



## Studies on antimicrobial activity of some home spices against *Salmonella* spp.

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### Abstract

Food borne Infections caused by *Salmonella* spp. such as typhoid, food poisoning remains a major public health problem, especially in developing countries. The increasing resistance of this pathogen to commonly used antibiotics has created an urgent need to explore alternative antimicrobial agents. Home spices such as lemon, garlic, ginger, and onion are widely used in daily diets and traditional medicine and were known to possess antimicrobial properties. In the present study, the antimicrobial activity of fresh juice extract of common household species viz. garlic (*Allium sativum*), lemon (*Citrus limon*), onion (*Allium cepa*) and ginger (*Zingiber officinale*) were tested by agar well diffusion method against the typhoid fever causing *Salmonella typhi* bacteria. A total of 22 *Salmonella* isolates were tested against three concentrations (100%, 50%, and 25%) of each extract. Lemon extract exhibited the highest antibacterial activity, with a maximum zone of inhibition of 23 mm, followed by garlic (20 mm), onion (21 mm), and ginger (12 mm). The effectiveness of all extracts decreased with decreasing concentration. Garlic and lemon extracts demonstrated strong inhibitory effects against most isolates, while onion showed moderate activity and ginger showed comparatively lower activity. Some isolates exhibited no sensitivity, particularly at lower concentrations. These findings suggest that lemon and garlic possess significant antimicrobial potential against *Salmonella typhi* and may serve as effective natural alternatives to conventional antibiotics.

**Keywords:** Antibacterial activity, Home spices, *Salmonella*

### Introduction

Particularly in developing nations like India, bacterial infectious diseases continue to be a serious health concern. Contaminated food and water are the main ways that these infections are transmitted (World Health Organization [WHO], 2023) <sup>[60]</sup>. Although antibiotics are frequently used to treat bacterial infections, antibiotic resistance has developed as a result of overuse and misuse. Common medications are no longer able to effectively control many bacteria because of this resistance. The need to look for safe, effective, and natural substitutes for synthetic antibiotics has been brought about by this circumstance.

*Salmonella typhi* is a Gram-negative, rod-shaped bacterium belonging to the family *Enterobacteriaceae* and is the causative agent of typhoid fever (Ryan & Ray, 2014) <sup>[51]</sup>. Typhoid fever is mainly transmitted through contaminated food and drinking water. It is more common in areas with poor sanitation and hygiene. Typhoid fever is mainly transmitted through the fecal-oral route. The infection spreads when food or drinking water becomes contaminated with feces or urine of an infected person or a carrier. Poor sanitation, unsafe drinking water, improper disposal of waste, and inadequate personal hygiene play a major role in the spread of the disease in widely distributed regions (Crump *et al.*, 2015) <sup>[11]</sup>.

Symptoms of typhoid fever include prolonged fever, headache, weakness, abdominal pain, diarrhea or constipation, and loss of appetite. If not treated properly, it can lead to serious complications such as intestinal perforation and septicemia may occur, which can be life-threatening (Crump *et al.*, 2015) <sup>[11]</sup>. In recent years, many strains of *Salmonella typhi* have become resistant to commonly used antibiotics, making treatment difficult. This has increased interest in finding alternative antimicrobial agents from natural sources.

Lemon (*Citrus limon*) is a small evergreen tree belonging to the family *Rutaceae*. It is believed to have originated in Northeast India, Northern Myanmar, and China, and is now widely cultivated in tropical and subtropical regions worldwide. The plant grows up to 3–6 meters in height and bears yellow, oval-shaped fruits with a characteristic sour taste. Lemon is widely used in culinary, medicinal, and traditional practices due to its rich nutritional and phytochemical composition. The fruit is an excellent source of vitamin C (ascorbic acid), citric acid, flavonoids, and essential oils such as limonene. These bioactive compounds contribute to its antioxidant, antimicrobial, and preservative properties (D. A. Brat *et al.*, 2006 <sup>[7]</sup>; Ghasemnezhad *et al.*, 2011) <sup>[23]</sup>. The acidic nature of lemon juice creates an unfavorable environment for microbial growth. Citric acid disrupts bacterial cell membranes, while flavonoids interfere with microbial enzyme activity. (Fisher & Phillips, 2009; Dhanavade *et al.*, 2011) <sup>[14, 21]</sup>.

