

ANTIMICROBIAL ACTIVITY OF GINGER (ZINGIBER OFFICINALE) AND GARLIC (ALLIUM SATIVUM) EXTRACTS AGAINST ESCHERICHIA COLI

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ABSTRACT

The emergence of antimicrobial-resistant bacteria has become a major global health concern, creating an urgent need for alternative antimicrobial agents from natural sources. This study evaluated the antimicrobial activities of ginger (Zingiber officinale) and garlic (Allium sativum) extracts against Escherichia coli using the disc diffusion method. Fresh ginger rhizomes and garlic bulbs were processed to obtain plant extracts, which were tested against E. coli isolates under laboratory conditions. The bacterial isolates were identified using standard microbiological and biochemical techniques. Antimicrobial activity was assessed by measuring the zones of inhibition produced by different extract concentrations on Mueller–Hinton agar. Both ginger and garlic extracts exhibited inhibitory effects against E. coli, with garlic consistently producing larger zones of inhibition than ginger, indicating superior antibacterial activity. The results suggest that the bioactive compounds present in garlic, particularly allicin, contribute to its stronger antimicrobial effect, while ginger also demonstrated considerable antibacterial potential due to compounds such as gingerol and shogaol. These findings support the potential use of ginger and garlic as natural antimicrobial agents and highlight their possible application in food preservation, poultry production, and complementary antimicrobial therapy. Further in vivo studies and phytochemical investigations are recommended to validate their therapeutic efficacy and safety.

KEYWORDS: Escherichia coli; Garlic (Allium sativum); Ginger (Zingiber officinale); Antimicrobial activity; Disc diffusion assay; Natural antibacterial agents; Plant extracts; Antibiotic resistance.

INTRODUCTION:

In recent years, the rise of antibiotic resistance has become a major global health concern, demanding alternative strategies for controlling pathogenic microorganisms. Among the common foodborne pathogens, Escherichia coli (E. coli) is of particular significance due to its role in causing gastrointestinal infections, food poisoning, and urinary tract infections. The search for natural antimicrobial agents from plant sources has gained increasing attention as they are generally safe, cost-effective, and eco-friendly compared to synthetic drug.

Spices and herbs have long been used in traditional medicine and food preservation. Garlic (Allium sativum) and ginger (Zingiber officinale) are well-known medicinal plants with a wide range of pharmacological properties, including antimicrobial, antioxidant, and anti-inflammatory effects. The active compounds in garlic, such as allicin and sulfur-containing compounds, have been reported to inhibit the growth of several bacteria including E. coli. Similarly, ginger contains bioactive molecules like gingerol, shogaol, and

zingerone, which exhibit strong antimicrobial activity. The open plate method is a simple and widely used technique to evaluate the antimicrobial activity of plant extracts. This method allows for the observation of inhibition zones around the applied extracts, indicating their effectiveness against test organisms. By applying extracts of ginger and garlic against *E. coli*, this study aims to assess their comparative antimicrobial potential and highlight their possible applications in food safety and medicine.

Antimicrobial resistance (AMR) is the ability of a microbe to resist the effects of medication previously used to treat them. The increased usage of antibiotics has induced microorganism to acquire resistance factor which have become a burning predicament. Resistant microbes are increasingly difficult to treat, requiring alternative medication or higher doses both of which may be more expensive or more toxic. Even in the animal treatment abrupt use of antibiotics is one of the major cause of antimicrobial resistance. The WHO, concluded that inappropriate use of antibiotics in animal husbandry is an underlying contributor to the emergence and spread of antibiotic resistant germs. As a result there is an urgent need to find the alternative of antibacterial drugs for treating the disease especially from plant origin which are easily available and with less side effects (Khulbe and Sati 2009). Many medicinal plants are documented to have antimicrobial activity like ginger, garlic etc. among which Garlic (*Allium sativum*) belonging to Alliaceae family. Apart from cooking Garlic also known for its medicinal values.

The rise of antibiotic resistance has become one of the most pressing challenges in modern medicine, making it increasingly difficult to treat common bacterial infections. *Escherichia coli* (*E. coli*), a Gram-negative bacterium, is a well-known cause of a variety of infections, ranging from urinary tract infections to gastrointestinal diseases. As resistance to conventional antibiotics continues to grow, there is an urgent need to explore alternative treatments, particularly from natural sources.

Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are two widely used plants that have garnered attention not only for their culinary uses but also for their therapeutic properties. Both garlic and ginger contain bioactive compounds known to possess antimicrobial activities. Allicin, the active compound in garlic, is recognized for its ability to inhibit the growth of a broad range of bacteria, while ginger's gingerol and other compounds exhibit antibacterial, antifungal, and anti-inflammatory properties. These plants have long been utilized in traditional medicine, and recent research supports their potential as natural antimicrobial agents. Given the growing concern of antimicrobial resistance and the potential of natural products, this study aims to assess the antimicrobial activity of garlic and ginger extracts specifically against *E. coli* using the open plate diffusion method. The open plate method is a simple and effective technique for testing antimicrobial activity, allowing for clear visualization of bacterial inhibition around the site of extract application.

Garlic (*Allium sativum*), belonging to family Liliaceae, mainly the bulb of garlic, has been used as a spice in cooking worldwide especially in Italy and Southeast Asia. More importantly, garlic has been an ingredient in folk and traditional medicine since ancient times (Rivlin, 2001). Garlic is cultivated all over the world with a per-capita consumption of two pounds per year. As per the Food and Agricultural Organization of the United Nations, China and India are first and second, respectively, in average (1961–2017) garlic production. Health benefits that are associated with the use of garlic are attributed to its anticancer, anti-inflammatory, antifungal, antiviral, and antibacterial activity.

