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EVALUATION OF ALLIUM SATIVUM (GARLIC) EXTRACT AS AN ALTERNATIVE TO ANTIBIOTICS AGAINST ESCHERICHIA COLI IN BROILER BIRDS

Malik Muhammad Sohail Elahi[1], Farhana Bano[2], Shehzad Zareen[*3], Shawana Nawaz[4], Ayesha Siddiqua[5], Muhammad Taimur[6], Syed Ali Abbas[7], Robi[8]

*[1,2,*3,4,6]Department of Zoology, Kohat University of Science and Technology, Kohat, 26000, Khyber Pakhtunkhwa, Pakistan.*

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

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

[8]Department of Botany, Khushal Khan Khattak University Karak, Khyber Pakhtunkhwa, Pakistan.

[1]maliksohail44889@gmail.com, [2]farhanabano865@gmail.com, [*3]shehzadzareen1@kust.edu.pk, [4]shawananawazkust@gmail.com, [*5]ayeshaphd.kust@gmail.com, [6]zo320211010@kust.edu.pk, [7]syedaliabbas552@gmail.com, [8]jasmee06@gmail.com

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Corresponding Authors: *
Shehzad Zareen
Ayesha Siddiqua
Shawana Nawaz

Abstract

Colibacillosis, a significant cause of morbidity and mortality in broiler poultry, is primarily induced by Escherichia coli. The overreliance on conventional antibiotics to manage this infection has contributed to the rising threat of antimicrobial resistance. This study evaluates the in vivo antibacterial efficacy of Allium sativum (garlic) aqueous extract as a natural alternative to conventional antibiotics in broiler chickens experimentally infected with E. coli. Bacterial isolates were obtained from naturally infected birds and biochemically confirmed as E. coli. A total of 20 broiler chickens were divided into four groups: a negative control (uninfected), a positive control (infected but untreated), and two experimental groups treated respectively with garlic extract and Moxifloxacin following E. coli inoculation. Results showed that all chickens treated with either A. sativum or Moxifloxacin recovered fully, while the untreated infected group exhibited high morbidity and mortality. Post-mortem examinations confirmed the absence of internal lesions in treated groups, validating the therapeutic effect of A. sativum. These findings suggest that garlic extract holds promise as a cost-effective, natural antimicrobial agent for managing colibacillosis in poultry, warranting further investigation for its integration into commercial poultry health practices.

INTRODUCTION

Colibacillosis is a common bacterial infection in poultry caused by *Escherichia coli*, affecting broiler chickens predominantly between 4–6 weeks of age. It contributes significantly to flock mortality worldwide. Clinical signs include respiratory distress, reduced feed intake, and stunted growth. Common post-mortem lesions include airsacculitis, pericarditis, perihepatitis, and peritonitis (Dho-Moulin et al.,

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1999). Beyond its pathogenic role, *E. coli* is a key model organism in biotechnology due to its ease of cultivation, fully sequenced genome, and ability to grow in both aerobic and anaerobic conditions. It is widely used in industrial and medical applications, especially recombinant DNA technology (Yoon et al., 2009). *E. coli* is a Gram-negative, motile, facultatively anaerobic rod. It comprises strains with varying pathogenic potential—some are harmless gut commensals, others cause infections in the gastrointestinal and urinary tracts, bloodstream, and central nervous system (Schaechter, 2009). The genus *Escherichia* was named after Dr. Theodor Escherich (Gould, 2011), and uropathogenic strains are the primary cause of *E. coli*-related UTIs (Gould, 2010).

Although *E. coli* is typically associated with the lower gastrointestinal tract, few studies have investigated its distribution across intestinal regions or the genetic diversity of region-specific strains (Bettelheim et al., 1992). Some studies indicate that faecal isolates reflect strains from other gut regions (Gordon et al., 2002), although this may not hold true for all species, such as *Streptococcus mitis* (Hohwy et al., 2001). In Pakistan, poultry contributes significantly to the national GDP (Hussain et al., 2015). However, the sector faces challenges including environmental stress and disease, particularly from *E. coli* (Manges, 2016). Its relevance to human health is amplified by rising antibiotic resistance, often mediated by horizontally acquired resistance genes (Matthew, 2010). Misuse of antibiotics in poultry exacerbates this issue, with Colibacillosis often resulting in high mortality (Dho-Moulin et al., 1999). Garlic (*Allium sativum*), a member of the Alliaceae family, is cultivated worldwide for culinary and medicinal use. It consists of a compound bulb with 10–16 cloves and is classified into hard neck (*Ophioscordon*) and soft neck (*Sativum*) types (Rehman, 2003). Garlic is traditionally used to treat gastrointestinal issues, infections, and cardiovascular conditions due to its hypocholesterolaemia properties. It is rich in calcium, phosphorus, and vitamin C (Borek, 2001).

