Policy Research Journal
ISSN (E): 3006-7030 ISSN (P) : 3006-7022 Volume 3, Issue 6, 2025

SCREENING THE ANTIMICROBIAL POTENTIAL OF FOUR ETHNOBOTANICALS AGAINST POULTRY-DERIVED *ESCHERICHIA COLI*: AN IN-VITRO STUDY

Farhana Bano[1], Malik Muhammad Sohail Elahi[2], Shehzad Zareen[*3], Shawana Nawaz[4], Mehtab Parveen[*5], Amina Nazar[*6], Syed Irfan Ullah Shah[7], Syed Ali Abbas[8], Mubasher Khan[9]

[1,2,3,4]*Department of Zoology, Kohat University of Science and Technology, Kohat, 26000, Khyber Pakhtunkhwa, Pakistan*

[*5,*6]*Department of Botany, Kohat University of Science and Technology, Kohat, 26000, Khyber Pakhtunkhwa, Pakistan*

[7,8,9]*Department of Microbiology, Kohat University of Science and Technology, Kohat, 26000, Khyber Pakhtunkhwa, Pakistan*

[1]farhanabano865@gmail.com, [2]maliksohail44889@gmail.com, [*3]shehzadzareen1@kust.edu.pk, [4]shawananawazkust@gmail.com, [*5]mehtabkhan11191@gmail.com, [*6]amnanazarktk@gmail.com, [7]irfanullah9154@gmail.com, [8]syedaliabbas552@gmail.com, [9]mubasherk410@gmail.com

DOI: https://doi.org/10.5281/zenodo.15719373

Keywords
Allium sativum, Escherichia coli, broiler chickens, antibacterial activity, agar well diffusion, medicinal plants, minimum inhibitory concentration

Article History
Received on 14 May 2025
Accepted on 14 June 2025
Published on 23 June 2025

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Corresponding Authors: *
Mehtab Parveen
Amina Nazar
Shehzad Zareen

Abstract

Antibiotic resistance among bacterial pathogens in poultry has become a critical concern, necessitating the exploration of alternative antimicrobial agents. This in-vitro study evaluated the antibacterial potential of four medicinal plants–Allium sativum (garlic), Piper betle (betel leaf), Phyllanthus emblica (Indian gooseberry), and Trachyspermum ammi (ajwain)–against Escherichia coli isolated from naturally infected broiler chickens in District Kohat, Khyber Pakhtunkhwa, Pakistan. The isolation and identification of E. coli were performed using standard microbiological techniques, including culturing on MacConkey and EMB agar, followed by Gram staining and biochemical tests such as citrate, catalase, and oxidase assays. Antibacterial activity was assessed using the agar well diffusion method. At a concentration of 0.5 mg/ml, A. sativum demonstrated the strongest inhibition zone (20 mm) among the plant extracts, second only to the standard antibiotic Moxifloxacin (31 mm). P. emblica showed moderate activity (10 mm), while T. ammi exhibited no observable inhibition. Due to its superior performance, A. sativum was selected for further concentration-dependent analysis, which revealed zones of inhibition ranging from 32.5 mm to 33.5 mm at higher concentrations (0.75–1.25 mg/ml), nearly matching or surpassing Moxifloxacin (33 mm). The minimum inhibitory concentration (MIC) of A. sativum was determined to be 0.050 mg/ml, at which it produced a significant average inhibition zone of 31 mm. These results suggest that A. sativum possesses potent antibacterial activity even at lower concentrations, making it a promising natural candidate for controlling E. coli infections in poultry. This study supports the potential of plant-based antimicrobials, particularly garlic, as effective alternatives to conventional antibiotics in the face of increasing antimicrobial resistance.

INTRODUCTION

Escherichia coli is a Gram-negative, facultatively anaerobic, motile, non-spore-forming rod-shaped bacterium belonging to the family *Enterobacteriaceae*. While many strains are harmless commensals of the mammalian intestinal tract, others possess distinct virulence factors that enable them to cause a wide spectrum of infections in both humans and animals, including gastrointestinal, urinary tract, bloodstream, and central nervous system infections (Schaechter, 2009). The genus *Escherichia* was named in honor of Dr. Theodor Escherich, who first isolated the organism (Gould, 2011). Among pathogenic variants, uropathogenic *E. coli* (UPEC) are the principal agents of urinary tract infections (Gould, 2010).

Avian pathogenic *E. coli* (APEC) strains are responsible for colibacillosis, a globally prevalent poultry disease that predominantly affects broiler chickens between 4 and 6 weeks of age. The disease contributes substantially to poultry mortality and economic losses. Clinical manifestations include respiratory distress, anorexia, and poor growth, while post-mortem lesions commonly observed are airsacculitis, pericarditis, perihepatitis, and peritonitis (Dho-Moulin et al., 1999). In addition to its clinical significance, *E. coli* is a cornerstone organism in biotechnology. Its rapid growth, well-characterized genetics, and adaptability to both aerobic and anaerobic conditions make it ideal for genetic engineering, including applications in recombinant DNA technology and industrial microbiology (Yoon et al., 2009). In Pakistan, the poultry industry, despite its rapid expansion and substantial contribution to the national economy, faces significant setbacks due to infectious diseases. Respiratory pathogens, particularly *E. coli*, are among the leading causes of morbidity and mortality in poultry flocks, leading to considerable economic losses through decreased productivity and increased treatment costs (Ali et al., 2000; Pang et al., 2002).

The growing concern regarding *E. coli* is amplified by the increasing prevalence of antimicrobial resistance (AMR), often mediated by horizontally acquired antibiotic resistance genes (ARGs) such as those carried on plasmids (Matthew, 2010). In poultry, infections caused by resistant *E. coli* strains often lead to colibacillosis, posing challenges to treatment and increasing mortality rates (Dho-Moulin et al., 1999). Transmission of *E. coli* is not limited to food or water; it may also occur via direct contact with infected animals or humans. This zoonotic potential underlines its importance in public health. The widespread use and, at times, misuse of antibiotics in both human and veterinary medicine have driven the emergence of resistant *E. coli* strains. Resistance mechanisms may be intrinsic—such as the presence of efflux pumps—or acquired via mutations or horizontal gene transfer. These resistance determinants, often located on plasmids or other mobile genetic elements, can be transferred between bacteria, compounding the challenge (Nataro et al., 1998). Antimicrobial resistance leads to prolonged illnesses, increased mortality, higher treatment costs, and a growing need for novel therapeutics—an especially dire concern for resource-limited settings.