Garlic (*Allium sativum*) is a bulbous herbaceous plant widely cultivated across Asia, Europe, and the Mediterranean region. It has been used since ancient times for both culinary and medicinal purposes. The plant consists of underground bulbs composed of multiple cloves covered by a thin papery skin. Garlic is rich in sulfur-containing compounds, particularly allicin, which is released when the cloves are crushed. These compounds are responsible for its strong aroma and potent antimicrobial, antifungal, antiviral, and antioxidant activities (Lawson, 1996; Ankri & Mirelman, 1999) <sup>[37]</sup>.

One of the most researched medicinal plants with strong antibacterial properties is garlic. Crushed garlic cloves release allicin, a bioactive compound with potent antiviral, antifungal, and antibacterial qualities. By interacting with thiol groups, allicin inhibits vital bacterial enzymes, impairing metabolism and causing cell death. Garlic is a

promising natural antimicrobial agent. (Ankri & Mirelman, 1999; Ross, 2001) [2, 50].

Ginger (*Zingiber officinale*) is a perennial herbaceous plant primarily cultivated in India, China, and Southeast Asia. The medicinally important part of the plant is its underground rhizome. Ginger has been widely used in traditional medicine systems such as Ayurveda and Chinese medicine. It contains bioactive compounds including gingerol, shogaol, and zingerone, which possess antioxidant, anti-inflammatory, and antimicrobial properties (Rahmani *et al.*, 2014; Rahman, 2003) [47, 49]. These substances prevent the synthesis of proteins and damage bacterial cell membranes. Numerous harmful bacteria, such as *Salmonella*, *Shigella*, and *Vibrio species*, have been shown to be inhibited in their growth by ginger. Its antimicrobial potential is further supported by its use as a traditional treatment for gastrointestinal infections (Jolad *et al.*, 2004; Sasidharan *et al.*, 2012) [31, 52].

Onion (*Allium cepa*) is a biennial herbaceous plant widely cultivated as an annual vegetable crop throughout the world. It is believed to have originated in Central Asia and is now extensively grown in India, China, the United States, and many other countries. The plant consists of a bulb formed by concentric fleshy leaf bases covered with dry outer scales. Onions are widely used in culinary preparations and traditional medicine due to their nutritional and medicinal value (Rabinowitch & Brewster, 1990) [46]. The antimicrobial properties of onions are attributed to their abundance of phenolic acids, flavonoids (quercetin), and organosulfur compounds. Onions and garlic both produce sulfur-containing compounds that disrupt the enzyme systems of bacteria growth (Ankri & Mirelman, 1999; Lanzotti V., 2006) [2]. Onion extracts have been shown to have an inhibitory effect on *Salmonella typhi* and other enteric pathogens (Dhanavade *et al.*, 2011) [14].

## Material and Methods

### Collection of Spices Sample

- Fresh samples of commonly used household spices, namely garlic (*Allium sativum*), lemon (*Citrus limon*), onion (*Allium cepa*), and ginger (*Zingiber officinale*), were collected from the local market under hygienic conditions. To reduce the possibility of contamination, sterile plastic containers were used to transport the samples to the lab. Before extraction, the spices were allowed to air dry at room temperature after being thoroughly cleaned with distilled water to get rid of any surface contaminants.

### Preparation of Spices Extract

- After being cleaned, the spice samples were cut into small pieces and, if needed, peeled. Each sample was ground separately using a sterile grinder to obtain a fine paste. A known amount (10 g) of each ground sample was combined with 25mL of sterile distilled water for aqueous extraction, and the mixture was thoroughly homogenized. To obtain a clear extract, The solid particles were removed from the mixture by filtering it through sterile muslin cloth. After being gathered in sterile containers, The filtrates were collected in sterile containers and stored in a refrigerator at 4°C until further use. As needed for antimicrobial testing, sterile distilled water was used to dilute the extracts into various concentrations.

## Determination of Antimicrobial Activity

- The antibacterial activity of the prepared spice extracts against *Salmonella spp* was measured using the agar well diffusion method. The standard bacterial suspension was added to Mueller-Hinton agar plates. A sterile cork borer was used to create uniformly sized wells in the agar. Each spice extract was added to its corresponding well in a (100 µL) measured volume. The plates were incubated at 37°C for 24 hours. A ruler was used to measure the zones of inhibition in millimeters. The size of the inhibition zone was used to calculate each spice extract's antimicrobial activity.