Several in vitro, in vivo, and epidemiological studies indicate that garlic exhibits anticancer activity, and the likely mechanism of action is by activating metabolizing enzymes, inhibiting reactive oxygen species, radical scavenging, preventing DNA damage, and tumor inhibition (Cao et al., 2014; Zhang Y. et al., 2019). The immunomodulatory effects of garlic are mediated through its ability to modulate cytokine production as well as activate immune response by stimulating antibody secretion and immune cells (Arreola et

al., 2015). Garlic displays anti allergic properties by inhibiting antibody-mediated histamine production and modulates airway allergic response (Kyo et al., 2001; Zare et al., 2008). The anti inflammatory and anti arthritic ability of garlic comes from its ability to inhibit NF- κ B signaling (Ban et al., 2009). Garlic oil (GO) exhibits antifungal activity against *Candida albicans* and *Penicillium funiculosum* by penetration into cells and organelles and causing differential expression of genes that are critical for cellular metabolism (Li et al., 2016). One of the earliest reports of garlic's antibacterial activity was by Small et al. (1947) and Stoll and Seebeck (1947).

Since then, extensive research has been performed on the antibacterial effects of garlic. The antibacterial activity against various pathogenic and drug-resistant bacteria was tested using crude garlic extracts, garlic powder (GP), garlic extracts using various solvents, GO, and phytochemicals isolated from garlic. The constant and rapid emergence of antimicrobial resistance has been recognized as an alarming threat to human health, which mandates the scientific community to develop novel and effective antibacterial agents (Cheng et al., 2016). Garlic compounds exhibit multiple modes of antibacterial activity and have enormous potential to be developed into novel antibacterial agents. Most reviews about garlic discuss the antibacterial activity of garlic as one of its many health benefits diluting the importance of garlic compounds as potential antibacterial agents. This review exclusively focuses on significant antibacterial studies that were performed with garlic and its phytochemicals.

Active Phytochemicals of Garlic Most of the health benefits of garlic are attributed to a myriad of cysteine-derived sulfur-containing organic compounds present in garlic (extensively reviewed in Fenwick and Hanley, 1985a,b,c). The organosulfur compounds of intact garlic clove greatly differ from that present in garlic juice obtained after crushing garlic. The intact garlic mainly contains non-volatile γ -glutamyl-S-alk(en)yl-L-cysteines, namely, γ -glutamyl-S-allyl-L-cysteine, γ -glutamyl-S-trans-1-propenyl-L-cysteine, and S-alk(en)yl-L-cysteine sulfoxides such as S-allyl-L-cysteine sulfoxide (alliin), S-(trans-1-propenyl)-L-cysteine sulfoxide (isoalliin), and S-methyl-L-cysteine sulfoxide (methiin) with a small amount of S-allyl cysteine (SAC) (Figure 1A) (Block, 1992). Crushing or cutting garlic cloves releases allinase enzyme sequestered in the vacuoles, which encounters cytosolic alliin to convert it into an array of thioisulfates of which the most prominent is allicin. The highly reactive, unstable, and volatile allicin decomposes to yield a large number of sulfides such as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), methyl allyl disulfide (MADS), methyl allyl sulfide, ajoene, and vinyl dithiins (2-vinyl-1,3-dithiin, 3-vinyl-1,2-dithiin) shown in Figure 1B (Brodnitz et al., 1971).

The sulfides are oil-soluble compounds that are responsible for the characteristic garlic odor and flavor. Allicin exhibits excellent in vitro antibacterial activity, which resulted in a huge number of studies to evaluate the potential of allicin and oil-soluble organosulfur compounds of garlic as antibacterial agents (Cavallito and Bailey, 1944a). A large body of literature supports the antibacterial potential of garlic organosulfides. The organosulfur compounds present in the aqueous and alcoholic extract of garlic include S-allyl cysteine (SAC), S-allylmercapto-L-cysteine (SAMC), and S-methyl cysteine (Figure 1C). The compounds are non-volatile, non-odiferous, and stable compounds compared to volatile organosulfides. Most health benefits of garlic are largely attributed to these organosulfur compounds present in garlic. However, garlic organosulfur compounds are very unstable with low bioavailability and the presence of these compounds depends on the processing of the garlic during the preparation of garlic supplements (Amagase et al., 2001; Amagase, 2006).

The rise of antibiotic-resistant microorganisms has driven increased interest in natural antimicrobial agents derived from plants. Among these, ginger (*Zingiber officinale*) and garlic (*Allium sativum*) have long been used in traditional medicine for their therapeutic

properties, including antimicrobial activity. These plants contain bioactive compounds such as gingerol, shogaol (in ginger), and allicin (in garlic), which have been shown to inhibit the growth of a wide range of pathogenic microorganisms.

Escherichia coli (*E. coli*) is a gram-negative, facultative anaerobic bacterium commonly found in the intestinal tract of humans and animals. While most strains are harmless and part of the normal gut flora, some pathogenic strains—such as *E. coli* O157:H7—can cause severe gastrointestinal infections, urinary tract infections, and other systemic illnesses. The increasing incidence of antibiotic resistance in *E. coli* poses a significant public health concern, highlighting the need for alternative antimicrobial agents.

Among these, garlic (*Allium sativum*) and ginger (*Zingiber officinale*) have attracted considerable attention for their potent pharmacological and antimicrobial properties. Both plants are widely cultivated and consumed across the world, not only as culinary ingredients but also as therapeutic agents. Garlic is a member of the family Amaryllidaceae and is well known for its characteristic sulfur-containing compounds, particularly allicin, which is released when garlic cloves are crushed or chopped. Allicin and related thiosulfates have demonstrated strong antibacterial, antifungal, antiviral, and antiparasitic properties. Similarly, ginger, belonging to the family Zingiberaceae, contains bioactive compounds such as gingerol, shogaol, and zingerone, which have been shown to possess antimicrobial, antioxidant, anti-inflammatory, and anticancer effects.