Medicinal plants are essential to healthcare due to their rich content of bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant properties. They serve as a foundation for drug discovery, offering affordable and accessible treatment options, especially in developing regions. With growing interest in natural therapies and rising antibiotic resistance, medicinal plants remain a valuable resource for both traditional and modern medicine. South Asia, particularly Pakistan, possesses a rich heritage of medicinal flora, with over 8,000 plant species identified as having therapeutic properties—1,000 of which are native to Pakistan (Bashir et al., 2015). Local communities, particularly in remote areas, continue to use indigenous knowledge for treating infections and ailments using these medicinal plants (Gul et al., 2012; Mahmood et al., 2011). Phytochemicals such as alkaloids, glycosides, saponins, resins, essential oils, and various phenolic compounds have been isolated from these species, demonstrating antimicrobial and other pharmacological activities (Shinwari et al., 2013; Singh et al., 2011).

Trachyspermum ammi commonly known as Ajwain, is native to Egypt but widely cultivated in Pakistan, India, and neighbouring regions (Bairwa et al., 2012). Its seeds are traditionally used for a variety of ailments including indigestion, respiratory distress, abdominal pain, and parasitic infections (Avesina, 1985; Murad et al., 2019). Veterinary applications by traditional

healers include usage as a stomachic and anthelmintic in the form of decoctions and medicinal balls (Jabbar et al., 2006).

Phyllanthus emblica (Indian Gooseberry / Amla) is a subtropical tree from the family *Euphorbiaceae*, used in traditional medicine for its anti-inflammatory, antipyretic, and antioxidant properties (Summanen, 1999). It has been employed in the treatment of disorders ranging from scurvy and cardiovascular disease to cancer and viral infections such as HIV and herpes (Khopde et al., 2000). Its leaves and fruit contain potent secondary metabolites with significant bioactivity (Calixto et al., 1998). Traditional uses across Asia include treating fevers, headaches, and digestive issues (Burkill, 1966; Perry et al., 1980).

Belonging to the family *Piperaceae*, *Piper betle (Betel Leaf)* is a dioecious climber traditionally used across South and Southeast Asia. The plant contains bioactive compounds with antiseptic, anti-inflammatory, wound-healing, and anticancer properties (Pradhan et al., 2013; Baliga et al., 2013). In many cultures, it is chewed with areca nut and lime, but it also features in traditional remedies for pharyngitis, abdominal swelling, malaria, and skin infections (Bhattacharya et al., 2007; Bhalerao et al., 2013).

Allium sativum (Garlic) a member of the *Alliaceae* family, is among the oldest cultivated medicinal plants. Known for its culinary and therapeutic applications, garlic contains organosulfur compounds such as allicin, which confer broad-spectrum antimicrobial activity (Rehman, 2003; Borek, 2001). Historically, garlic has been used to combat infections, cardiovascular diseases, and metabolic disorders (Rivlin, 2001; Goncagul et al., 2010). Its medicinal use is widespread across cultures, including for cancer symptom relief and hypertension management (Devrim et al., 2007; Ishtiaq et al., 2007; Sandhu et al., 2005).

MATERIALS AND METHODS

Isolation of Bacteria from Infected Broiler Chickens

The bacteria were isolated from naturally infected broiler chickens using sterile cotton swabs, collected from various poultry shops in District Kohat, Khyber Pakhtunkhwa. Samples were obtained by swabbing the mouth and nasal cavities of symptomatic birds. To minimize contamination, the swabs were immediately wrapped in aluminum foil and transported promptly to the Microbiology Laboratory at Kohat University of Science and Technology for further analysis (Figure-1).



Figure-1: Sample Collection from Infected Broiler Chickens

Sterilization by Autoclaving

Prior to the commencement of the experiment, all glassware, culture media, and instruments were thoroughly sterilized using an autoclave to eliminate any potential microbial contaminants. Autoclaving was carried out at 121°C under 15 psi pressure for 15–20 minutes, ensuring aseptic conditions were maintained throughout the experimental procedures. This step was critical to prevent cross-contamination

and to ensure the reliability and accuracy of the microbiological analyses (Figure-2).