## Results & Discussion

In the present study a total of 22 *Salmonella spp* which were isolated from previous study were used to check their sensitivity against some fresh aqueous extract *viz* ginger, garlic, lemon, onion.

In the study, 100%, 50% & 25% concentration of garlic extract was checked against 22 *Salmonella* isolates (Figure 1). It was found that highest zone 20 mm was observed for isolate S13 followed by 19mm for isolates S6,S7,S11, while for 17 mm for isolates S4,S9,16 mm for isolates S5, S8, S17, S21, S22, 15 mm for isolates S3, S12, S18, S20, & 14mm for isolates S2, S10, S14, S19.

For 50% aqueous garlic extract highest zone of inhibition 10 mm was obtained against S13 (Figure 1) followed by 9mm for isolates S11 while for 8mm for isolates S4, S9, 8 mm for isolates S5, S8,S17,S21,S22, 7 mm for isolates S7,S13,S18. However, some isolates, namely S3, S4, S9, S14, S18 and S20, did not show sensitivity towards any extract.

For 25% aqueous garlic extract highest zone of inhibition 7mm was obtained in S13 followed by 6mm for isolates S1, S4, S5, S8, S9, S17, S21, S22, (Figure 1). However, no antibacterial activity was observed against some isolates, including S3, S4, S9, S14, S15, S16, S18, S19 and S20.

In the study, 100%, 50% & 25% concentration of lemon extract was checked against 22 *Salmonella* isolates (Figure 2). It was found that highest zone (23mm) was observed for isolate S6 followed by 22 mm for isolates S2, S15, 21mm for isolates S3, 20 mm for isolates S4, S14,19mm for isolates S18, while for 18 mm for S1, S11, S13, 17 mm for isolates S10, 16 mm for isolates S5, S7, S17, S19, 15 mm for isolates S8, S15, & 14mm for isolates S9, S21, S22. However, no antibacterial activity was observed against one isolate, S12.

For 50% aqueous lemon extract highest zone of inhibition 11mm was obtained against S2, S6, S15 (Figure 2) followed by 10mm for isolates S4, S12, S14, while for 9mm for S1, S11, S13, 8mm for isolates S5, S7, S16, S17&7mm for isolates S8, S21, S22. Although, antibacterial activity was observed against some isolates, including S2, S8, S9, and S15.

For 25% aqueous lemon extract highest zone of inhibition 7mm was obtained in S3, S4, S14, followed by 6mm for isolates S5, S7, S14 (Figure 2). However, no antibacterial activity was observed against some isolates, including S1, S2, S7, S8, S9, S10, S11, S12, S14, S15, S17, S18, S19 and S20.

In the study, 100%, 50% & 25% concentration of onion extract was checked against 22 *Salmonella* isolates (Figure 3). It was found that highest zone was (21mm) observed for isolate S12 followed by 20 mm for isolates S3, S8,while for 19 mm to isolates S6,S11,18mm for isolates S6,15 mm

