Original Article

Antimicrobial Activity of Zingiber officinale (Ginger) and Allium sativum (Garlic) Against *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*

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ABSTRACT

Objective: To evaluate the antimicrobial activity of ethanol extracts of ginger and garlic against *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* and compare them with antibiotics used to treat infections caused by these bacteria.

Methodology: It was a quasi-experimental study conducted at the Microbiology Department & Biochemistry Laboratory of Hazrat Bari Imam Sarkar Medical and Dental College, Islamabad from October 2021 to January 2022. After getting ethical approval from the Institutional Review Board, ethanol extracts of ginger and garlic were prepared and tested against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.* The isolates were also tested against antimicrobials; ceftriaxone, oxacillin, and piperacillin/tazobactam for *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa*, respectively. Data analysis was done using the Statistical Package for the Social Sciences (SPSS) version 25.

Results: Among all ginger & garlic extracts and antimicrobials, the most significant zone of inhibition was produced by ginger day 3 extract against all bacterial isolates. The one-way ANOVA test showed a significant difference in the zone diameters of day 3 ginger extract compared to other extracts and antimicrobials (p-value <0.01).

Conclusion: Ethanol extracts of ginger and garlic have significant antimicrobial activity and inhibit the growth of *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. The most effective is the day 3 ginger extract producing the most significant zone of inhibition against all bacterial isolates as compared to day 3 garlic extract, day 9 garlic & ginger extracts, and antimicrobials.

Keywords: *Antimicrobial activity. Garlic. Ginger.*

INTRODUCTION

Plants have been in continuous use for centuries for essential oil preparation because of their application in medicine, pharmaceutics, food industries, and biotechnology.[1,2] Ginger and garlic are not only used as spices in food but also in medicine due to the presence of various bioactive substances.[3] Besides, these two herbs are cheap, easy to obtain and have almost no profound side effects documented. Ginger and garlic have antioxidant, antifungal, and antibacterial properties.[4]

Many studies found that garlic and ginger have a beneficial effect in reducing pain, inflammation, fever, and ulcers. They also decrease serum cholesterol and gastric motility in nausea. They have an effective role in allergies, thrombosis, osteoporosis, osteoarthritis, rheumatoid arthritis, degenerative diseases, atherosclerosis, and coronary diseases. Because of their activity against various viral, bacterial and parasitic pathogens, these herbs are found to be effective against these infectious agents.[5]

Antibiotic resistance has emerged as a global health concern due to the overuse of antibiotics. It is a major threat because a few options of antimicrobials are left for treatment. The new antimicrobials are not being synthesized at the same rate that bacteria are developing resistance to them.[6] Various non-antibiotic approaches can be used to treat and prevent various infections, for example, probiotics, bacteriophages, phytomedicines, and plants (herbs). The use of plants and herbs in medicine is not a new concept, but they have been used to cure different infectious and non-infectious diseases for a long time globally.[7] The World Health Organization reported that almost 80% of people all around the world are dependent on traditional medicine in which different plant extracts and herbs are used.[3] Ginger and garlic are also among these plant extracts.[8]

Based on the antimicrobial properties of garlic and ginger, we hypothesized that their antimicrobial activity would be beneficial in our geographical region. A few studies have been done in Pakistan to observe the antimicrobial spectrum of ginger and garlic. Therefore, we aimed our study to use 95% ethanol for the preparation of ginger and garlic extracts and study their antimicrobial activity against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Pseudomonas aeruginosa* (*P. aeruginosa*). Their antimicrobial activity was compared with antibiotics used to treat infections caused by these bacteria.

METHODOLOGY

It was a quasi-experimental study conducted at the

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Microbiology Department & Biochemistry Laboratory of Hazrat Bari Imam Sarkar Medical and Dental College, Islamabad after approval from the Institutional Review Board (Letter No: EC, 01/27/09/2021, 27-09-2021). Fresh garlic and ginger were obtained. Their raw forms were peeled, cut into tiny pieces, and air-dried at room temperature for 7 days. Then they were grounded by mortar and pestle. Two preparations of ginger were made by putting 20 grams of ginger in 400 ml of 95% ethanol in each beaker. Similarly, two garlic preparations were made by adding 20 grams of garlic in 400 ml of 95% ethanol in each beaker. The preparations were stirred intermittently with a glass rod in a dark place at room temperature. One of the ginger and garlic preparations was filtered on the 3rd day and the other on the 9th day using a Whatman filter paper number 1. The supernatant filtrate of extractions was labeled and used for the present study.