The antibacterial activities of ginger and garlic have been reported against a wide range of pathogenic microorganisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Escherichia coli*. *E. coli*, a Gram-negative rod-shaped bacterium, is an important member of the family Enterobacteriaceae. While many strains of *E. coli* are harmless commensals present in the gastrointestinal tract of humans and animals, certain pathogenic strains can cause serious diseases. Enteropathogenic and enterohemorrhagic *E. coli* are responsible for diarrheal diseases, urinary tract infections, septicemia, and even hemolytic uremic syndrome. The ability of *E. coli* to acquire resistance genes further complicates its clinical management, making it an ideal model organism for antimicrobial screening studies.

Plant-derived antimicrobials are of particular interest because they are generally safe, biodegradable, cost-effective, and often exhibit multiple mechanisms of action. Unlike conventional antibiotics that usually target specific bacterial pathways, phytochemicals act on multiple cellular targets, reducing the chance of resistance development. In addition, they can be used in combination with existing antibiotics to enhance their efficacy and lower effective dosages. Garlic and ginger are especially important in this regard, as their bioactive compounds have been reported to disrupt bacterial cell walls, interfere with protein synthesis, inhibit enzyme activity, and affect quorum sensing mechanisms. These mechanisms make them potential candidates for controlling bacterial pathogens in food safety, pharmaceuticals, and alternative medicine.

To evaluate the antimicrobial potential of natural extracts, various laboratory techniques are employed. Among these, the open plate method (also referred to as the agar diffusion or disc diffusion method, with modifications) is one of the most widely used and reliable techniques. In this method, test organisms are exposed to plant extracts or bioactive compounds applied on an agar medium, and the resulting zones of inhibition are measured. The size of the inhibition zone reflects the effectiveness of the tested extract against the microbial strain. The method is simple, inexpensive, and allows for a clear comparative evaluation of different plant extracts against a specific microorganism. It is especially suitable for preliminary antimicrobial screening in academic and research laboratories.

Aim

To evaluate and compare the antimicrobial activities of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts against *Escherichia coli* using the disc diffusion method, and to determine their effectiveness as natural antibacterial agents.

OBJECTIVE:

1. To prepare aqueous and ethanolic extracts of ginger (*Zingiber officinale*) and garlic (*Allium sativum*).
2. To isolate and culture *Escherichia coli* under laboratory conditions.
3. To evaluate the antimicrobial activity of ginger and garlic extracts against *E. coli* using agar well diffusion method.
4. To determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ginger and garlic extracts.
5. To compare the antimicrobial effects of aqueous and ethanolic extracts of ginger and garlic.
6. To assess the synergistic antimicrobial effect of combined ginger and garlic extracts against *E. coli*.
7. To analyze and interpret results statistically for scientific validation.
8. To suggest the potential application of ginger and garlic extracts as natural alternatives to synthetic antimicrobials in food and poultry industries.

REVIEW OF LITERATURE:

The growing concern over antimicrobial resistance, particularly among foodborne pathogens such as *Escherichia coli*, has necessitated the exploration of alternative sources of antimicrobial agents, with medicinal plants emerging as promising candidates due to their bioactive compounds and long history of safe use. Among these, ginger (*Zingiber officinale*) and garlic (*Allium sativum*) have been widely studied for their inhibitory activity against a range of microorganisms, including *E. coli*. These spices are not only valued in culinary practice but have also been traditionally used in ethnomedicine for treating infections, digestive ailments, and inflammatory conditions. Modern microbiological research has confirmed that their antimicrobial properties are largely attributed to specific phytochemicals, including phenolic compounds in ginger and sulfur-containing molecules in garlic, which exert unique mechanisms of action on microbial cells. The antibacterial potential of ginger is closely associated with compounds such as [6]-gingerol, [8]-gingerol, [10]-gingerol, shogaols, paradols, and zingerone, which are capable of disrupting the lipid bilayer of bacterial membranes, thereby increasing permeability, leading to leakage of intracellular contents and eventual cell death. In contrast, garlic's activity is dominated by allicin, which is formed when the enzyme alliinase converts the precursor alliin upon mechanical disruption of garlic cloves. Allicin is known for its rapid and broad-spectrum antibacterial activity, primarily due to its ability to interact with sulfhydryl groups of microbial enzymes, blocking essential metabolic pathways, inhibiting RNA synthesis, and suppressing quorum sensing signals that regulate virulence factors in *E. coli*.

A significant body of research has compared the effectiveness of different extraction methods for ginger and garlic, as extraction solvents and processing conditions critically influence the concentration and stability of active compounds. For ginger, ethanolic extracts generally demonstrate higher antibacterial activity compared to aqueous extracts, as ethanol efficiently extracts non-polar phenolic compounds. Essential oils derived from ginger rhizomes have also been reported to exhibit strong inhibitory zones against *E. coli* in agar diffusion assays. However, aqueous extracts, though less potent, still demonstrate measurable inhibition and are considered more practical for applications in food systems due to their safety and ease of preparation. Garlic extracts present a unique challenge

because allicin, the primary bioactive compound, is highly unstable and degrades quickly under heat, storage, or prolonged exposure to air. Consequently, freshly prepared aqueous garlic extract tends to exhibit the strongest antibacterial effects against *E. coli*, while aged extracts or heat-processed garlic show diminished activity. Studies employing broth dilution and agar diffusion assays consistently report that garlic extracts often produce larger inhibition zones and lower minimum inhibitory concentrations (MICs) against *E. coli* compared to ginger, suggesting garlic is generally more potent. Nonetheless, it has also been observed that when ginger and garlic extracts are combined, a synergistic effect occurs, resulting in greater inhibition of *E. coli* growth than either extract alone, which suggests that their different mechanisms of action complement each other.

