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Original Research Article

# EVALUATION OF ANTIBACTERIAL ACTIVITY OF GARLIC (*ALLIUM SATIVUM*), UTAZI (*GONGRONEMA LATIFOLIUM*) AND GINGER (*ZINGIBEROF FICINALE*) AGAINST MULTI-DRUG RESISTANT *ESCHERICHIA COLI* AND *SALMONELLA SPP*

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## ABSTRACT

The spread of multi-drug resistant strains due to increased abuse of antibiotics has necessitated the search for alternative, novel antibacterial agents from natural sources. This study investigated the antibacterial efficacy of garlic, Utazi and ginger against Salmonella and multi-drug resistant *Escherichia coli*. Agar well diffusion tests were conducted to evaluate the extracts' inhibitory effects. Ceftazidime and Amoxicillin-clavulanate served as positive controls via disc diffusion test. The results showed that all extracts exhibited a dose-dependent inhibitory activity, especially at 200 mg/kg. Methanolic extracts demonstrated superior inhibitory activity, compared to aqueous extracts, particularly, against *E.coli*. This study, therefore highlights the potential of these herbs as natural antimicrobial agents against food-borne pathogens, offering a promising alternative to synthetic antibiotics that are safe, easily accessible, and affordable.

Keywords: agar well diffusion, *Escherichia coli*, garlic, ginger, multi-drug resistant Salmonella, utazi

## INTRODUCTION

The alarming increase in the emergence of multi-drug resistant strains of bacteria as a result of indiscriminate use of antibiotics is a global health concern due to persistent ineffective therapies. The increasing threat of antimicrobial resistance is the main hindrance to the successfully treating infectious diseases. Therefore, the continual spread of multi-drug resistant strains due to increased abuse of antibiotics has necessitated the search for alternative, novel antibacterial agents from natural sources (Fu *et al.*, 2007).

Medicinal plants have been revered for their therapeutic properties, particularly in African traditional medicine. They have been used to treat various ailments, including bacterial infections. These plants contain phytochemicals that are effective in therapy.

They are highly regarded as excellent alternatives due to their cost-effectiveness, ready availability, ease of use, low toxicity, rapid degradability, and environmentally friendly nature (Al sheikh *et al.*, 2020).

*Escherichia coli* (*E.coli*) and Salmonella are gram-negative bacteria frequently associated with food-borne illnesses,



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urinary tract infections and other life-threatening conditions such as septicaemia. Their increasing resistance to antibiotics underscores the urgent need for innovative antimicrobial strategies. Garlic (*Allium sativum),* Utazi (*Gongronema latifolium)* and Ginger (*Zingiberof ficinale*) are widely used herbs that have gained recognition as safe, natural alternatives to conventional treatments for various health disorders such as diabetes, hypertension, inflammatory, renal, and dental disorders (Gull *et al.,* 2012). Other studies conducted elsewhere have reported on the antimicrobial properties of these herbs (Mohamedin & Ashraf, 2018; Singh, 2018). These herbal spices are rich in compounds such as flavonoids, alkaloids, gingerols and sesquiterpenes with established antimicrobial properties (Singh, 2018; Dini, 2018).While evidence supporting the antibacterial efficacy of garlic, utazi and ginger, there is still insufficient information regarding their effectiveness against multi-drug resistant (MDR) pathogens in poultry. Therefore, our study aimed to assess the antibacterial potential of aqueous and methanolic extracts of garlic, utazi and ginger against MDR bacteria isolated from poultry, as well as contribute to the development of plant-based antimicrobial agents suitable as antibiotic alternatives against MDR bacteria.

## MATERIALS AND METHODS

### SOURCING OF THE HERBS AND SPICES

The utazi leaves, ginger and garlic were sourced from the local Orie Ugba market and were properly identified in the Department of Crop Science, Michael Okpara University of Agriculture, Umudike, Abia State.

### PREPARATION OF AQUEOUS EXTRACTS

The utazi leaves, ginger and garlic were thoroughly washed, peeled and cut into smaller sizes and air-dried at room temperature. The dried herbs and spices were then ground to powder using a dry laboratory blender.