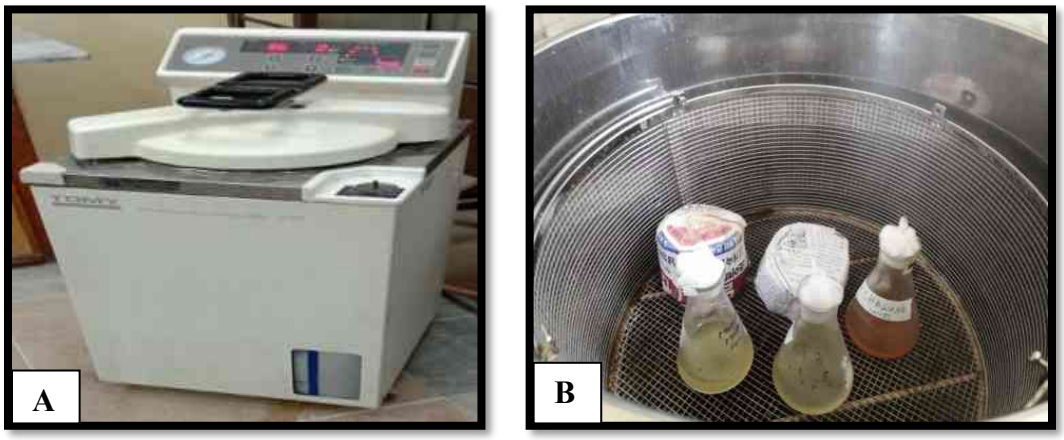
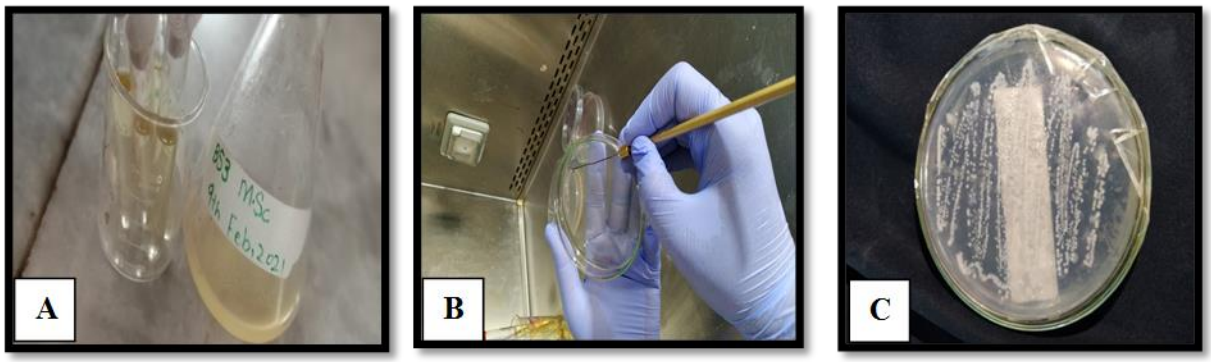


Figure-2: A) High Pressure Steam Sterilizer (TOMY ES-215), B) Sterilization Chamber

Preparation of Nutrient Agar and bacterial lawn

All collected samples were subjected to standard microbiological procedures for culturing, isolation, and identification of *Escherichia coli*. Initially, nutrient agar was prepared and sterilized by autoclaving at 121°C for 15–20 minutes. The sterilized medium was then aseptically poured into sterile Petri dishes inside a laminar flow safety cabinet. Once the agar solidified (after approximately 10 minutes), the swab samples were streaked onto the agar surface using the streak plate method to promote isolated colony formation. The inoculated plates were incubated at 37°C for 24 hours, after which bacterial growth was observed in the form of distinct colonies, indicating successful culture of the target organism (Figure-3)..

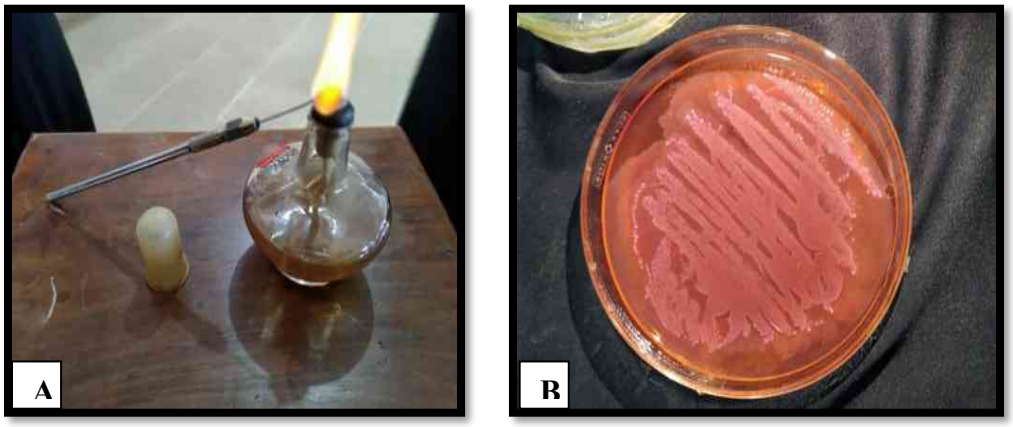
Figur-3: Preparation of Nutrient Agar, B) Streaking of Bacteria on Agar Plate, C) Bacterial Colony on Nutrient Agar



Preparation of Mackonkey Agar

MacConkey agar was prepared and sterilized by autoclaving at 121°C for 15–20 minutes to ensure complete elimination of contaminants. The sterilized medium was then poured into sterile Petri plates under a laminar flow safety cabinet. Once solidified (after about 10 minutes), bacterial colonies previously grown on nutrient agar were aseptically transferred using a sterile wire loop and streaked onto the MacConkey agar to support selective growth and facilitate further identification of *Escherichia coli*. The plates were incubated at 37°C for 24 hours. Following incubation, characteristic light pink colonies

appeared on the medium, indicative of lactose-fermenting *E. coli* (Figure-4).

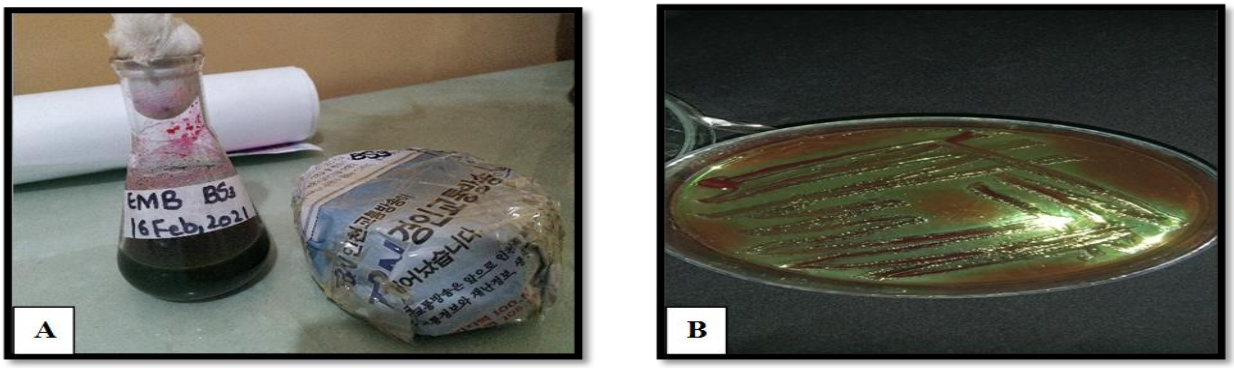


Figur-4: A) Sterilization of Wire Loop for Streaking, B) *E. coli* colony on Mackonkey Agar