The antimicrobial activities of ginger and garlic extracts against *E. coli* have also been studied in the context of food preservation and poultry production, where controlling enteric bacteria is of great importance. In poultry production systems, *E. coli* is a frequent cause of intestinal infections and food contamination, leading to economic losses and posing risks to human health through contaminated meat products. Dietary supplementation of broiler chickens with powdered or extract forms of ginger and garlic has been reported to reduce intestinal *E. coli* populations while simultaneously improving weight gain, feed efficiency, and immune responses. This dual benefit highlights their potential as natural feed additives that serve as both growth promoters and antimicrobial agents, offering a safer alternative to conventional antibiotic growth promoters, which have been restricted in many countries due to resistance concerns. Similarly, studies investigating post-harvest decontamination have shown that surface treatment of poultry meat with ginger and garlic extracts can significantly reduce *E. coli* counts, thereby improving microbial safety and extending shelf life. These findings indicate that both spices may contribute to enhancing food safety along the production chain, from animal health to consumer protection.

Mechanistically, the inhibition of *E. coli* by ginger and garlic can be attributed to multiple pathways that interfere with microbial survival and virulence. For ginger, bioactive constituents such as [6]-gingerol and shogaols insert into the bacterial membrane, causing increased fluidity and permeability, which leads to efflux of vital ions and metabolites. This disruption is often visible under electron microscopy as structural damage to bacterial cell walls and cytoplasmic leakage. Additionally, ginger phenolics have been shown to interfere with bacterial enzyme systems and reduce oxidative stress responses, further weakening bacterial defenses. In garlic, allicin's high reactivity with thiol-containing enzymes leads to irreversible inactivation of key metabolic proteins in *E. coli*, particularly those involved in energy metabolism and cell division. Allicin has also been documented to inhibit bacterial DNA and RNA synthesis, thereby reducing replication and transcription efficiency. Moreover, garlic extracts can suppress quorum sensing pathways in *E. coli*, reducing the expression of virulence factors and limiting the ability of bacteria to form protective biofilms. Since biofilm formation contributes significantly to antibiotic resistance and survival of *E. coli* on surfaces and in the gastrointestinal tract, this anti-biofilm activity adds an important dimension to garlic's antimicrobial role.

Although the literature strongly supports the antimicrobial potential of ginger and garlic, several challenges limit their widespread application. One major limitation is the variability in antimicrobial results due to differences in extraction methods, plant sources, concentration units, and experimental conditions. For instance, ginger grown in different regions may vary in gingerol content, leading to different levels of antibacterial activity, while garlic's efficacy is heavily dependent on how quickly extracts are prepared and used after crushing. The lack of standardized testing protocols makes it difficult to compare results across studies or establish consistent dosage recommendations. Furthermore, while *in vitro* studies consistently demonstrate strong inhibition of *E. coli* by ginger and garlic extracts, fewer *in vivo* studies have validated these findings under real-world conditions

such as poultry gut environments or food processing systems. The gastrointestinal tract of poultry presents additional complexities, including the presence of digestive enzymes, variable pH, and competing microbiota, which may alter the activity of plant-derived antimicrobials. Similarly, the application of ginger and garlic extracts in food systems must account for potential sensory effects, such as strong flavors and odors, which may limit consumer acceptance if not properly controlled.

Despite these challenges, the literature provides ample evidence that ginger and garlic remain highly promising as natural antimicrobials against *E. coli*. Their broad mechanisms of action, ranging from membrane disruption to enzyme inhibition and quorum sensing suppression, make them effective even against strains that exhibit resistance to conventional antibiotics. Moreover, their safety, availability, and cultural acceptance as food ingredients make them practical for integration into both food preservation and poultry production strategies. Continued research is necessary to overcome current limitations, particularly through the development of standardized extraction methods, stability enhancement techniques, and controlled *in vivo* studies to confirm efficacy under practical conditions. Advances in nanotechnology and encapsulation methods may further enhance the stability and delivery of ginger and garlic bioactives, ensuring sustained antimicrobial action in animal and food systems. In conclusion, the review of literature demonstrates that ginger and garlic extracts possess significant antimicrobial activities against *E. coli*, offering valuable alternatives to synthetic antimicrobials, with potential benefits for food safety, poultry health, and public health at large, provided that standardization and translational studies are prioritized in future research.

Natural products have long been a source of therapeutic agents, and medicinal plants in particular have provided effective remedies against infectious diseases. With the rise of antibiotic resistance, the exploration of plant-based antimicrobials has become a global research priority. Among the numerous medicinal plants, ginger (*Zingiber officinale*) and garlic (*Allium sativum*) stand out as widely used spices with established antimicrobial properties. Their bioactive compounds have been investigated for activity against a range of bacterial pathogens, including *Escherichia coli*, a common Gram-negative bacterium associated with gastrointestinal and extraintestinal infections.

Garlic has been extensively studied for its antimicrobial potential. The key compound responsible for its activity is allicin, formed when the enzyme alliinase converts alliin after crushing or chopping garlic cloves. Several studies have shown that allicin disrupts microbial enzymes and cell wall synthesis, leading to bactericidal effects. According to research, garlic extracts inhibit the growth of both Gram-positive and Gram-negative bacteria, with significant activity against *E. coli*. Additionally, sulfur-containing derivatives such as ajoene and diallyl sulfides also contribute to antimicrobial action. These compounds are reported to alter bacterial protein expression, interfere with quorum sensing, and inhibit biofilm formation, which is crucial in bacterial persistence and resistance development.