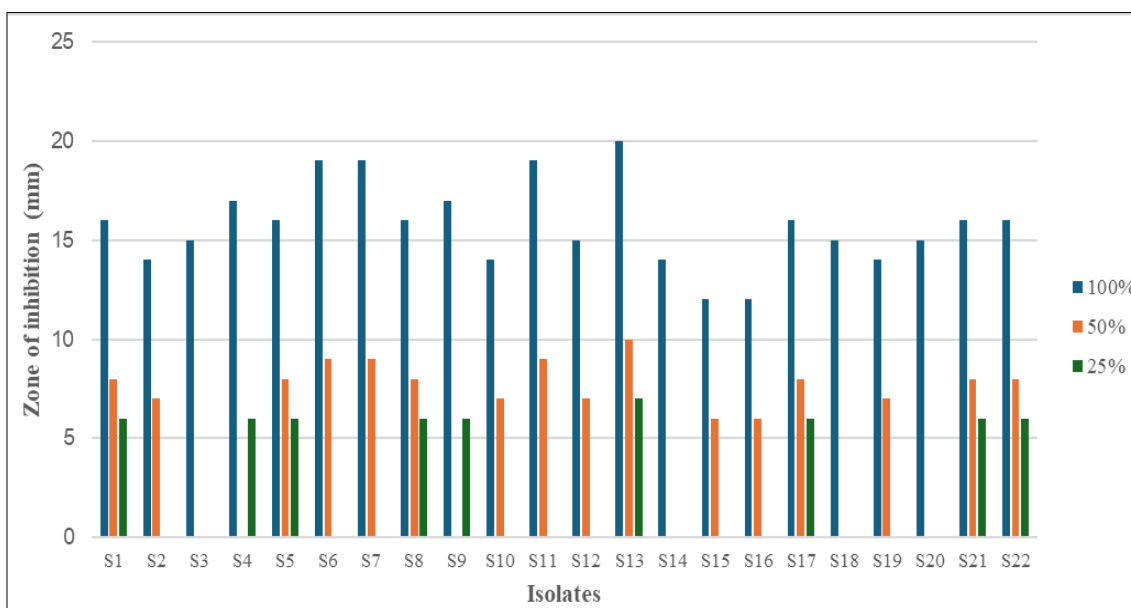
for isolates S10, S20, 14 mm for isolates S17, 13 mm for isolates S1, S16, S19, 12mm for isolates S4, S9, S18, S13, & 11mm for isolates S15, S22. However, no antibacterial activity was observed against some isolates, S2, S5, S7, S9, S14, and S21.

For 50% aqueous onion extract highest zone of inhibition 10 mm was obtained against S3, S8, (Figure 3) followed by 9 mm for isolates S6, S14, 7mm for isolates S7, S15, S17, S22 while for 6 mm for isolates S4, S13 & However, no antibacterial activity was observed against some isolates, S1, S2, S5, S9, S10, S11, S12, S16, S18, S19, S20 and S21.

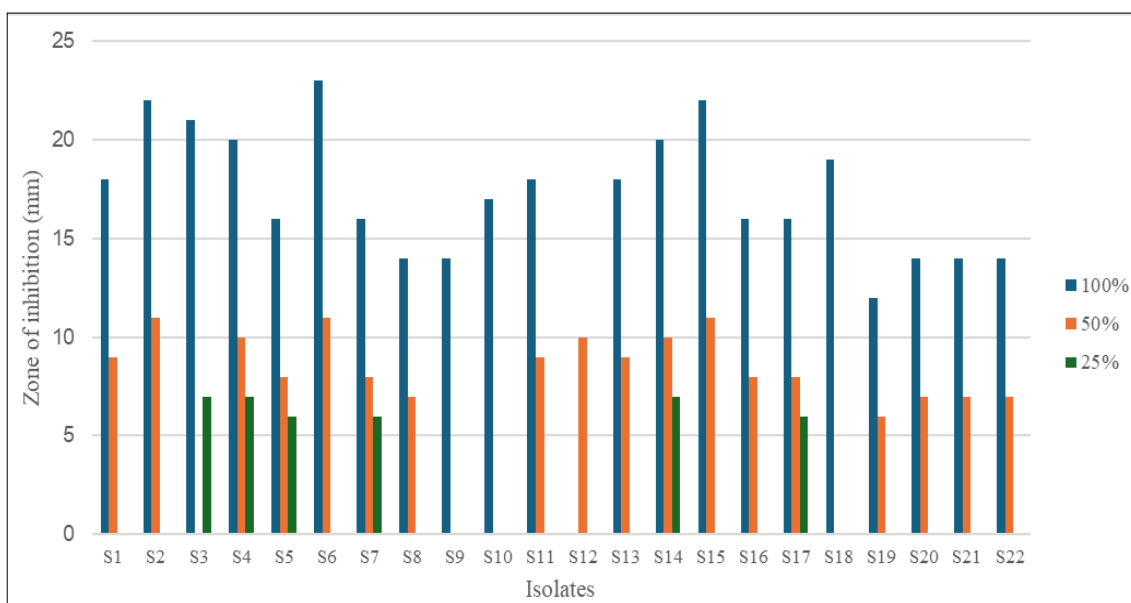
25% aqueous onion extract highest zone of inhibition 7mm was obtained against S2, S8, S12, 6mm for isolates S6, S11, S14 (Figure 3). Although, against some isolates, namely S1, S3, S4, S5, S7, S9, S10, S13, S15, S16, S17, S18, S19, S20, S21 and S22, no any antibacterial activity was recorded.