Preparation of EMB Media

Eosin Methylene Blue (EMB) agar was prepared and sterilized by autoclaving at 121°C for 15–20 minutes. The sterile medium was then aseptically poured into Petri plates within a laminar flow safety cabinet and allowed to solidify. Once set, a bacterial colony previously cultured on MacConkey agar was carefully transferred using a sterile wire loop and streaked onto the EMB agar surface. The plates were then incubated at 37°C for 24 hours. After incubation, the appearance of characteristic metallic green sheen colonies confirmed the presence of *Escherichia coli*, as this distinctive sheen is a hallmark of lactose-fermenting *E. coli* on EMB medium (Figure-5).

Figur-5: A) Preparation of EMB media, B) *Escherichia coli* colony on EMB media



Biochemical Identification of *Escherichia coli*

Accurate identification of *Escherichia coli* was a crucial component of this study. To confirm the bacterial identity, several standard biochemical tests were performed, including Gram staining, citrate utilization, catalase activity, and oxidase testing.

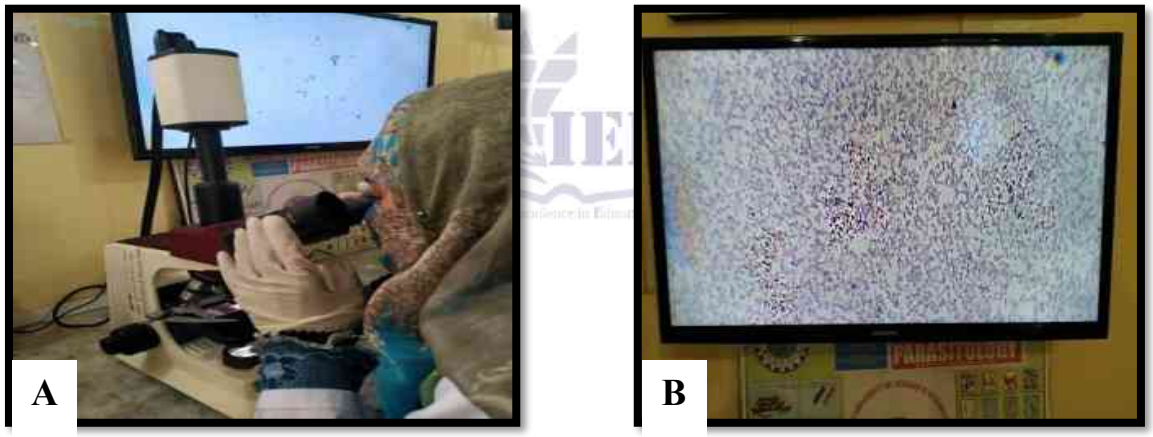
Gram Staining

The Gram staining technique, based on the method of Beveridge et al. (1983), was used to determine the Gram reaction of the isolated bacterium. A bacterial smear was prepared by mixing a colony from EMB agar with a drop of saline on a clean glass slide, which was then air-dried and heat-fixed. The smear was stained sequentially with crystal violet for 15–20 seconds, followed by iodine for one minute. After

rinsing, ethanol was applied for one minute to decolorize the sample, and finally, safranin was used as a counterstain for 20 seconds. Microscopic observation revealed pink-colored, rod-shaped bacteria, confirming the presence of Gram-negative bacilli consistent with *E. coli* (Figure-6,7).

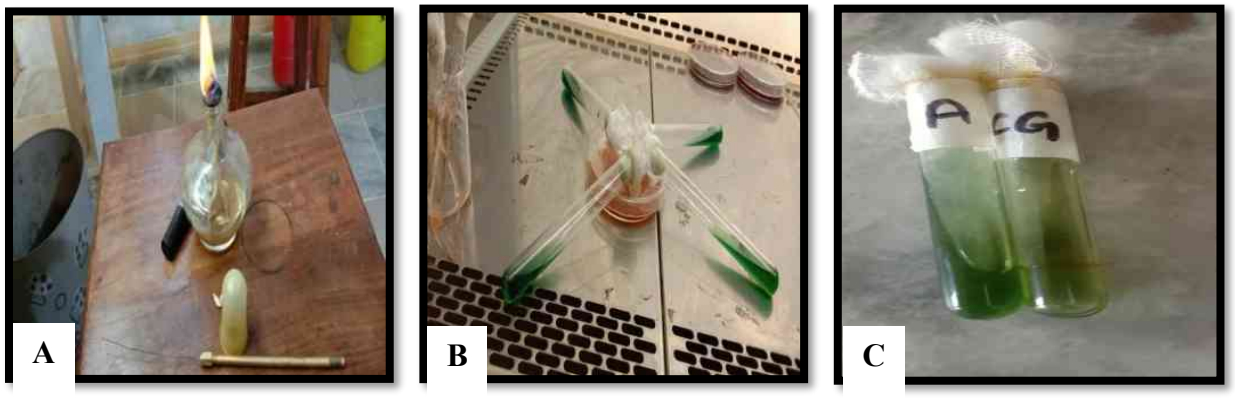
Figur-6: A) Making Smear, B) Inoculation of Bacteria, C) Dyes for Gram Staining





Figur-7: A) Microscopy, B) Microscopy Results

Figur-8: A) Sterilization of straight Wire Loop for Citrate Test, B) Citrate Media, C) Citrate Test Results



Citrate Test

The citrate test was performed as described by MacWilliams (2009). Simmons' citrate agar was prepared, sterilized in slant tubes, and inoculated with a single colony using a sterile wire loop. After 24 hours of incubation at 37°C, no growth and no color change were observed, indicating a negative result. This confirmed the presence of *E. coli*, a citrate-negative, Gram-negative bacterium (Figure-8).

