1 and PW2, while ineffectiveness was shown against *E. coli*. A minimum inhibitory concentration (MIC) of 0.15% against PW1 and 0.625% against PW2 isolates was observed in the ethanol extract of mango leaves. Phytochemical analysis of mango plant extract revealed the presence of alkaloids, saponins, phenols, flavonoids, proteins, and amino acids, while triterpenes and carbohydrates were absent. The present study suggested that the isolation of bacteria and *In vivo* study of bioactive compounds will be done before using as antimicrobial agent in place of commercially available antibiotics.

Conflict of Interest

The authors declare that they have no competing interests

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References

- Abdullah, S. S. S., & Mazlan, A. N. (2020). Quantification of polyphenols and antioxidant activity in several herbal and green tea products in Malaysia. *Materials Today: Proceedings*, 31, A106-A113. <https://doi.org/10.1016/j.matpr.2020.12.1083>
- Abiala, M., Olayiwola, J., Babatunde, O., Aiyelaagbe, O., & Akinyemi, S. (2016). Evaluation of therapeutic potentials of plant extracts against poultry bacteria threatening public health. *BMC Complementary and Alternative Medicine*, 16(1), 417. <https://doi.org/10.1186/s12906-016-1399-z>
- Abreu, R., Semedo-Lemsaddek, T., Cunha, E., Tavares, L., & Oliveira, M. (2023). Antimicrobial drug resistance in poultry production: Current status and innovative strategies for bacterial control. *Microorganisms*, 11(4), 953. <https://doi.org/10.3390/microorganisms11040953>
- Agyare, C., Boamah, V. E., Zumbi, C. N., & Osei, F. B. (2018). Antibiotic use in poultry production and its effects on bacterial resistance. *Antimicrobial resistance—A global threat*, IntechOpen. <https://doi.org/10.5772/INTECHOPEN.79371>
- Ajila, C. M., Naidu, K. A., Bhat, S. G., & Rao, U. P. (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry*, 105(3), 982-988. <https://doi.org/10.1016/j.foodchem.2007.04.052>
- Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M., & Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Sci Res Essay*, 2(5), 163-166. https://academicjournals.org/article/article1380191019_Akinmoladun%20%20et%20al.pdf
- Akond, M. A., Alam, S., Hassan, S. M. R., & Shirin, M. (2009). Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. *American Journal of Environmental Sciences*, 11. <https://doi.org/10.3844/ajessp.2009.47.52>
- Alzoreky, N. S., & Nakahara, K. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology*, 80(3), 223-230. [https://doi.org/10.1016/s0168-1605\(02\)00169-1](https://doi.org/10.1016/s0168-1605(02)00169-1)
- Aneja, K. R. (2007). *Experiments in microbiology, plant pathology and biotechnology*. New Age International. New Delhi, India. <https://books.google.com.np/books?id=QYl4xk9kOIMC&printsec=frontcover#v=onepage&q&f=false>
- Anizoba, N. W., Ugwu, S. O., Ndofor-Foleng, H. M., Onyimonyi, A. E., Ikeh, N. E., Ezenwosu, C., ... & Machebe, N. S. (2024). Effects of aqueous extracts of neem leaf and ginger rhizome on growth performance and haematological parameters of pure and crossbred chickens. *Journal of World's Poultry Research*, 14(3), 273-281. <https://dx.doi.org/10.36380/jwpr.2024.28>
- Aslam, J., Ali, H. M., Hussain, S., Ahmad, M. Z., Siddique, A. B., Shahid, M., ... & Alarjani, K. M. (2024). Effectiveness of cephalosporins in hydrolysis and inhibition of *Staphylococcus aureus* and *Escherichia coli* biofilms. *Journal of Veterinary Science*, 25(3), e47. <https://doi.org/10.4142/jvs.23258>
- European Food Safety Authority (2017). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal*, 15(12), e05077. <https://doi.org/10.2903/j.efsa.2017.5077>
- Eid, S., & Erfan, A. M. (2013). Characterization of *E. coli* associated with high mortality in poultry flocks. *Assiut Veterinary Medical Journal*, 59(139), 51-61. <https://doi.org/10.21608/avmj.2013.171926>
- Enejiyon, S., Abdulrahman, A. H., Adedeji, A., Abdulsalam, R., & Oyedum, M. (2020). Antibacterial activities of the extracts of *Allium sativum* (Garlic) and *Allium cepa* (Onion) against selected pathogenic bacteria. *Tanzania Journal of Science*, 46(3), 914-922. <https://doi.org/10.4314/tjs.v46i3.29>
- Gržinić, G., Piotrowicz-Cieślak, A., Klimkowicz-Pawlas, A., Górný, R. L., Ławniczek-Wałczyk, A., Piechowicz, L., ... & Wolska, L. (2023). Intensive poultry farming: A review of the impact on the environment and human health. *Science of the Total Environment*, 858, 160014. <https://doi.org/10.1016/j.scitotenv.2022.160014>
- Hooda, P. S., Edwards, A. C., Anderson, H. A., & Miller, A. (2000). A review of water quality concerns in livestock farming areas. *Science of The Total Environment*, 250(1-3), 143-167. [https://doi.org/10.1016/S0048-9697\(00\)00373-9](https://doi.org/10.1016/S0048-9697(00)00373-9)