Garlic's bioactivity is attributed to over 200 compounds, including sulfur-containing molecules like alliin, allicin, and ajoene; enzymes (e.g., alliinase); minerals (e.g., selenium); and amino acids such as cysteine and methionine. It also contains flavonoids (e.g., quercetin), vitamins C, E, A, B1, B2, niacin, and β-carotene (Ayaz et al., 2007). Alliin, an inactive precursor, is enzymatically converted to allicin upon crushing, which imparts garlic's characteristic smell and therapeutic properties (Aviello et al., 2009). Keeping in view the above facts the current study was designed to evaluate the in vivo antibacterial potential of *Allium sativum* (garlic) against *Escherichia coli*-induced Colibacillosis in poultry, with the aim of exploring its efficacy as a natural alternative to conventional antibiotics.

Materials And Methods

Bacterial Isolation Procedure

Bacterial isolates were obtained from naturally infected broiler chickens using sterile cotton swabs. Sampling was conducted at various poultry shops across District Kohat, Khyber Pakhtunkhwa. Swabs were gently rubbed over the oral and nasal cavities of visibly infected birds to collect specimens. To minimise contamination, the swabs were immediately wrapped in sterile aluminium foil. The collected samples were then promptly transported to the Microbiology Laboratory at Kohat University of Science and Technology (KUST), Kohat, for further microbiological analysis (Figure-1).

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Figure 1: Bacterial Isolation from Infected Broiler Chickens

Biochemical Identification of Bacteria

Biochemical identification of *Escherichia coli* was a critical component of this study, carried out through a series of standard tests including Gram staining, citrate utilization, catalase, and oxidase tests. Gram staining, following the protocol by Beveridge et al. (1983), was the initial method used to determine Gram characteristics. A smear was prepared by mixing a bacterial colony from EMB agar with a drop of sterile saline on a clean slide. The smear was fixed and subjected to crystal violet staining for 15–20 seconds, followed by iodine for 1 minute, ethanol decolorization for 1 minute, and counterstaining with safranin for 20 seconds. After drying, microscopic examination revealed pink, rod-shaped bacteria, confirming the presence of Gram-negative organisms consistent with *E. coli* morphology. This initial identification was further supported by results from subsequent biochemical tests.

Citrate Test

The citrate utilization test was performed following the protocol described by MacWilliams (2009) to assess the ability of the bacterial isolate to use citrate as a sole carbon source. Simmon's citrate agar (5 mL per tube) was prepared and dispensed into test tubes to form slants. Using a sterile straight wire loop, a single colony of the suspected *E. coli* isolate was lightly streaked across the slant surface. The tubes were then incubated at 37°C for 24 hours. Following incubation, no bacterial growth was observed and the color of the medium remained green, indicating a negative citrate reaction. This result supported the identification of the isolate as *E. coli*, which is typically citrate-negative and Gram-negative.

Catalase Test

The catalase test was conducted to biochemically confirm the identity of *Escherichia coli*, following the protocol described by Reiner (2010). A clean microscopic slide was used to prepare a smear by transferring a bacterial colony from EMB agar using a sterile wire loop. A drop of hydrogen peroxide (H_2O_2) was then carefully added to the smear without mixing. The immediate formation of oxygen bubbles indicated a positive catalase reaction, which is characteristic of *E. coli*, thereby supporting its identification.

Oxidase Test

The oxidase test was performed following the protocol described by Kovacs (1956) to detect the presence of cytochrome c oxidase, an enzyme involved in the bacterial electron transport chain. A dry filter paper was moistened with two drops of oxidase reagent. A bacterial colony was then picked using a sterile wire loop and smeared onto the treated area of the filter paper. The absence of any color change indicated a negative oxidase reaction, consistent with the biochemical profile of *Escherichia coli*, which lacks cytochrome c oxidase.

Collection of Medicinal Plant

Fresh bulbs of *Allium sativum* (garlic) were purchased from the local market in District Kohat, Khyber Pakhtunkhwa, Pakistan. The plant material was identified based on morphological characteristics and used for further phytochemical and antimicrobial analysis. (Figure-2).