Catalase Test

The catalase test was conducted following the protocol by Reiner (2010) to confirm *E. coli* identification. A bacterial smear from EMB media was prepared on a clean microscopic slide using a sterile wire loop. A drop of hydrogen peroxide (H_2O_2) was then added to the smear without mixing. The immediate formation of bubbles indicated a positive catalase reaction, confirming that the bacterium was catalase-positive, consistent with *E. coli* (Figure-9).



Figure-9: Bubbles indicate Catalase Positive

Oxidase Test

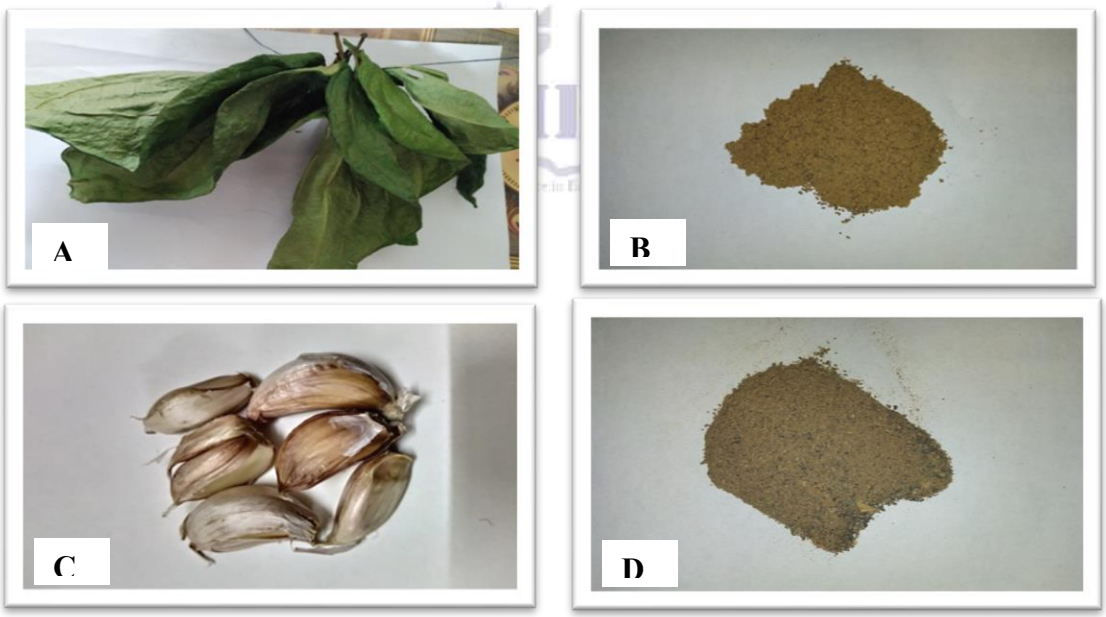
The oxidase test was performed following the protocol by Kovacs (1956) to detect the presence of cytochrome C oxidase enzyme. A dry filter paper was moistened with two drops of oxidase reagent, and a bacterial colony was applied using a sterile wire loop. The absence of color change indicated a negative result, confirming that *E. coli* was oxidase-negative (Figure-10).

Figure 10: A) Adding drop of Oxidase Reagent on Filter paper, B) Inoculation of Bacteria on Filter paper through Sterile Wire Loop, C) No coloration indicates Oxidase Negative



Collection of Plant Materials

Fresh bulbs of *Allium sativum* (garlic) and leaves of *Piper betle* (betel leaf) were collected from local areas of District Kohat. Seed powder of *Trachyspermum ammi* (ajwain) and *Phyllanthus emblica* (Indian gooseberry or amla) were purchased from local markets in the same district to ensure the authenticity and quality of the plant materials used in the study (Figure-11).



Figur-11: A) ***Piper betle*** **(betel leaf), B)** ***Trachyspermum ammi*** **(ajwain), C)** ***Allium sativum*** **(garlic), D)** ***Phyllanthus emblica*** **(amla)**

Preparation of Aqueous Extract

Extract was prepared using the protocol of Arora *et al.*, 2007. Firstly the leaves of *P. betle* were shade dry and then grinded. Seeds of *T. ammi* and *P. emblica* were grinded. Also *A. sativum* was grinded to obtain the extract. The grinded powder was added into test tubes. 5 ml of distilled water was added in each test tube & left for 7 days at room temperature. This mixture was then filtered through filter paper and extract was obtained (Figure-12).



Figure-12: Aqueous Extracts of Selected Plants

In-vitro Antibacterial Assay

The in-vitro antibacterial activity of all four medicinal plants was evaluated using the Agar Well Diffusion Method, following the protocol described by Valgas et al. (2007).

Preparation of Nutrient Broth

To revive *Escherichia coli* cultures prior to antibacterial testing, nutrient broth was prepared by dispensing it into sterile test tubes followed by autoclaving to ensure sterility. Using a sterile wire loop, a bacterial colony of *E. coli* was aseptically inoculated into the autoclaved broth. The inoculated broth was then incubated at 37°C for 24 hours to obtain a refreshed bacterial suspension for subsequent assays.

Preparation of Mueller Hinton Agar (MHA) Media

Mueller Hinton Agar (MHA) was employed to evaluate the antibacterial activity of plant extracts. The powdered MHA medium was dissolved in distilled water and autoclaved to achieve sterility. Under aseptic conditions in a laminar flow hood, the sterilized MHA was poured into petri plates and allowed to solidify. After approximately 10 minutes, a uniform lawn culture of *E. coli* was prepared by dipping a sterile cotton swab into the refreshed broth and evenly spreading it across the surface of the solidified MHA plates (Figure-13,14).

Agar Well Diffusion Method

The antibacterial activity of plant extracts was assessed using the Agar Well Diffusion Method. Five wells were aseptically created on each inoculated MHA plate using sterile blue pipette tips. The first well served as the control, while the remaining four wells were filled with different plant extracts using sterile yellow tips and a micropipette. The plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured to determine antibacterial efficacy.