In the study 100%, 50% and 25% Concentration of ginger extract was checked against 22 found *Salmonella* isolates (Figure 4) It was found that highest zone (12mm) was observed for isolates for S9, S22, while 11mm for isolates S1, S20, 10mm for isolates S8, S15, 8mm for isolates S12, S18, 7 mm for isolates for S6, S7, S17, S18, & 6mm for isolates S3. However, against some isolates such as S2, S4, S5, S10, S11, S14, S16, S19, and S21 no any antibacterial activity was not recorded.

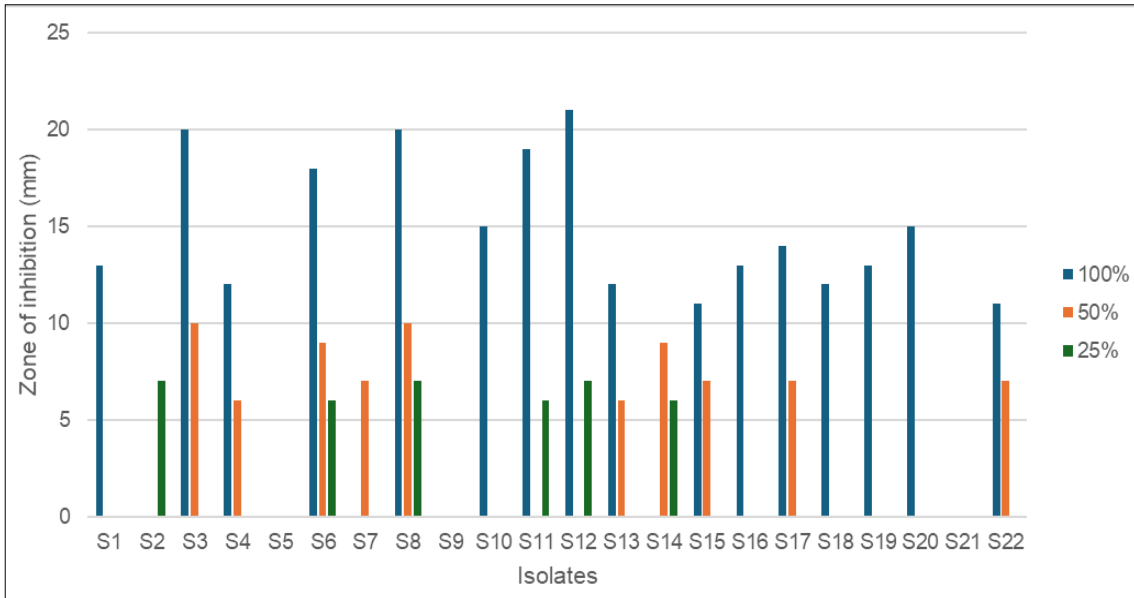
For 50% aqueous ginger extract highest zone of inhibition 7 mm for isolates S1, S9, S20, and S21, 6mm for isolates S10, S12, S17 (Figure 4) However, against other isolates S2, S3, S4, S5, S6, S7, S8, S11, S14, S15, S16, S18, S19 and S21 did not show any antibacterial activity was not observed. For 25% aqueous ginger extract no zone of inhibition was observed in (Figure 4) for all 22 *Salmonella* isolates