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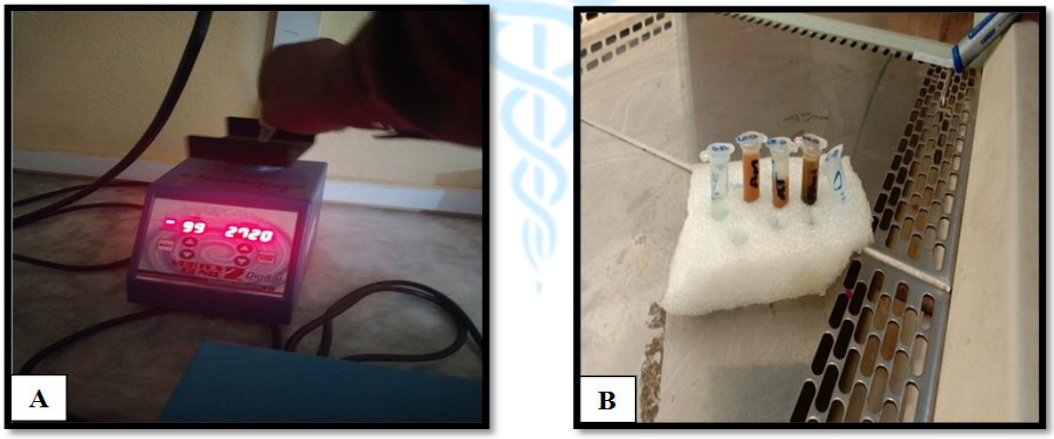
Figure 2: Allium Sativum

Preparation of Aqueous Extract

The garlic (*Allium sativum*) extract was prepared following the protocol of Arora et al. (2007). Fresh garlic cloves were ground into a fine paste, and the resulting material was transferred into sterile test tubes. Each tube received 5 mL of distilled water and was left undisturbed at room temperature for seven days to allow extraction of the active compounds. After the incubation period, the mixture was filtered using standard filter paper, and the aqueous extract was collected for further experimental use (Figure-3).

Figure 3: A) Vortex machine B) Aqueous Extracts of Selected Plants



***In-vivo* Assay**

The in vivo assay was conducted following the protocol described by Elamary et al. (2018), using 20 broiler chickens reared in the animal house of the Department of Zoology, Kohat University of Science and Technology (KUST). The birds were acclimatized for four days to stabilise environmental conditions. Subsequently, the chickens were randomly divided into four groups, each consisting of five birds. To ensure group identification, three groups were marked on the head with different food-grade dyes—red, brown, and yellow—while the fourth group remained unmarked and served as the negative control. Each group was housed separately in isolated compartments to prevent cross-contamination and ensure experimental integrity (Figure-4).

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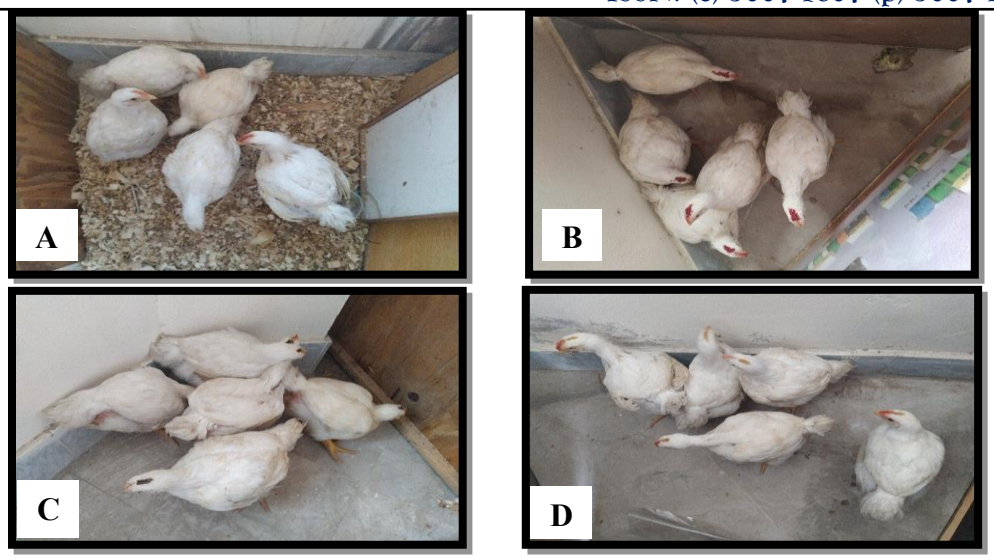

Inoculation of Bacterial Infection

Fresh Broth of *E. coli* was prepared in the microbiology laboratory. Broth was diluted in distilled water and 3 drops were inoculated into the trachea of each experimental chicken through a dropper (Figure-5).