Sterile filter paper discs were purchased. Stock solutions of ginger and garlic were made by dissolving 0.5 g of each ginger and garlic in 5 ml of dimethyl sulphoxide. The stock solution had a concentration of 10,000 mg/mL. The extracts of 100 µg/ml concentration were prepared from the stock solution. The sterile discs were dipped in the extracts overnight and then put in a sterile petri dish for drying. In addition to these discs, the antibiotics used were ceftriaxone (1 µg) for *E. coli*, oxacillin (30 µg) for *S. aureus*, and piperacillin/tazobactam (100/10 µg) for *P. aeruginosa*.

The antimicrobial properties of ginger and garlic extract were tested against three bacterial species: *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli,* and *Pseudomonas aeruginosa* (Gram-negative bacteria). The cultures were taken from the Microbiology Department of Pathology Laboratory of HBS Hospital by non-probability sampling technique. Informed consent was taken from the patients whose samples were used in the study. The bacterial strains were preserved in agar slants and stored in the refrigerator at 4°C.

The antimicrobial activity was evaluated using the Kirby-Bauer method. The suspension of each organism was made by mixing 2-3 well-isolated colonies into a tube of normal saline using a sterile wire loop. The turbidity of each suspension was then matched to the 0.5 McFarland turbidity standard. Next, the suspension was inoculated into the nutrient agar plate, and then discs were applied. The plates were incubated aerobically at 35-37°C for 24 hours. After 24 hours of incubation, inhibition zones were measured in millimeters.[9]

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25.0. The zones of inhibition of day 3 ginger & garlic extracts, day 9 ginger & garlic extracts, and antimicrobials were calculated as mean and standard deviation (SD). One-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test was applied to compare the means of different groups. A p-value of ≤0.05 was considered statistically significant.

RESULTS

Our results showed that the greatest zone of inhibition was produced by ginger day 3 extract against all bacterial isolates among all ginger and garlic extracts. The zone of inhibition of ginger day 3 extract was even greater than that of antimicrobials. The mean zone of inhibition of ginger day 3 extract was 41.4±2.8 mm as compared to the mean zone of inhibition of ceftriaxone (33.5±1.6 mm) for *E. coli*. The mean zone of inhibition of ginger day 3 extract was 44.8±3.8 mm as compared to the mean zone of inhibition of oxacillin (36.7±3 mm) for *Staphylococcus aureus*. Similarly, for *Pseudomonas aeruginosa*, ginger day 3 extract exhibited a zone diameter of 34.6±2.6 mm, whereas the strain was resistant to piperacillin/tazobactam showing a 0 mm zone diameter. The one-way ANOVA test showed a significant difference in the zone diameters of day 3 ginger extract compared to day 3 garlic extract, day 9 garlic & ginger extract, and antimicrobials with a p-value of <0.01. The mean zones of inhibition and one-way ANOVA test results for all the bacterial isolates are shown in Table 1.

Post-hoc Tukey's analysis showed that the ginger day 3 extract has the highest antimicrobial activity against *E. coli* than the ceftriaxone, ginger day 9 extract, garlic day 3, and day 9 extract ($p < 0.01$). There is no significant difference between the antimicrobial activity of garlic day 3 extract with ginger day 9 extract ($p > 0.05$). All others have significant differences from each other ($p < 0.01$) (Figure 1).

One-way ANOVA followed by post-hoc Tukey's analysis showed that the ginger day 3 extract has the highest antimicrobial activity against *S. aureus* than oxacillin, ginger day 9 extract, garlic day 3 extract, and garlic day 9 extract ($p < 0.01$). There is no significant difference between ginger day 9 extract and garlic day 9 extract ($p > 0.05$). All others have significant differences from each other ($p < 0.01$) (Figure 2).

Data analysis showed ginger day 3 extract has the highest antimicrobial activity against *P. aeruginosa* than the piperacillin/tazobactam, ginger day 9 extract, garlic day 3 extracts, and garlic day 9 extract ($p < 0.01$). Piperacillin/tazobactam has no antimicrobial activity. There is no significant difference between the antimicrobial activity of ginger day 9 extract with garlic day 9 extract against *P. aeruginosa* ($p > 0.05$). All others have significant differences from each other ($p < 0.01$) (Figure 3).