Figur-13: A) Picking bacterial colony, B) Inoculation of Bacteria, C) Solidified MHA

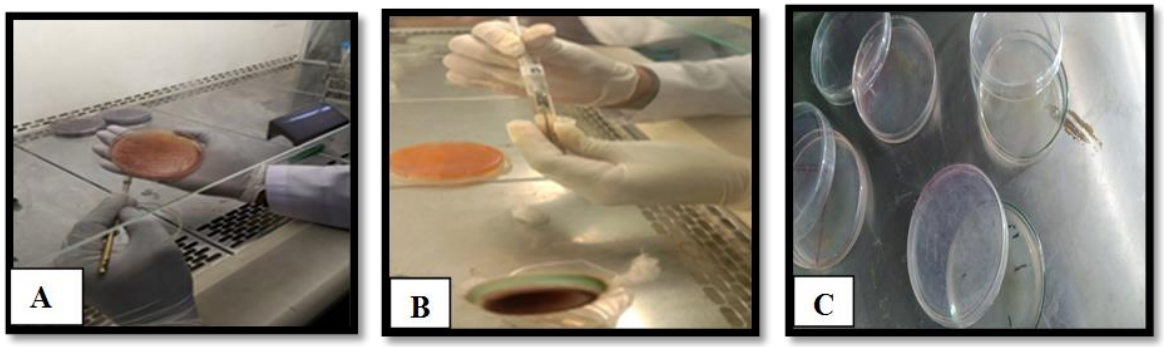
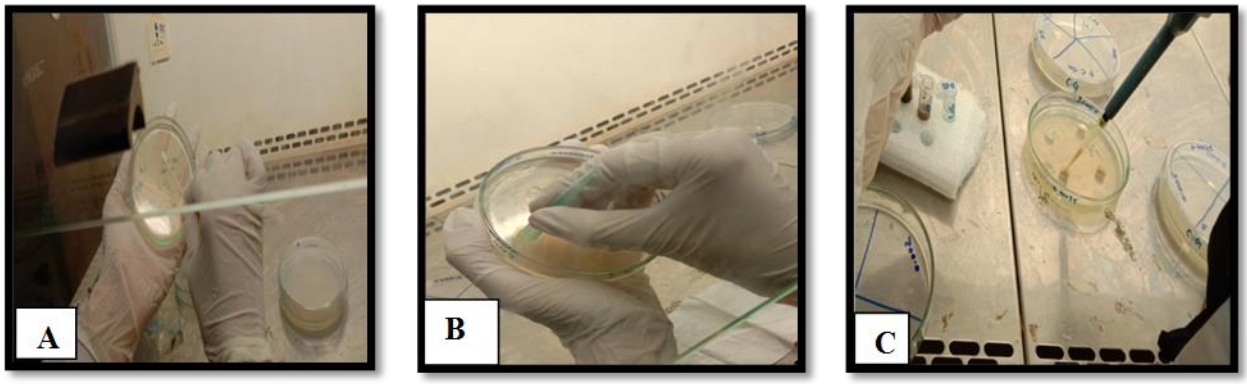


Figure-14: A) Making Lown on MHA media, B) Making Wells for in vitro assay, C) Filling Wells by using pipette



RESULTS

Comparative Antibacterial Analysis

A comparative evaluation was conducted to assess the antibacterial activity of *Allium sativum*, *Piper betle*, *Phyllanthus emblica*, *Trachyspermum ammi*, and the standard antibiotic Moxifloxacin against *E. coli*. Using the agar well diffusion method, 0.5 mg/mL of each test substance was introduced into separate wells. The first well, containing Moxifloxacin as the control, exhibited the largest inhibition zone of 31 mm. *Allium sativum*, tested in the second well, produced a zone of inhibition measuring 20 mm. *Phyllanthus emblica*, placed in the third well, demonstrated moderate activity with a 10 mm inhibition zone. In contrast, *Trachyspermum ammi*, tested in the fourth well, showed no observable zone of inhibition, indicating a lack of antibacterial activity at the given concentration (Figure-15,16).



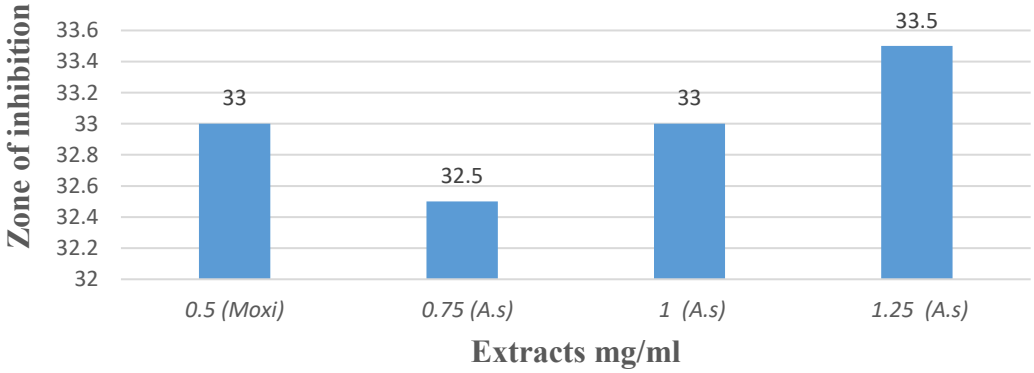
Figure-16: Comparative Antibacterial Analysis of Selected Medicinal Plants with Moxifloxacin Against *Escherichia coli*

In this study, *Allium sativum* (garlic) exhibited notable and comparable antibacterial activity against *Escherichia coli*, producing a clear zone of inhibition second only to the standard antibiotic Moxifloxacin. Due to its promising results, *A. sativum* was selected for further in-vitro antibacterial analysis. To evaluate its efficacy in greater detail, four different concentrations of its extract were prepared and tested to observe the dose-dependent antibacterial effect.