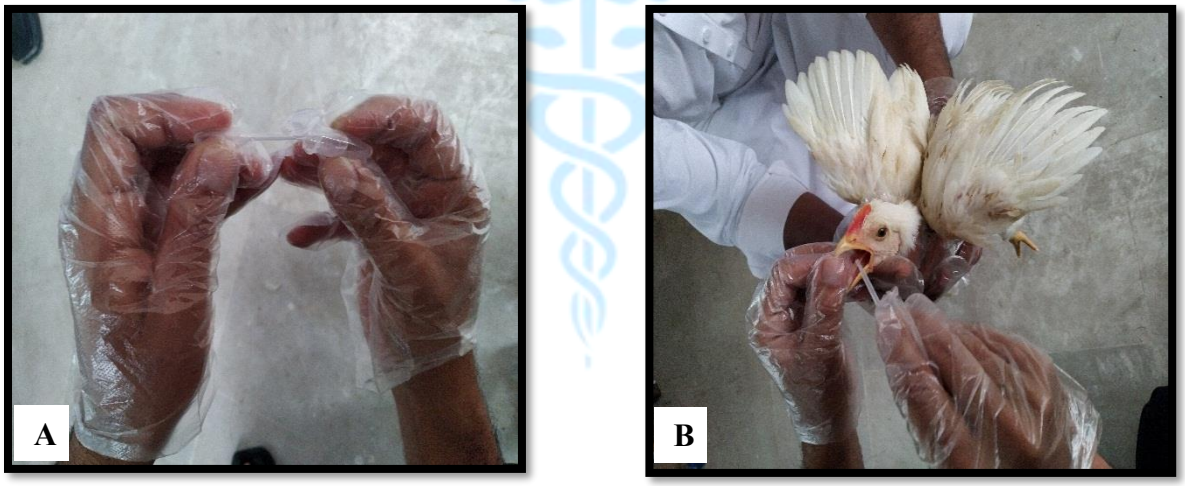

Figure-5: a) Isolation Of Bacteria Through Dropper
b) Inoculation Of Bacteria Through Dropper

Results

Observations

Negative Control Group (Unstained)

The unmarked group, which served as the negative control, was not inoculated with Escherichia coli and received no treatment. These chickens were maintained on a standard poultry feed under controlled environmental conditions. Throughout the duration of the experiment, all five birds in this group remained healthy, exhibiting no clinical symptoms of illness. Their stable condition confirmed that the experimental environment was not a confounding factor and validated the health baseline for comparison with the infected groups (Figure-6).

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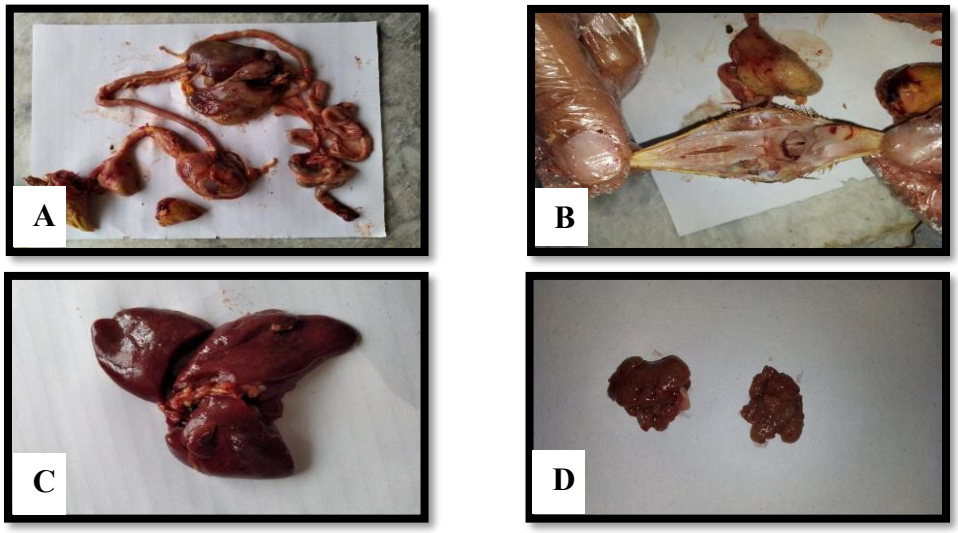

Figure-6: Negative Control Group (Unstained): Negative Control A) Digestive system B) Dissected Oral Cavity, C) Liver, D) Lungs

Positive Control Group (Red-Stained) The red-stained group was designated as the positive control. All five chickens in this group were inoculated intra-tracheally with E. coli to establish infection. Within two days post-inoculation, clinical symptoms began to appear, including lethargy, respiratory distress, and the development of white patches in the oral cavity. As this group was not given any treatment, the disease progressed naturally. By the end of the experiment, two chickens had died due to severe infection, while the remaining three showed significant signs of illness. Post-mortem examination revealed visible lesions in the liver, lungs, and upper respiratory tract, confirming the severity of the bacterial infection (Figure-7).