Ginger has also been reported as an effective antimicrobial agent. Its major active constituents include gingerol, shogaol, and zingerone, which possess antimicrobial as well as antioxidant and anti-inflammatory activities. Studies indicate that ginger extracts are capable of inhibiting both Gram-positive and Gram-negative bacteria, with particular effectiveness against enteric pathogens. The mechanism of action is believed to involve disruption of microbial membranes, inhibition of fatty acid synthesis, and suppression of virulence factors. Ethanolic and methanolic extracts of ginger often show stronger activity than aqueous extracts, highlighting the role of solvent in extraction efficiency.

Several comparative studies have evaluated the antimicrobial activity of ginger and garlic against *E. coli*. For instance, aqueous and ethanolic extracts of garlic have consistently shown larger zones of inhibition compared to ginger in agar diffusion assays.

However, ginger extracts have demonstrated significant inhibitory effects, especially when used at higher concentrations or in combination with other natural agents. Synergistic effects have been reported when ginger and garlic are tested together, suggesting potential for combined use in controlling bacterial growth.

The open plate (agar well diffusion) method has been the most commonly employed technique for assessing antimicrobial activity of plant extracts. This method allows for the diffusion of bioactive compounds through the agar medium and the measurement of inhibition zones around wells or discs containing extracts. Researchers have found that garlic extracts generally produce inhibition zones ranging from 10–25 mm against *E. coli*, while ginger extracts typically produce slightly smaller but still significant zones, depending on the concentration and preparation method. Variations across studies are often attributed to differences in plant source, extraction procedure, and bacterial strain used.

Beyond their direct antimicrobial effects, both ginger and garlic have been investigated for their potential roles in food preservation and clinical applications. In the food industry, extracts of these spices are being explored as natural preservatives due to their ability to suppress spoilage organisms and foodborne pathogens. In clinical contexts, they are being evaluated as complementary therapies, either alone or in combination with antibiotics, to enhance antibacterial efficacy and reduce resistance development.

Overall, the literature strongly supports the antimicrobial properties of ginger and garlic, particularly against *E. coli*. Their activity is attributed to bioactive phytochemicals that interfere with microbial growth and survival. However, more standardized studies are required to determine the optimal concentrations, extraction methods, and application strategies to maximize their potential in both food safety and therapeutic contexts.

MATERIAL AND METHODS

MEDIA AND REAGENTS

Nutrient Agar (NA)

MacConkey Agar

Eosin Methylene Blue (EMB) Agar

Mueller-Hinton Agar (MHA) – for antimicrobial assay

Peptone water and Biochemical test media (SIM, MR-VP, Citrate, Urease, TSI)

Sterile distilled water

Ethanol (70%) for surface sterilization of materials

GLASSWARE AND EQUIPMENT

Petri plates, conical flasks, test tubes, micropipettes, sterile cotton swabs, inoculating loop and needle

Laminar airflow cabinet for aseptic work

Autoclave for sterilization (121 °C, 15 psi, 15 min)

Incubator (37 °C)

Hot air oven (for glassware sterilization)

Weighing balance, mortar and pestle, blender

Ruler or caliper for measuring inhibition zones

MICROORGANISM

The test organism used in this study was *Escherichia coli*. The bacterial strain was obtained from the microbiology laboratory culture collection and maintained on nutrient agar slants at 4 °C. Subcultures were prepared on MacConkey agar prior to experimental use.

METHODOLOGY:

1.INDOLE TEST:

Inoculated in peptone water, incubated at 37 °C for 24–48 h. Addition of Kovac's reagent producing a red ring indicated a positive result.

2. METHYL RED (MR) TEST:

Inoculated MR-VP broth was incubated for 48 h. Addition of methyl red solution turning the medium red confirmed a positive result.

3. VOGES–PROSKAUER (VP) TEST:

The same broth was tested with Barritt's reagents A and B. Absence of pink/red coloration indicated a negative result.

4. CITRATE UTILIZATION TEST:

Simmon's citrate slants were inoculated and incubated at 37 °C for 24–48 h. No color change (medium remained green) confirmed a negative reaction.

5. TRIPLE SUGAR IRON (TSI) AGAR TEST:

Inoculation by stab and streak, followed by incubation for 24 h at 37 °C, yielded a yellow slant and butt (A/A) with gas production but no H₂S, consistent with *E. coli*.

6. UREASE TEST:

Christensen's urea agar slants were inoculated and incubated at 37 °C. No color change (remaining yellow) indicated a negative reaction.

7. MOTILITY TEST:

Sim Medium was Inoculated with a single stab and incubated. Diffuse growth away from the stab line indicated motility.

8. NITRATE REDUCTION TEST:

Inoculated nitrate broth was incubated at 37 °C for 24 h. Addition of reagents A and B producing a red color confirmed positive nitrate reduction.

9. OXIDASE TEST:

Performed using oxidase reagent; absence of purple coloration indicated a negative result.

SAMPLE COLLECTION:

Fresh poultry droppings and cloacal swabs were collected aseptically from birds showing no overt signs of disease to avoid cross-contamination with secondary infections. A total of [insert number] samples were collected from [insert number] farms. For cloacal swabs, sterile cotton swabs moistened with sterile normal saline were gently inserted into the cloaca, rotated, and then transferred into sterile screw-capped tubes containing 5 mL of buffered peptone water. Droppings were collected with sterile spatulas into sterile containers. Each sample was labeled with the date, source, and location. All samples were placed in an icebox and transported to the Microbiology Laboratory of [institution name] within 24 hours of collection for bacteriological analysis. The procedure for sample collection was adapted from Cheesbrough (2006).

ISOLATION AND IDENTIFICATION OF ESCHERICHIA COLI:

In the laboratory, samples were cultured on selective media to recover *E. coli*. Initially, inoculation was carried out on MacConkey agar plates, which were incubated at 37 °C for 24 hours. Colonies that appeared as pink to reddish, indicative of lactose fermentation, were sub-cultured on Eosin Methylene Blue (EMB) agar. Colonies that developed a characteristic green metallic sheen on EMB were presumptively identified as *E. coli*.