Comparative Analysis of *Allium sativum* at Varying Concentrations with Moxifloxacin

A comparative in-vitro analysis was conducted to evaluate the antibacterial efficacy of *Allium sativum* (garlic) at different concentrations in relation to the standard antibiotic Moxifloxacin. In the first well, Moxifloxacin at a concentration of 0.5 mg/ml demonstrated a zone of inhibition measuring 33 mm. In the second well, *A. sativum* at a concentration of 0.75 mg/ml produced a zone of inhibition of 32.5 mm. When the concentration of *A. sativum* was increased to 1.0 mg/ml in the third well, the inhibition zone measured 33 mm. Finally, in the fourth well, *A. sativum* at 1.25 mg/ml showed a slightly larger inhibition zone of 33.5 mm. These results indicate that *A. sativum* exhibits strong and concentration-dependent antibacterial activity comparable to that of Moxifloxacin (Figure-17,18).

Figure-17: Comparative Analysis of *Allium sativum* at Varying Concentrations with Moxifloxacin



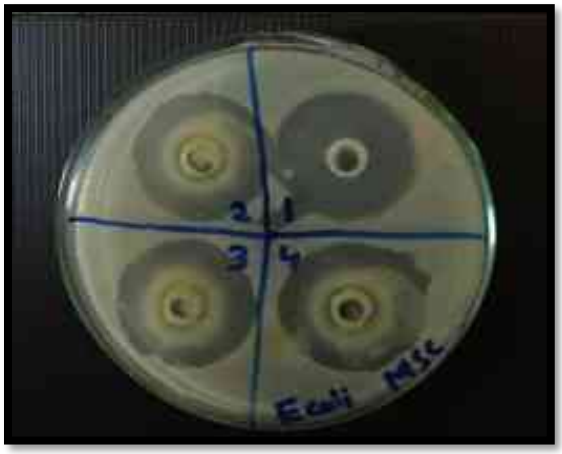


Figure-18: Comparative Analysis of *Allium sativum* at Varying Concentrations with Moxifloxacin

Determination of Minimum Inhibitory Concentration (MIC) of *Allium sativum*

Following the promising antibacterial activity of *Allium sativum* at higher concentrations, further dilutions of the extract were prepared to determine its minimum inhibitory concentration (MIC) against *Escherichia coli*. A comparative analysis with Moxifloxacin was conducted using the agar well diffusion method in triplicates. In the first well, Moxifloxacin at a concentration of 0.025 mg/ml served as the control and showed an average zone of inhibition of 33 mm. In the second well, *A. sativum* at the same concentration (0.025 mg/ml) produced an average inhibition zone of 25.16 mm. The third well, containing 0.050 mg/ml of *A. sativum*, demonstrated an average zone of inhibition of 31 mm, while the fourth well with a concentration of 0.075 mg/ml showed an average inhibition zone of 31.76 mm. These results suggest that *A. sativum* exhibits considerable antibacterial activity even at lower concentrations, indicating its potential as an effective natural antimicrobial agent.

Based on the findings of this study, the minimum inhibitory concentration (MIC) of *Allium sativum* extract against *Escherichia coli* was determined to be 0.050 mg/ml. At this concentration, the extract exhibited a significant average zone of inhibition measuring 31 mm, which was comparable to that of the standard antibiotic control, Moxifloxacin. This indicates that *A. sativum* possesses strong antibacterial potential even at relatively low concentrations. Therefore, 0.050 mg/ml is concluded as the MIC for *Allium sativum* against *E. coli* in this in-vitro study (Figure-19, 20).

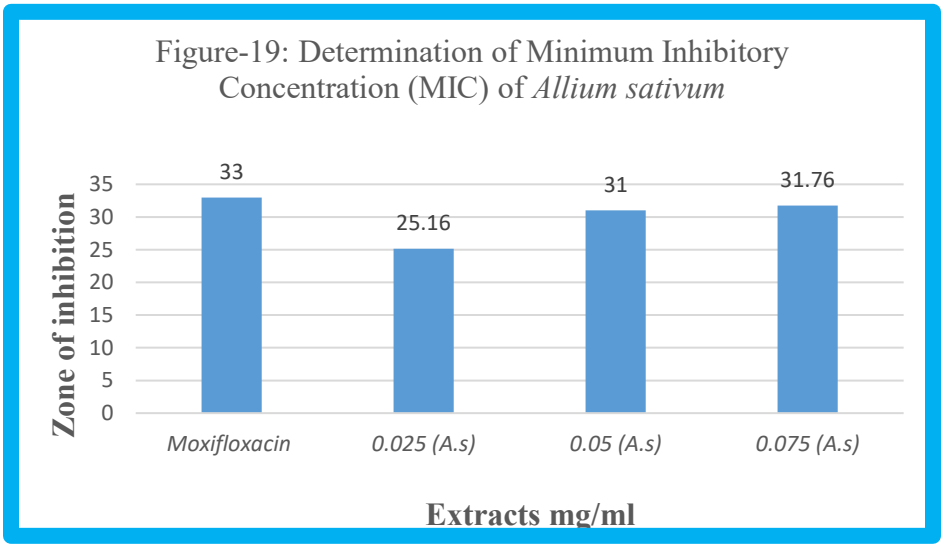


Figure-19: Determination of Minimum Inhibitory Concentration (MIC) of *Allium sativum*

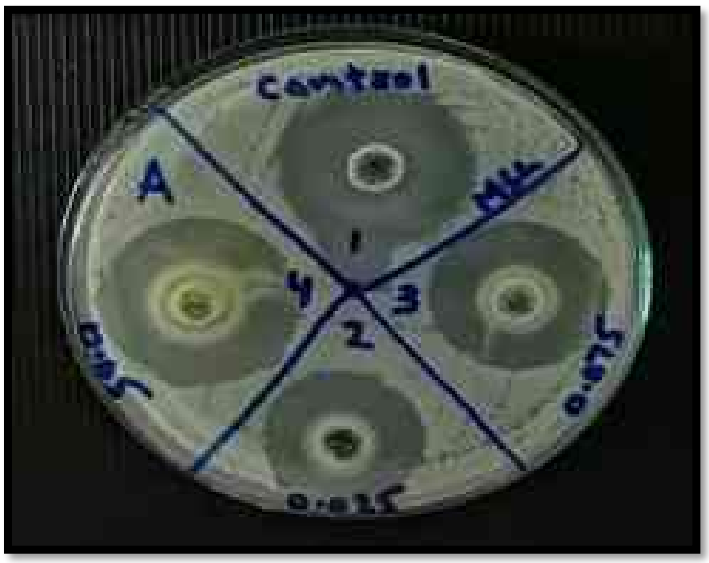


Figure-20: Comparative Analysis of Different Concentrations of *Allium sativum* Against *Escherichia coli*