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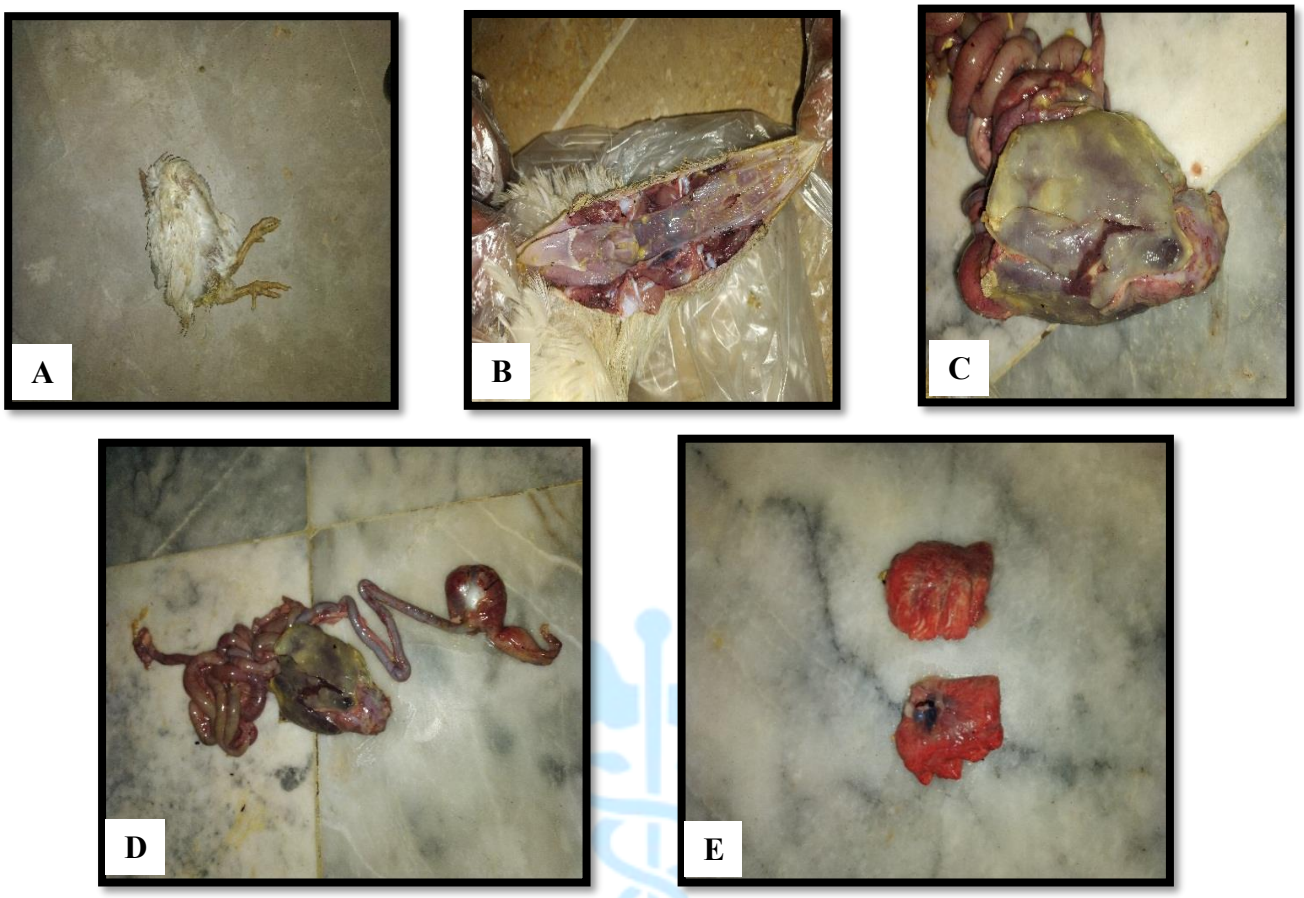

Figure 7: Red Stained: Positive Control A) Dead Chicken B) Dissected Oral Cavity C) Infected Liver D) Infected Digestive System E) Infected Lungs

Experimental Group 1 (Brown-Stained, Treated with Allium Sativum)

The brown-stained group served as an experimental group to evaluate the in vivo antibacterial potential of Allium sativum (garlic). All chickens were inoculated with E. coli in the same manner as the positive control. After the onset of clinical symptoms, each bird was isolated and administered the garlic extract at varying frequencies—one to six times per day across the five chickens. Over a three-day treatment period, visible signs of infection began to subside. At the conclusion of the treatment, one chicken was dissected for internal examination, and no pathological changes were observed in its organs, indicating recovery. The results suggested that A. sativum has promising antibacterial effects against E. coli infection in poultry (Figure-8).