Table 1: Mean Zones of Inhibition & One-Way ANOVA Test Results for all Bacterial Isolates

Escherichia coli					
	Garlic (Mean±SD)	**Ginger (Mean±SD)**	**Ceftriaxone (Mean±SD)**	**f (6.77)**	**p-value**
Day 3	13.1±2.1 mm	41.4±2.8 mm	33.5±1.6 mm	580	<0.01***
Day 9	20±3.5 mm	11.8±1.6 mm	34.8±1.9 mm		
Staphylococcus aureus					
	Garlic (Mean±SD)	**Ginger (Mean±SD)**	**Oxacillin (Mean±SD)**	**f (6.77)**	**p-value**
Day 3	12.2±1.5 mm	44.8±3.8 mm	36.7±3 mm	431	<0.01***
Day 9	14.8±2 mm	20.2±1.9 mm	37.2±4.3 mm		
Pseudomonas aeruginosa					
	Garlic (Mean±SD)	**Ginger (Mean±SD)**	**Tazobactam (Mean±SD)**	**f (6.77)**	**p-value**
Day 3	9.3±1.9 mm	34.6±2.6 mm	0±0 mm	626	<0.01***
Day 9	21.6±3.4 mm	19.8±1.8 mm	0±0 mm		

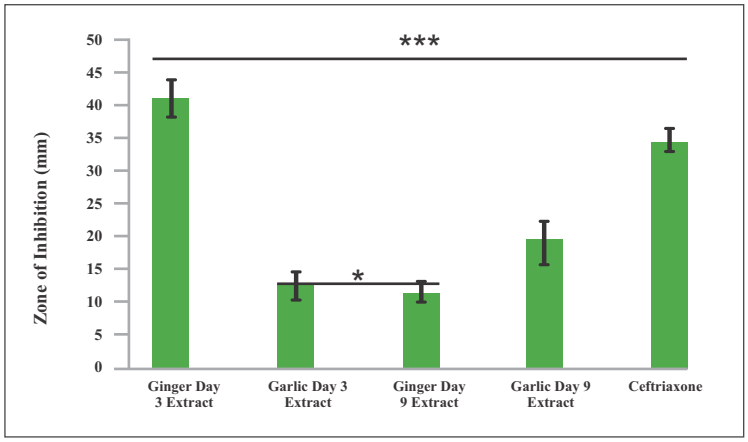

Figure 1: Bar Chart of the Mean Zone of Inhibition (mm) of Ginger & Garlic Extracts and Ceftriaxone Against *E. coli*

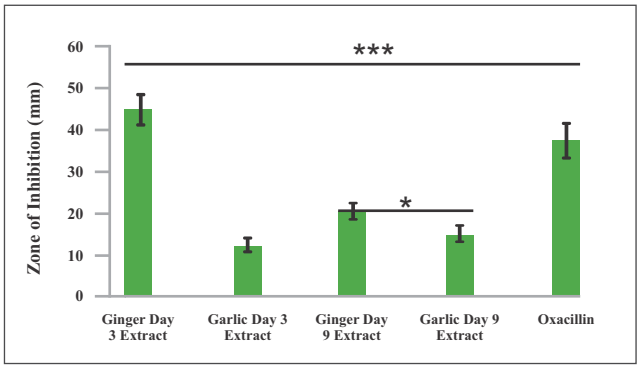

Figure 2: Bar Chart of the Mean Zone of Inhibition (mm) of Ginger & Garlic Extracts and Oxacillin Against *S. aureus*

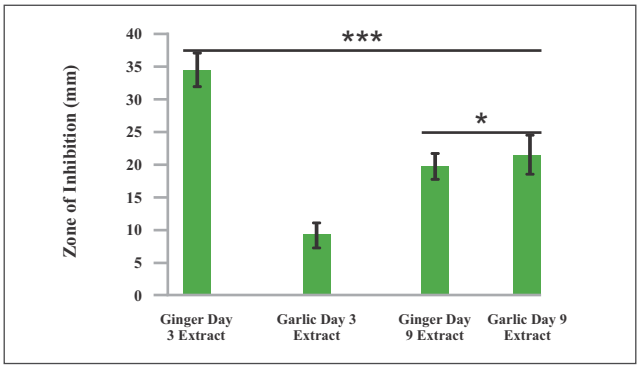

Figure 3: Bar Chart of the Mean Zone of Inhibition (mm) of Ginger & Garlic Extracts and Piperacillin/Tazobactam Against *P. aeruginosa*