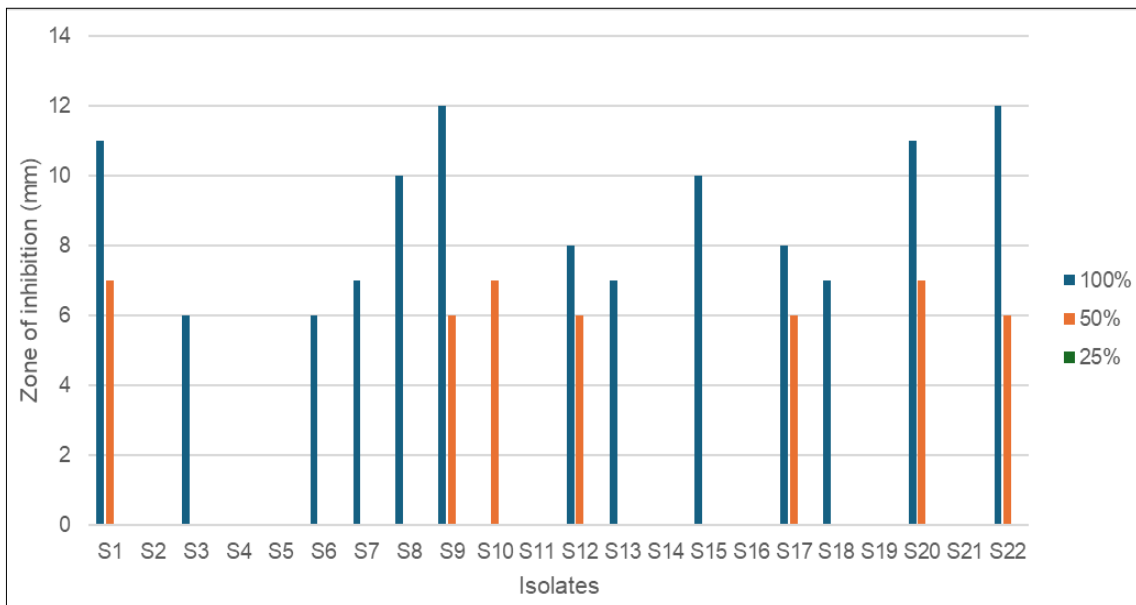
**Fig 1:** Determination of antibacterial potential of garlic extract against *Salmonella* spp.