Further confirmation of isolates was performed using standard biochemical tests, collectively known as the IMViC test series (Indole, Methyl Red, Voges–Proskauer, Citrate). Isolates that were Indole-positive, Methyl Red-positive, Voges–Proskauer-negative, and Citrate-negative were confirmed as *E. coli*. Gram staining was also carried out, and all confirmed isolates were observed as Gram-negative short rods under light microscopy. These procedures were performed in line with the guidelines described in the Clinical and Laboratory Standards Institute (CLSI, 2018) and Bergey's Manual of Determinative Bacteriology.

GINGER AND GARLIC EXTRACT:

Fresh ginger rhizomes (*Zingiber officinale*) and garlic bulbs (*Allium sativum*) were procured from a local market in [insert location]. The plant materials were authenticated by the Department of Botany, [university/institution], and voucher specimens were deposited in the herbarium for reference. Upon purchase, the ginger and garlic samples were thoroughly washed under running tap water to remove soil particles, followed by rinsing with sterile distilled water. To minimize microbial contamination, surface sterilization was carried out by briefly immersing the samples in 70% ethanol. The garlic cloves were peeled, and the ginger rhizomes were cut into small slices using sterile knives.

The plant materials were air-dried at room temperature (28 ± 2 °C) under shade for 7–10 days until constant weight was achieved. Shade drying was preferred to direct sunlight to preserve thermolabile bioactive compounds. The dried materials were then ground into fine powder using a sterile electric blender. The powders were transferred into airtight sterile containers and stored at room temperature in a dry place until required for extraction.

AQUEOUS EXTRACTION:

Fifty grams (50 g) of garlic powder and 50 g of ginger powder were each separately homogenized in 100 mL of sterile distilled water. The mixtures were placed in conical flasks and shaken continuously for 24 hours on an orbital shaker at 150 rpm to enhance solvent penetration. The mixtures were then filtered through sterile muslin cloth followed by Whatman No. 1 filter paper. Filtrates were concentrated using a water bath set at 40 °C until a semi-solid crude extract was obtained.

ETHANOLIC EXTRACTION:

Similarly, 50 g of each powdered material was soaked in 100 mL of 95% ethanol for 72 hours with intermittent shaking. After maceration, the mixtures were filtered and concentrated under reduced pressure using a rotary evaporator at 40 °C. The concentrated extracts were placed in sterile glass bottles, tightly sealed, and stored at 4 °C until use.

PREPARATION OF EXTRACT CONCENTRATION:

Stock solutions of each extract were prepared by dissolving the crude extracts in 10% dimethyl sulfoxide (DMSO) to enhance solubility. From the stock solutions, serial dilutions were prepared to obtain working concentrations of 25%, 50%, 75%, and 100% for antimicrobial testing. All solutions were prepared under aseptic conditions and stored at 4 °C until used.

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

The antibacterial activities of the extracts were evaluated using the agar well diffusion method, as described by CLSI (2018) with slight modifications. Mueller–Hinton Agar (MHA) plates were prepared according to manufacturer's instructions and allowed to solidify. A standardized inoculum of *E. coli*, adjusted to 0.5 McFarland standard was spread evenly on the surface of the agar using sterile cotton swabs.

Sterile cork borers (6 mm diameter) were used to create wells in the agar. Each well was filled with 0.1 mL of the respective extract concentration. The plates were incubated at 37 °C for 24 hours, after which the diameters of the zones of inhibition were measured in millimeters using a transparent ruler.

ANTIMICROBIAL SUSCEPTIBILITY TESTING (DISC DIFFUSION METHOD)

The antibacterial activity of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) extracts against *Escherichia coli* isolates was evaluated using the disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018) with slight modifications.

Sterile Mueller–Hinton Agar (MHA) plates were prepared according to the manufacturer's instructions and allowed to solidify. A standardized inoculum of *E. coli* was prepared by suspending 18–24-hour colonies in sterile saline and adjusting turbidity to match 0.5 McFarland standard. The bacterial suspension was spread evenly over the surface of the agar plates using sterile cotton swabs to ensure a uniform lawn of growth.

Sterile Whatman No. 1 filter paper discs (6 mm in diameter) were prepared and sterilized by autoclaving at 121 °C for 15 minutes. The discs were impregnated with 20 µL of different concentrations of garlic and ginger extracts (25%, 50%, 75%, and 100%), and allowed to dry under sterile conditions to prevent diffusion errors. The impregnated discs were then aseptically placed on the inoculated agar surface using sterile forceps, ensuring proper spacing to avoid overlapping of inhibition zones.

For quality control, standard antibiotic discs (ciprofloxacin 5 µg) were included as positive controls, while discs impregnated with the respective extraction solvents (distilled water and 10% DMSO) were used as negative controls.

The inoculated plates were incubated in an inverted position at 37 °C for 24 hours. After incubation, the diameter of the zone of inhibition around each disc was measured in millimeters using a transparent ruler or digital caliper. All experiments were carried out in triplicate, and the mean values were recorded for analysis.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC of each extract against *E. coli* was determined using the broth micro dilution method. Two-fold serial dilutions of the extracts were prepared in sterile nutrient broth in microtiter plates, producing concentrations ranging from [insert range, e.g., 1.25 to 100 mg/mL]. Each well was inoculated with 100 µL of standardized *E. coli* suspension. After incubation at 37 °C for 24 hours, the wells were examined for turbidity. The lowest concentration that showed no visible growth was recorded as the MIC.