** p-value >0.05*
**** p-value <0.01*

DISCUSSION

Ginger and garlic are of particular interest to scientists for ages and are gaining popularity due to their antimicrobial activities.[10] The World Health Organization (WHO) reported that almost 80% of people are using traditional medicine based on plant-based medications.[11] Ginger contains various compounds such as zingerone, zingiberene, camphene, and phellandrene which contribute to its antimicrobial properties.[3] The antimicrobial activity of garlic is chiefly due to sulphur containing compound, Allicin. When garlic is crushed or cut into tiny pieces, the alliinase enzyme converts alliin into allicin.[12] The antimicrobial activity of ginger and garlic depends on the solvent and method used for extraction and the amount of bioactive compounds.[8,10]

Our study investigated the antimicrobial activity of ethanol extracts of ginger and garlic. Our results showed that day 3 ginger extract showed the maximum antimicrobial activity against *E. coli*, *S. aureus*, and *P. aeruginosa*. The mean zone of inhibition of ginger day 3 extract was 41.4±2.8 mm for *E. coli*, 44.8±3.8 mm for *S. aureus,* and 34.6±2.6 mm for *P. aeruginosa*. Antimicrobial activity of Allium sativum of garlic and Zingiber officinale of ginger against both clinical and laboratory isolates of *Staphylococcus aureus* were studied in Madonna University Teaching Hospital, Nigeria. The study showed that both garlic and ginger extract was effective against *Staphylococcus aureus*. However, ginger produced a greater zone of inhibition for *Staphylococcus aureus* (16.0±0.21 mm) than garlic (11.5±0.69 mm) at the concentration of 100 µg/ml.[13] Sikrodia et al. reported that the aqueous extract of ginger showed zones of inhibition of 20 mm for *E. coli* and 15 mm for *Staphylococcus aureus*. In contrast, garlic extracts did not show any zone of inhibition. The zones of inhibition of gentamicin, ampicillin plus sulbactam, and ciprofloxacin were 20, 16, and 30 mm for *E. coli*, and 17, 22, and 20 for *Staphylococcus*, respectively.[9]

Some studies reported contradictory results as compared to other studies. A study conducted by Emmanuel et al. revealed that aqueous garlic extract had the highest antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa* whereas ethanol extract of ginger showed the highest zone of inhibition against *E. coli.* Overall, the aqueous, methanol, and ethanol extracts of ginger showed better inhibition zones than various extracts of garlic except the aqueous extract of garlic. The aqueous extract of garlic was most effective as compared to other garlic extracts whereas the ethanol extract of ginger showed the highest antimicrobial activity among all the ginger extracts.[14] A study conducted in Bangladesh evaluated the antimicrobial activity of aqueous, methanol, and propranolol extracts of garlic and ginger against *Staphylococcus aureus* and *Klebsiella pneumoniae.* The greatest antibacterial activity was exhibited by the aqueous extract of garlic against *Staphylococcus aureus* (25 mm).[15] A study was conducted in Nigeria to assess the antimicrobial activity of n-hexane and methanol extracts of ginger and garlic against *E. coli, Shigella*, *Salmonella*, and *Klebsiella.* They found that all organisms were susceptible to extracts of ginger and garlic due to the presence of bioactive compounds in them. The study also reported that garlic extracts had higher antimicrobial activity for *E. coli* than the hexane and methanol extracts of ginger.[16] Another study revealed that aqueous extract of garlic exhibited a zone diameter of 15 mm against *E. coli*, 21 mm for *Staphylococcus aureus*, and 19 mm against *Pseudomonas*. The aqueous extract of garlic exhibited a stronger antimicrobial effect as compared to ginger & turmeric against *Staphylococcus, E. coli* & *Pseudomonas*.[17]

CONCLUSION

Ethanol extracts of ginger and garlic have significant antimicrobial activity and inhibit the growth of *E. coli*, *S. aureus,* and *P. aeruginosa*. The most effective is the day 3 ginger extract producing the greatest zone of inhibition against all bacterial isolates as compared to day 3 garlic extract, day 9 garlic & ginger extract; and antimicrobials. This is a finding which guides us toward the pattern of behaviors of bioactive compounds in ginger.

LIMITATIONS & RECOMMENDATIONS

The study did not compare the antimicrobial activity of ethanol extracts of ginger and garlic with other ginger and garlic extracts such as aqueous or methanol extracts. The antimicrobial activity of various dilutions of ginger & garlic extracts was not evaluated in the study. Further studies can guide clinicians towards better use of the antimicrobial activity of these herbs. However, the side effects and pharmacokinetic properties of these plants should also be explored.

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