**Fig 2:** Determination of antibacterial potential of lemon extract against *Salmonella* spp.



**Fig 3:** Determination of antibacterial potential of onion extract against *Salmonella spp.*

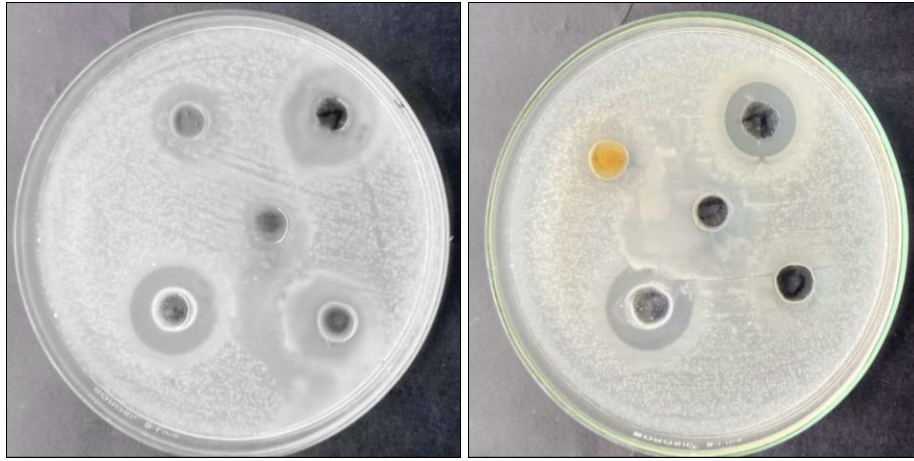


**Fig 4:** Determination of antibacterial potential of ginger extract against *Salmonella spp.*

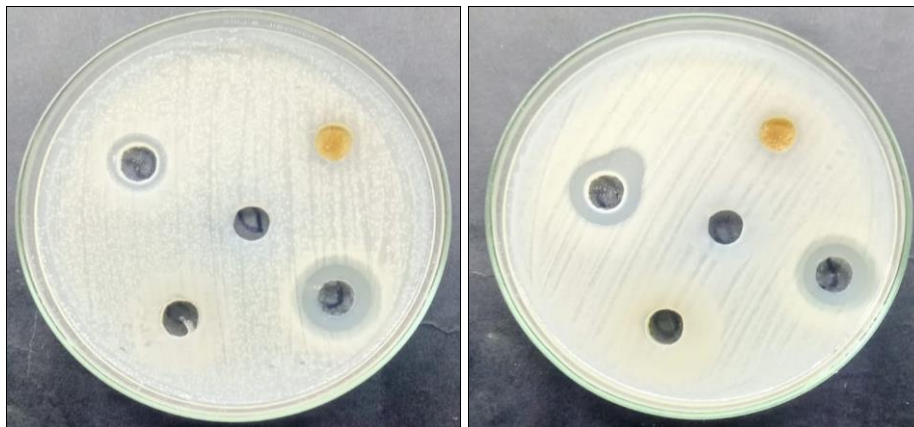
**Results & Discussion**



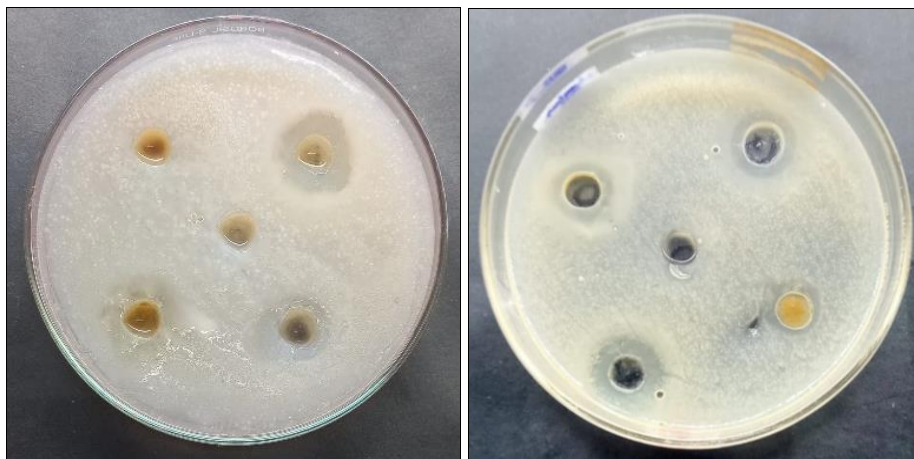
**Photo 1:** Antibacterial activity of garlic against *Salmonella spp.*



**Photo 2:** Antibacterial activity of lemon against *Salmonella spp*



**Photo 3:** Antibacterial activity of onion against *Salmonella spp*.



**Photo 4:** Antibacterial activity of ginger against *Salmonella spp*.

### Discussion

In the present study, 100% concentration of garlic extract exhibited excellent antibacterial activity while 50% concentrations garlic extract exhibited moderate antibacterial activity and 25% garlic concentrations showed minimum antibacterial activity against *Salmonella spp*. Previous studies have also described the antibacterial activity of garlic extract against microorganisms Chand 2013<sup>[9]</sup>. In addition, garlic was shown to have antimicrobial activity against *Salmonella typhi*, Shobana *et al.*, (2009)<sup>[56]</sup> Ankri and Mirelman, (2011) showed that the Allicin, one of the active principles of freshly crushed Garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure

form was found to exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, multidrug-resistant including multidrug resistant enterotoxigenic strains. Allicin is an organo-sulfur compound found in garlic (the active ingredient), it shows inhibitory effect on some pathogenic bacteria. *Allium sativum* could manage and regulate the oxidative stress status by trapping (binding and subsequent deactivating) the harmful oxidant agent (free radical) Eja *et al* (2007)<sup>[15]</sup> showed the bacteriostatic and bactericidal activities of Garlic extract towards *E. coli* and *S. enterica Enteritidis* *Escherichia coli* were more sensitive than *S. enterica*. Garlic reduced the viable cells of *S. enterica* serovar *Enteritidis*,

Celiini *et al.* (1996) showed the Garlic extracts exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Streptococcus*, *Bacillus*, and *Clostridium*. Hamza, (2015) [27] also reported garlic was effective against a plethora of gram-positive and gram harmful bacteria such as *S. aureus*, *Proteus*, *Pseudomonas*, *E. coli*, *Salmonella*, and *Klebsiella*

In the present study, 100% concentration lemon extract exhibited excellent antibacterial activity while the 50% concentrations lemon extract showed moderate antibacterial activity and the 25% concentrations lemon extract observed very low antibacterial activity against *Salmonella spp.* The high antibacterial activity exhibited by garlic compared to lemon may be attributed to sulfur-based compounds such as alliin, which possess strong antibacterial activities (Larkcom 1976; Bocchini *et al.* 2001) [6, 36]. Earlier studies on their activity show that they had antifungal and antibacterial agents at concentrations of 50 µL and 100 µL (Gopalan *et al.* 2000; Jayaprakasha *et al.* 2002 [30]; Fisher and Phillips 2006 [20, 25], Bocchini *et al.* (2001) [6] showed that the lemon/garlic/turmeric blend gave inhibition zones against *E. coli* and *S. typhi* that are substantially different from pure garlic ( $p < 0.05$ ) From the mean inhibition zones, the lemon/garlic blend has lower activity on *S. typhi* (12.0±0.0) compared to pure garlic (17.7±2.5). This may be due to the deactivating effect of citric acid on alliinase, an enzyme that converts alliin to allicin.