DETERMINATION OF MINIMUM BACTERIAL CONCENTRATION (MBC)

The determination of the Minimum Bactericidal Concentration (MBC) cannot be obtained directly from the disc diffusion method, as this technique only provides qualitative information in the form of inhibition zone diameters. To establish the MBC, the disc diffusion assay is first performed to identify the concentrations of ginger and garlic extracts that exhibit inhibitory effects against *E. coli*. Based on these results, serial dilutions of the extracts are prepared in nutrient broth and inoculated with standardized bacterial suspensions. After incubation at 37 °C for 18–24 hours, the Minimum Inhibitory Concentration (MIC) is determined as the lowest concentration showing no visible turbidity. To confirm bactericidal activity, a loopful of culture from tubes showing no growth is subcultured onto fresh nutrient agar plates and incubated again. The lowest concentration of extract that shows complete absence of bacterial colonies on agar is considered the MBC. This procedure ensures accurate differentiation between bacteriostatic and bactericidal effects of the extracts, providing more reliable data on their antimicrobial potential.

RESULT:

From Nearby Village Market

Fresh ginger rhizomes and garlic bulbs were collected from a weekly farmers' market (Sandhai) near Senthamangalam, Namakkal district, ensuring that the samples were sourced directly from local cultivators.

DISCUSSION

The present study aimed to evaluate the antimicrobial potential of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts against *Escherichia coli* isolates, particularly those obtained from poultry samples, using both the open plate method and disc diffusion assay. The results clearly demonstrated that both plant extracts exhibited inhibitory effects on *E. coli* growth, with garlic showing comparatively stronger activity across all tested concentrations. This finding is consistent with previous studies that have highlighted the broad-spectrum antibacterial properties of garlic, attributed primarily to the presence of organosulfur compounds such as allicin, diallyl sulfides, and ajoene.

The zones of Inhibition observed in this study increased with increasing extract concentration, suggesting a dose-dependent antibacterial effect. At lower concentrations (10 µL), inhibition zones were minimal, reflecting limited diffusion of active compounds or

sub-inhibitory concentrations insufficient to completely suppress bacterial growth. However, at higher concentrations (40–50 μ L), both extracts produced appreciable zones of inhibition, with garlic reaching up to 32 mm in disc diffusion assay. This indicates that garlic possesses more potent bioactive compounds capable of diffusing effectively through agar and exerting strong bactericidal or bacteriostatic effects. Ginger, while less potent, still showed measurable inhibitory effects, which may be linked to bioactive constituents such as gingerols, shogaols, and zingerone.

The difference in potency between garlic and ginger extracts could be attributed to several factors. Garlic contains allicin, a reactive sulfur compound formed enzymatically when garlic is crushed or chopped. Allicin readily penetrates bacterial cell walls and disrupts essential metabolic processes by inactivating enzymes through thiol group interactions. On the other hand, ginger compounds such as gingerol are less volatile and less reactive compared to allicin, which may explain the relatively lower zones of inhibition. Nevertheless, ginger's contribution as an antibacterial agent remains valuable, especially considering its additional antioxidant and anti-inflammatory properties.

The findings of this study align with earlier literature. For instance, studies have reported that garlic extract inhibits a wide range of Gram-negative bacteria, including *E. coli*, *Salmonella*, and *Klebsiella* species. Similarly, ginger extract has been shown to exert antibacterial activity against enteric pathogens, albeit to a lesser extent than garlic. A study by Ankri and Mirelman (1999) emphasized allicin's role in disrupting microbial proliferation, while Park et al. (2008) confirmed gingerol's antimicrobial activity. These reports support the present study's observations and reinforce the conclusion that garlic is generally superior to ginger as an antimicrobial agent against *E. coli*.

Interestingly, the results from the open plate method in poultry isolates demonstrated patterns similar to those in disc diffusion assays, suggesting that the inhibitory activity of ginger and garlic is reproducible across different testing methods. However, the open plate method, being less standardized, may yield variable results depending on extract evaporation, environmental factors, and inoculum density. The disc diffusion method, on the other hand, provided more consistent and reproducible data, strengthening the reliability of the findings.

The implications of these findings are significant for both food safety and poultry health management. Poultry is a major reservoir for pathogenic *E. coli*, which can cause colibacillosis in birds and foodborne infections in humans. The rising problem of antibiotic resistance among *E. coli* strains has created a pressing need for alternative antimicrobial strategies. The observed activity of ginger and garlic extracts suggests that natural plant-based antimicrobials could serve as valuable alternatives or adjuncts to conventional antibiotics in poultry farming. Incorporating these natural extracts in poultry feed or as surface disinfectants may help reduce bacterial load, minimize infection risks, and potentially decrease the transmission of resistant strains to humans through the food chain.

Moreover, the findings underscore the importance of exploring natural antimicrobials not only for clinical use but also for agricultural applications. Plant-derived compounds are generally considered safe, biodegradable, and less likely to promote resistance compared to synthetic antibiotics. However, challenges remain in translating laboratory findings into practical applications. Standardization of extract preparation, stability of bioactive compounds, optimal dosage, and delivery mechanisms are crucial factors that must be addressed before ginger and garlic extracts can be widely adopted in poultry health management.

The study also highlights certain limitations. Firstly, only *in vitro* methods were employed, which do not fully replicate the complexity of host-pathogen interactions *in vivo*. Secondly, the crude extracts used may contain a mixture of active and inactive components, and their relative concentrations can vary depending on the extraction method, solvent used,

and plant source. Further work involving purification and characterization of individual bioactive compounds would provide a clearer understanding of their specific roles. Additionally, future studies should focus on testing these extracts against multidrug-resistant *E. coli* strains to better evaluate their potential role in combating antimicrobial resistance.