Discussion

The present in-vitro study investigated the antibacterial potential of four medicinal plants—*Allium sativum* (garlic), *Piper betle* (betel leaves), *Phyllanthus emblica* (Indian gooseberry), and *Trachyspermum ammi* (ajwain)—against *Escherichia coli* isolated from infected broiler chickens in District Kohat. The isolation and identification of *E. coli* were conducted through culturing on MacConkey and EMB agar, followed by standard biochemical tests including Gram staining, Citrate, Catalase, and Oxidase tests. These methods successfully confirmed the presence of *E. coli* and were consistent with previously established protocols, such as those described by Ali Saadi et al. (2017).

The significance of *E. coli* as an opportunistic pathogen in poultry is well documented, being the main causative agent of colibacillosis in broiler chickens, particularly between 4 to 6 weeks of age (Dho-Moulin et al., 1999). The study began with screening the antibacterial activity of the plant extracts using the agar well diffusion method. Among the four plants tested at a concentration of 0.5 mg/ml, *Allium sativum* demonstrated the strongest antibacterial activity with a zone of inhibition measuring 20 mm, followed by *Phyllanthus emblica* at 10 mm. *Piper betle* showed moderate inhibition, whereas *Trachyspermum ammi* exhibited no antibacterial activity against *E. coli* at the tested concentration.

Given its superior efficacy, *Allium sativum* was selected for further analysis. Additional concentrations (0.75, 1.0, and 1.25 mg/ml) were tested in comparison with the standard antibiotic Moxifloxacin (0.5 mg/ml). The results revealed that *A. sativum* produced zones of inhibition (32.5–33.5 mm) nearly identical to that of Moxifloxacin (33 mm), confirming its potent antibacterial activity. These findings are in agreement with those of Yousufi (2012), who also reported strong antibacterial properties of garlic extract. The preparation method in the current study—using fresh bulbs and distilled water—differs from the frozen extraction method employed by Wilson et al. (1997), yet yielded highly effective results.

To determine the minimum inhibitory concentration (MIC), the extract of *A. sativum* was further diluted to 0.025, 0.050, and 0.075 mg/ml. The MIC was found to be 0.050 mg/ml, at which the average zone of inhibition was 31 mm—comparable to that of the standard antibiotic. This demonstrates that even at low concentrations, *A. sativum* maintains considerable antibacterial efficacy.

Antibiotic susceptibility testing of the isolated *E. coli* strains revealed resistance to Linezolid and Clarithromycin, with only mild sensitivity to Amoxicillin. In contrast, Moxifloxacin exhibited strong inhibitory activity, consistent with findings

reported by Rodriguez-Cerrato et al. (2001). The growing resistance of *E. coli* to conventional antibiotics observed in this study reflects global trends and reinforces the need for effective alternative treatments.

In conclusion, while all four plant extracts exhibited some level of antibacterial activity, *Allium sativum* stood out as the most effective against *E. coli* in-vitro. Its consistent performance across various concentrations and comparison with a potent synthetic antibiotic highlight its potential as a natural alternative for antibacterial therapy in poultry. This study supports the continued exploration of medicinal plants, especially garlic, as promising candidates in combating bacterial infections in the face of rising antibiotic resistance.

Conclusion

The present in-vitro study demonstrated that *Allium sativum* (garlic) possesses strong antibacterial activity against *Escherichia coli* isolated from naturally infected broiler chickens. Among the four medicinal plants tested—*Allium sativum*, *Piper betle*, *Phyllanthus emblica*, and *Trachyspermum ammi*—*A. sativum* exhibited the most significant inhibitory effect, with results comparable to the standard antibiotic Moxifloxacin. The minimum inhibitory concentration (MIC) of *A. sativum* was determined to be 0.050 mg/ml, confirming its potent action even at lower concentrations. These findings highlight the potential of *A. sativum* as a natural alternative to conventional antibiotics in combating *E. coli* infections in poultry. Moreover, the study underscores the importance of exploring plant-based antibacterial agents in response to rising antibiotic resistance. Further research is encouraged to optimize extraction methods and assess the broader antimicrobial spectrum of *A. sativum* in veterinary microbiology.

Recommendation

Based on the findings of this in-vitro study, it is recommended that *Allium sativum* be further explored as a natural antibacterial agent against *Escherichia coli*, given its strong and consistent activity across various concentrations. Its potential use as a herbal alternative in poultry health management, particularly for controlling colibacillosis, could help reduce dependency on synthetic antibiotics and mitigate antibiotic resistance. Further studies should focus on standardizing extract preparation, exploring other extraction methods for the remaining tested plants (*Piper betle*, *Phyllanthus emblica*, and *Trachyspermum ammi*), and investigating the underlying mechanisms of action. Additionally, continued monitoring of antibiotic resistance patterns in poultry pathogens is essential to guide future treatment strategies.

Acknowledgment

We are deeply grateful to **Dr. Shehzad Zareen** for his valuable supervision, guidance, and continuous support throughout the course of this research. His insight and expertise greatly contributed to the planning and successful execution of the study.

Special thanks are also extended to **Miss Shawana Nawaz** for her invaluable assistance in the identification of *Escherichia coli* and in performing the in vitro antibacterial activity testing. Her dedication and technical support were instrumental in achieving the objectives of this research.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

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