In the present study, 100% concentration onion extract exhibited strong antibacterial activity while the 50% concentrations onion extract showed medium activity of antibacterial and the 25% concentration observed to showed very low antibacterial activity against this may be due to the *Salmonella spp.* The Onion bulbs contains numerous organic sulfur compounds, including trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, S-propylcysteine sulfoxide and cycloalliin; flavonoids; phenolic acids; sterols including cholesterol, stigma sterol, b-sitosterol; saponins; sugars and a trace of volatile oil composed mainly of sulfur compounds, so the antibacterial activity of Onion attributed to its content: Alkaloids act through penetration via cell membrane deeply inside the cell and interacts with DNA, while flavones act through its conjugation with bacterial adhesions at the bacterial cell surface and form complex with bacterial cell wall (Cushnie and Lamb, 2005; Cowan, 1999; Daglia, 2012) [10, 12, 13], Adeshina *et al.* (2011) [1] which showed the susceptibility of the tested bacteria especially *P. aeruginosa* and *S. typhi* to the onion juice is encouraging because of the health crisis caused by these organisms all over the world. Hamza, (2015) [27] Showed the maximum inhibition zone of Gram positive bacteria to Garlic-Onion combination extract was observed against *S. epidermidis* (40 mm), and the minimum was against *Strep. Viridians* (30 mm) while the maximum inhibition zone of Gram negative bacteria to same extract was observed against *Salmonella typhi* (40 mm).

In the present study, 100% concentration of ginger extract showed moderate antibacterial activity while for 50% concentrations ginger extract observed very low antibacterial activity and 25% concentrations ginger extract doesn't showed antibacterial activity against *Salmonella spp.* Some other studies have also reported ginger having weak antimicrobial effects (Indu *et al.*, 2006; Eruteya and

Odufa, 2009; Esimone *et al.*, 2010; Silva and Frenandes, 2010) [18, 19, 28, 53]. Singh *et al.*, (2008) [58] have reported ginger extract had lower zone of inhibition which ranged from 6.67 plus/minus 0.57 to no inhibition and *Salmonella typhi* were completely resistant to all the ginger extract samples tested. The antimicrobial activity of ginger may be due to the considerable amounts of phenolic compounds present in ginger (Singh *et al.*, 2008) [58]. In ginger, the gingerol related components have been found to antimicrobial activities (Park *et al.*, 2008) [43]. There are several reports of the inhibitory effect of ginger in the form of extract in several bacteria Akoachere *et al.*, 2002 [2]; Karuppiah and Rajaram, 2012 [32]; Gull *et al.*, 2012 [26] [Moderate to good antimicrobial properties of ginger were shown in previous (Bansal and Bansal, 2011 [5]; Iwalokun *et al.*, 2004 [29]; Srinivasan, (2005) [59]. However some studies have reported as ginger having weak antimicrobial effects (Shelef, 1983; Kim *et al.*, 1995; Shukla and Singh, 2007; Indu *et al.*, 2006) [33, 28, 55, 57] and it compares well with present study where *S. enteritidis* and *Salmonella typhi* were resistant towards all ginger extract samples, Similar results were reported in which ginger did not show any antibacterial activity against the multidrug resistant bacteria viz: *Escherichia Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* (Hamza, 2015) [27]

## Conclusion

In the present study garlic and lemon fresh aqueous extracts showed prominent antibacterial activity against *Salmonella spp.* Onion had shown moderate activity against *Salmonella spp.* while ginger was not as effective as other. Overall a 100% concentration of aqueous extracts of all the home spices were shown prominent activity as compared to the dilution 25% and 50%. These home spices are readily available in day to day life and consumption of this home spices regularly can be beneficial in inhibition of typhoid and food poisoning causing *Salmonella spp.* However further studies need to be conducted to determine if better antibacterial activity is achieved by combining these home spices.

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