- Ofongo, R. T., Ohimain, E. I., & Iyayi, E. A. (2021). Qualitative and quantitative phytochemical screening of bitter and Neem leaves and their potential as antimicrobial growth promoter in poultry feed. *European Journal of Medicinal Plants*, 32(4), 38-49. <https://doi.org/10.9734/ejmp/2021/v32i430383>
- Jamil, M., Aleem, M. T., Shaukat, A., Khan, A., Mohsin, M., Rehman, T. U., ... & Li, K. (2022). Medicinal plants as an alternative to control poultry parasitic diseases. *Life*, 12(3), 449. <https://doi.org/10.3390/life12030449>
- Katariya, C., & Arjunker, R. (2019). Antimicrobial effect of curry leaves on *Staphylococcus aureus*—An In vitro Study. *Research Journal of Pharmacy and Technology*, 12(7), 3318-3322. <https://doi.org/10.5958/0974-360X.2019.00559.6>
- Khanal, T., Raut, S. B., & Paneru, U. (2017). Study of antibiotic resistance on *Escherichia coli* in commercial poultry of Nepal. *Nepalese Veterinary Journal*, 34, 6-17. <https://doi.org/10.3126/nvj.v34i0.22859>
- Masibo, M., & He, Q. (2008). Major mango polyphenols and their potential significance to human health. *Comprehensive Reviews in Food Science and Food Safety*, 7(4), 309-319. <https://doi.org/10.1111/j.1541-4337.2008.00047.x>
- Mohammed, M. T. (2023). Analytical detection of phytochemical compounds in *Cinnamomum zeylanicum* bark extract. *Egyptian Journal of Chemistry*, 66(4), 265-273. <https://doi.org/10.21608/ejchem.2022.146416.6366>
- Morton, N. (2022). Poultry Drinking Water Primer The importance of water, factors affecting water consumption, water quality and management tips are reviewed by Brian D. Fairchild and Casey W. Ritz, Extension Poultry Scientists at the University of Georgia. *Health*, 4, 5. https://secure.caes.uga.edu/extension/publications/files/pdf/b%201301_4.pdf
- Mukhtar, M. D., Rufa'i, F. A., Yola, A. U., Babba, N. I., & Baecker, D. (2023). Evaluating the potency of selected antibiotic medications dispensed in community pharmacies in Gwale, Kano, Nigeria. *Antibiotics*, 12(11), 1582. <https://doi.org/10.3390/antibiotics12111582>
- Nguyen, H. T., Miyamoto, A., Hoang, H. T., Vu, T. T. T., Pothinuch, P., & Nguyen, H. T. T. (2024). Effects of maturation on antibacterial properties of vietnamese mango (*Mangifera indica*) leaves. *Molecules*, 29(7), 1443. <https://doi.org/10.3390/molecules29071443>
- Nrior, R. R., Ugboma, C. J., Lugbe, Q., & Ogbonna, D. N. (2023). Susceptibility of *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* to extracts of mango (*Mangifera indica*). *Journal of Advances in Microbiology*, 23(5), 46-57. <https://doi.org/10.9734/jamb/2023/v23i5725>
- Nwafor, M. N., Anosike, A. C., Onodugo, C. A., Ya'u, M., & Babandi, A. (2022). Antioxidant, Phytochemical Composition and Proximate Analysis of *Mangifera haden* Seeds. *International Journal of Pharmaceutical Sciences and Research*, 1(01), 011-023. <https://doi.org/10.56781/ijrcrp.2022.1.1.0022>
- Okafor, U. C., & Ugwuegbulem, P. M. (2022). Antimicrobial susceptibility evaluation of microorganisms isolated from poultry water in awka metropolis. *Journal of Biochemistry International*, 9(4), 1-9. <https://doi.org/10.56557/job/2022/v9i47581>
- Olasehinde, G. I., Sholotan, K. J., Openibo, J. O., Taiwo, O. S., Bello, O. A., Ajayi, J. B., ... & Ajayi, A. A. (2018). Phytochemical and antimicrobial properties of *Mangifera indica* leaf extracts. *Covenant Journal of Physical and Life Sciences*, 16(1), 55-63. <https://journals.covenantuniversity.edu.ng/index.php/cjpls/article/view/934/608>
- Ritchie, H., Rosado, P., & Roser, M. (2019). *Meat and Dairy Production*. Our World in Data. <https://ourworldindata.org/meat-production>
- Rodrigues, G., Souza Santos, L., & Franco, O. L. (2022). Antimicrobial peptides controlling resistant bacteria in animal production. *Frontiers in Microbiology*, 13, 874153. <https://doi.org/10.3389/fmicb.2022.874153>
- Rouger, A., Tresse, O., & Zagorec, M. (2017). Bacterial contaminants of poultry meat: sources, species, and dynamics. *Microorganisms*, 5(3), 50. <https://doi.org/10.3390/microorganisms5030050>
- Samad, A., Hamza, M., Muazzam, A., Ahmer, A., Tariq, S., Shahid, M. J., ... & Din, F. U. (2022). Overview of Bacterial Diseases In Poultry And Policies To Control Disease And Antibiotic Resistance. *Bullet: Jurnal Multidisiplin Ilmu*, 1(01), 19-25. <https://www.neliti.com/publications/591942/overview-of-bacterial-diseases-in-poultry-and-policies-to-control-disease-and-an#cite>
- Seidavi, A., Tavakoli, M., Slozhenkina, M., Gorlov, I., Hashem, N. M., Asroosh, F., ... & Swelum, A. A. (2021). The use of some plant-derived products as effective alternatives to antibiotic growth promoters in organic poultry production: A review. *Environmental Science and Pollution Research*, 28(35), 47856-47868. <https://doi.org/10.1007/s11356-021-15460-7>
- Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D., & Kamal, M. A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi Journal of Biological Sciences*, 22(1), 90-101. <https://doi.org/10.1016/j.sjbs.2014.08.002>
- Sharma, L., & Pundir, R. K. (2018). Evaluation of antimicrobial activity of *emblica officinalis* against skin associated microbial strains. *Current Trends in Biotechnology and Pharmacy*, 12(4), 355-366.