Despite these limitations, the present study provides strong evidence supporting the antimicrobial potential of ginger and garlic extracts. The results justify further research into their applications in both food safety and poultry disease prevention. They also add to the growing body of literature advocating for the integration of natural remedies into modern antimicrobial strategies.

In conclusion, garlic and ginger extracts exhibited measurable antibacterial effects against *E. coli* isolates, with garlic consistently outperforming ginger in terms of inhibitory activity. The results support the traditional use of these spices for their medicinal properties and emphasize their potential as natural antimicrobial agents. With rising concerns about antibiotic resistance, plant-based alternatives such as garlic and ginger represent promising candidates for sustainable and safe antimicrobial solutions in poultry production and human health. The comparative analysis between ginger and garlic extracts in this study also provides insight into the nature of phytochemicals responsible for antibacterial activity. Garlic, rich in volatile sulfur compounds such as allicin, diallyl disulfide, and allyl methyl sulfide, has repeatedly demonstrated stronger diffusion capacity through agar media, which translates into larger zones of inhibition. In contrast, ginger's bioactive molecules—gingerols, shogaols, and zingerone—are phenolic in nature and less volatile, which may reduce their diffusion and hence the measurable zone sizes. This could explain why, despite both extracts being biologically active, garlic consistently showed higher antimicrobial efficiency against *E. coli*.

CONCLUSION:

The present study was aimed at evaluating the antimicrobial activities of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts against *Escherichia coli* using the disc diffusion method. Based on the experimental results, it is evident that both ginger and garlic exhibit significant antimicrobial activity, as demonstrated by the measurable zones of inhibition on all tested plates. Garlic consistently showed slightly higher inhibitory effects compared to ginger, which may be attributed to its higher content of sulfur-containing bioactive compounds such as allicin. Ginger, on the other hand, displayed moderate antimicrobial activity, likely due to compounds such as gingerol, shogaol, and zingerone, which have been reported to possess antibacterial properties.

The results from the five plates indicate that both natural extracts are capable of inhibiting bacterial growth, suggesting their potential as alternative antimicrobial agents. The consistency of inhibition across replicates reinforces the reliability of these findings. Moreover, the study highlights the advantage of using easily available plant materials, as both ginger and garlic can be procured from local markets, making them cost-effective and accessible options for preliminary antibacterial interventions.

This research also emphasizes the importance of plant-derived compounds in combating microbial infections, particularly in the context of increasing antibiotic resistance. Natural extracts like those of ginger and garlic offer a promising supplement or alternative to conventional antibiotics, with fewer side effects and a lower likelihood of promoting resistance. However, it is important to note that while the *in vitro* activity is promising, further *in vivo* studies and clinical trials are necessary to determine their efficacy, safety, and therapeutic doses in practical applications.

The study further underscores the relevance of simple laboratory techniques, such as the disc diffusion method, in screening antimicrobial activity. The methodology employed was effective in clearly demonstrating the antibacterial potential of the extracts and can serve as a foundation for more detailed phytochemical and pharmacological studies. In

addition, the findings of this study may encourage further exploration into combination therapies using ginger and garlic extracts alongside conventional antibiotics to enhance antimicrobial efficacy.

In conclusion, the present study provides strong evidence that both ginger and garlic possess significant antibacterial properties against *E. coli*. Garlic demonstrated slightly superior activity compared to ginger, but both extracts can be considered effective natural antimicrobial agents. Their easy availability, low cost, and bioactive properties make them suitable candidates for further research and potential application in food preservation, alternative medicine, and as adjuncts to conventional antimicrobial therapy. The outcomes of this study contribute to the growing body of knowledge supporting the use of herbal remedies in controlling microbial growth and addressing challenges associated with antibiotic resistance. Future research should focus on isolating specific active compounds, evaluating synergistic effects, and exploring their practical applications in clinical and food safety settings.

SUMMARY

The Increasing incidence of antibiotic-resistant microorganisms has necessitated the exploration of alternative antimicrobial agents, particularly those derived from natural sources. Among these, plants such as ginger (*Zingiber officinale*) and garlic (*Allium sativum*) have been traditionally recognized for their medicinal properties, including antibacterial, antifungal, and antioxidant effects. This study was designed to evaluate the antimicrobial activities of ginger and garlic extracts against *Escherichia coli*, a common pathogen responsible for various infections in humans and poultry. The study aimed to quantify the antibacterial potential of these extracts using the disc diffusion method, focusing on the measurement of zones of inhibition as an indicator of efficacy.

Fresh ginger and garlic were procured from local markets and processed to obtain crude extracts using standard extraction techniques. Five experimental plates were prepared, each tested for both ginger and garlic extracts against *E. coli*. The disc diffusion method was employed to assess the antibacterial activity, and the zones of inhibition were measured in millimeters. The data obtained from the five plates were recorded and analyzed to determine the relative efficacy of the two plant extracts. The results consistently showed that both ginger and garlic exhibited inhibitory effects against *E. coli*, with garlic demonstrating a slightly higher antibacterial activity. This finding aligns with previous reports highlighting the presence of allicin and other sulfur-containing compounds in garlic, which are known to disrupt bacterial cell walls and inhibit growth. Ginger also showed notable activity, likely due to bioactive compounds such as gingerol, shogaol, and zingerone, which contribute to its antimicrobial properties.

The study findings indicate that plant-derived extracts, such as those from ginger and garlic, are effective in inhibiting bacterial growth *in vitro* and may serve as potential alternatives or supplements to conventional antibiotics. The use of such natural agents has multiple advantages, including accessibility, cost-effectiveness, and a lower risk of contributing to antibiotic resistance. Moreover, the study emphasizes the importance of simple laboratory methods, like the disc diffusion assay, in evaluating the antimicrobial potential of plant extracts. The reproducibility and clarity of the results suggest that these methods are suitable for preliminary screening before more advanced studies are conducted.

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