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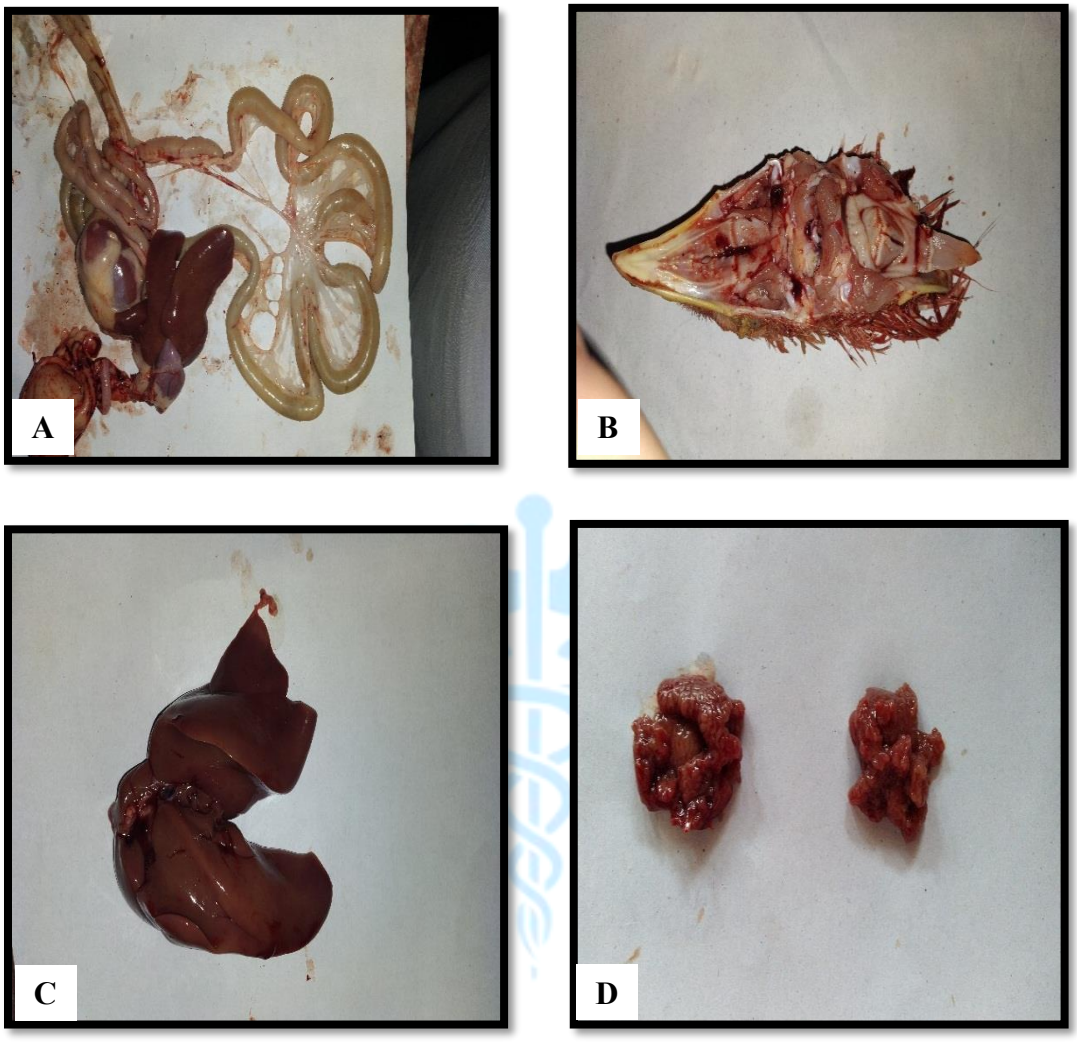
Figure 8: Experimental Group 1 (Brown-Stained, Treated with Allium sativum):

A) Digestive system, B) Dissected oral cavity, C) Liver, D) Lungs



Experimental Group 2 (Yellow-Stained, Treated with Moxifloxacin)

The yellow-stained group was used as a second experimental group to compare the efficacy of A. sativum with a standard antibiotic. Like the other experimental groups, these chickens were inoculated with *E. coli*. Following the appearance of symptoms, all birds in this group were treated with Moxifloxacin, a broad-spectrum fluoroquinolone antibiotic commonly used in veterinary medicine. All five chickens in this group recovered fully, showing complete resolution of clinical signs. These results provided a benchmark for assessing the performance of A. sativum, which showed comparable therapeutic effects under controlled conditions (Figure-9).