- Singh, S., Kriti, M., Sharma, P., Pal, N., Sarma, D. K., Tiwari, R., & Kumar, M. (2025). A one health approach addressing poultry-associated antimicrobial resistance: human, animal and environmental perspectives. *The Microbe*, 100309. <https://doi.org/10.1016/j.microb.2025.100309>
- Somkuwar, D. O., & Kamble, V. A. (2013). Phytochemical screening of ethanolic extracts of stem, leaves, flower and seed kernel of *Mangifera indica* L. *International Journal of Pharma and Bio Sciences*, 4(2), 383-389. <https://ijpbs.net/abstract.php?article=MjEzOQ==>
- Stromberg, Z. R., Johnson, J. R., Fairbrother, J. M., Kilbourne, J., Van Goor, A., Curtiss 3rd, R., & Mellata, M. (2017). Evaluation of *Escherichia coli* isolates from healthy chickens to determine their potential risk to poultry and human health. *PLoS one*, 12(7), e0180599. <https://doi.org/10.1371/journal.pone.0180599>
- Tripathy, B., & Sahoo, S. K. (2024). Biological evaluation of nutritional traditional fruit *Mangifera camptosperma* against diabetes and hyperlipidemia. *Pharmacological Research-Modern Chinese Medicine*, 11, 100436. <https://doi.org/10.1016/j.prmcm.2024.100436>
- Ustundag, A. O., & Ozdogan, M. (2015). Usage possibilities of mulberry leaves in poultry nutrition. *Scientific papers. series D. Animal Science*, 58, 170-178. <https://animalsciencejournal.usamv.ro/pdf/2015/Art28.pdf>
- Wardhana, D. K., Haskito, A. E. P., Purnama, M. T. E., Safitri, D. A., & Annisa, S. (2021). Detection of microbial contamination in chicken meat from local markets in Surabaya, East Java, Indonesia. *Veterinary world*, 14(12), 3138. <https://doi.org/10.14202/vetworld.2021.3138-3143>
- Woodford, N., & Livermore, D. M. (2009). Infections caused by Gram-positive bacteria: a review of the global challenge. *Journal of Infection*, 59, S4-S16. [https://doi.org/10.1016/s0163-4453\(09\)60003-7](https://doi.org/10.1016/s0163-4453(09)60003-7)
- Yao, H., Liu, J., Jiang, X., Chen, F., Lu, X., & Zhang, J. (2021). Analysis of the clinical effect of combined drug susceptibility to guide medication for carbapenem-resistant *Klebsiella pneumoniae* patients based on the Kirby–Bauer disk diffusion method. *Infection and Drug Resistance*, 79-87. <https://doi.org/10.2147/idr.s282s386>