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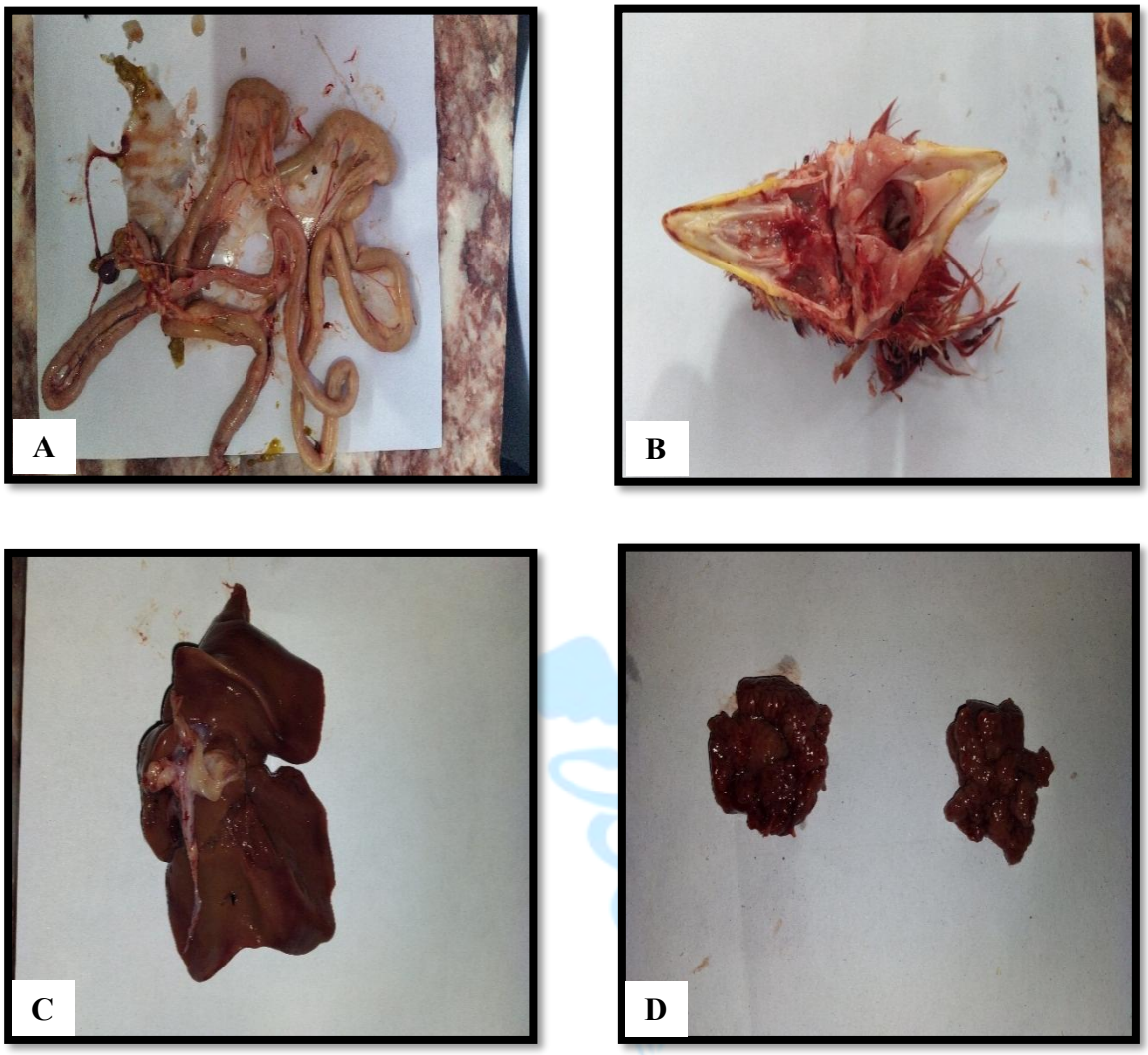

Figure 9: Experimental Group 2 (Yellow-Stained, Treated with Moxifloxacin
A) Digestive system, B) Dissected Oral cavity, C) Liver, D) Lungs

Table-1 Experiment Summary

Group No.	Identification Color	Status	Symptoms After 2 Days	Treatment	Outcome
1	No Color (White)	Negative Control (Uninfected)	None	Normal poultry feed only	All chickens remained healthy throughout the study.
2	Red stained	Positive Control (*E. coli* Infected)	White patches in oral cavity, lethargy, respiratory distress. 2 chickens died.	No treatment administered	2 chickens died, 3 remained severely infected.

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			Severe internal infections observed post-mortem.		
3	Brown stained	Experimental Group (*E. coli* Infected)	White patches in oral cavity, lethargy.	Garlic (*Allium sativum*) extract: 1st chicken – 2×/day 2nd – 3×/day 3rd – 4×/day 4th – 5×/day 5th – 6×/day	All chickens recovered. No internal symptoms observed after dissection.
4	Yellow stained	Experimental Group (*E. coli* Infected)	White patches in oral cavity, lethargy.	Moxifloxacin: 3 drops orally per day	All chickens recovered. No internal symptoms observed after dissection.

Discussion

This study explored the in vivo antibacterial potential of *Allium sativum* (garlic) aqueous extract against *Escherichia coli* infection in broiler chickens. The extract was prepared by grinding fresh garlic bulbs and mixing with 1 mL of distilled water, followed by vortexing. This simple and direct extraction method aligns closely with the procedure described by Yousufi (2012), who also utilized a fresh aqueous extract to evaluate garlic's antimicrobial properties. However, this method differs significantly from the protocol reported by Wilson et al. (1997), who froze garlic bulbs at –20°C for 48 hours prior to defrosting, straining, and filtering the extract into dark bottles to protect from light. Unlike Wilson's complex method involving cold preservation, the current approach used freshly prepared extract, supporting the notion that garlic retains its bioactive components effectively without the need for freezing.

In the in vivo part of the experiment, 20 broiler chickens were reared under controlled conditions and divided into four distinct groups. The red-stained group, serving as the positive control, was infected with *E. coli* but received no treatment. Within two days, typical symptoms of colibacillosis—such as lethargy, white oral patches, and respiratory distress—were observed. Post-mortem examination revealed severe infections in the lungs, liver, and upper respiratory tract. Two of the five chickens died, while the remaining three showed pronounced signs of internal infection, confirming the pathogenic impact of *E. coli* when untreated.

The brown-stained group was the experimental group treated with *A. sativum* extract following infection. Chickens in this group received varying doses, ranging from two to six administrations per day. Remarkably, all chickens in this group recovered within three days of treatment. Post-treatment dissections showed no signs of infection or organ damage, strongly indicating the therapeutic effectiveness of the garlic extract. These findings are in full agreement with the study of Elamary et al. (2018), who also reported the curative effects of garlic extract in *E. coli*-infected poultry.

The yellow-stained group, also infected with *E. coli*, was treated with Moxifloxacin, a broad-spectrum antibiotic. Similar to the garlic-treated group, all chickens recovered, and no symptoms or internal pathology were noted upon dissection. The comparable outcomes between the garlic-treated group and the antibiotic-treated group suggest that *Allium sativum* possesses a potent antimicrobial effect, potentially rivaling that of conventional antibiotics.

The white (unstained) group served as the negative control, remaining uninfected and untreated. These chickens remained healthy and symptom-free throughout the experiment, confirming that

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environmental conditions were well-maintained and that infection and recovery patterns were treatment-dependent.

In summary, the current study demonstrates that freshly prepared aqueous garlic extract is highly effective in treating *E. coli* infection in vivo. The extract led to full recovery in infected chickens, with no residual pathology, making it a promising natural alternative to synthetic antibiotics. Given the growing concern over antibiotic resistance and the need for safer, more sustainable treatments in poultry farming, *Allium sativum* may serve as a valuable antimicrobial agent in veterinary practice.

Conclusion and Recommendations

The findings of the present study clearly demonstrate that *Allium sativum* (garlic) exhibits significant in vivo antibacterial activity against *Escherichia coli* infections in broiler chickens. The aqueous extract of *A. sativum*, prepared through a simple method involving grinding fresh garlic and adding distilled water, proved effective in eliminating clinical symptoms of colibacillosis and preventing internal organ damage. Chickens treated with the garlic extract showed complete recovery, comparable to those treated with Moxifloxacin, a commonly used broad-spectrum antibiotic. These findings highlight the therapeutic potential of *A. sativum* as a natural, cost-effective, and accessible alternative to conventional antimicrobial agents in poultry health management. Given the increasing concern regarding antimicrobial resistance, particularly in pathogens like *E. coli*, the routine use of synthetic antibiotics should be minimized to preserve their efficacy. Instead, the incorporation of natural agents such as *A. sativum* into poultry feed could serve as a preventive strategy against bacterial infections. It is recommended that garlic extract be further explored and standardized for use in commercial poultry farming as a sustainable and biologically safe antimicrobial feed additive. Additionally, future studies should focus on optimizing dosage, delivery methods, and evaluating long-term effects to ensure safety and efficacy across different poultry systems.

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