Aqueous extracts of these herbs and spices were prepared by soaking 100 g of each of the powdered samples in 500 mL of distilled water for 72 h. The solution was then carefully filtered through Whatman number 1 filter paper. The residue was discarded and the filtrate evaporated to dryness over a water bath.

### PREPARATION OF METHANOLIC EXTRACTS

The fresh, washed herbs were dried at room temperature and pulverized using an electric blender. The ground leaves were then extracted in 80% methanol using asoxhlet apparatus as described by Habour (1998). The extract was dried using the hot air oven at the temperature of 35°C to obtain a yield percentage of the extract. The percentage yield of the sample was determined as follows:

Percentage yield (%) = weight of the extract × 100/ Weight of the dried powder

### PREPARATION OF DIFFERENT CONCENTRATIONS OF THE *ZINGERBAROF FICINALE, ALLIUM SATIVUM, GONGRONEMA LATIFOLIUM*

Stock solutions of the prepared extracts were made by aseptically weighing 1g of the extract and dissolving in 5 mL of Dimethylsulfoxide (DMSO) to make a 20 % (200 mg/ml) solution. Different working concentrations (25, 50, 100, and 200 mg/mL) of methanolic and aqueous extracts of each herb were prepared from the stock solution, as described by Harbour (1998), before using for antimicrobial sensitivity.

### BACTERIAL STRAINS

The strains of *E. coli* and Salmonella *spp* isolated from poultry were acquired from the Department of Veterinary Microbiology, Michael Okpara University of Agriculture, Umudike. Both strains were revived and verified from the stock solution as *E. coli* and *Salmonella spp*. on eosin methylene blue agar and MacConkey Agar, respectively.

### PREPARATION OF TEST ORGANISMS

A loopful of the test organisms were inoculated in Nutrient broth and incubated overnight at 37° C and its turbidity was adjusted to 0.5 McFarland standards.

### ANTIBACTERIAL ASSAY

Antibacterial activities of extracts of *Zingerbarofficinale*, *Alliumsativum* and *Gongronemalatifolium* were performed using the agar well diffusion method as described by Srinivasan *et al.* (2001). The disk diffusion test assessed the antibacterial activity of the selected herbs against MDR *E. coli* and *Salmonella spp*

The Mueller Hinton Agar (MHA) plates were inoculated with standardized inoculum (0.5 McFarland) of test bacteria by swabbing small volumes of the microbial broth on the plates and then evenly seeded and streaked onto the agar plate surface using sterile cotton swab and incubated for an hour at 37°C. Afterwards, wells of 6 mm were bored in the inoculated media using a sterile cork borer (6 mm in diameter). With the aid of a micropipette, the different concentrations of the plant extract solutions (25 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml) were dispensed in their respective wells in the plate. Each sample was tested in triplicates.

### ANTIBIOGRAM

Antimicrobial susceptibility test for the bacterial isolates was also carried out with commercial antibacterial agents: Amoxicillin-Clavulanic acid (0.03mg), Chloramphenicol (0.03mg), Ciprofloxacin (0.005mg), using the disk diffusion

test on Mueller Hilton media (Hi-Media, India), to compare their antimicrobial potency with that of the plant extracts.

**TABLE I: MEAN CONCENTRATION OF THE DIFFERENT ZONES OF INHIBITION (ZOI) OF AQUEOUS AND METHANOLIC EXTRACTS OF GINGER AGAINST *SALMONELLA SPP AND ESCHERICHIA COLI***

| Ginger concentration (mg/kg) | Inhibition zones (mm) of Aqueous extracts | | Inhibition zones Methanolic extracts | |
|---|---|---|---|---|
| | ***Salmonella spp.*** | ***Escherichia coli*** | ***Salmonella spp.*** | **E*scherichia coli*** |
| 25 | 8.00±1.00[a] | 10.33±3.21[a] | 3.33±3.33 [a] | 10.67±1.53 [a] |
| 50 | 9.33±0.58[a] | 12.33±1.53 [a] | 10.33±1.16 [a] | 11.67±0.58 [a] |
| 100 | 12.33±1.53[a] | 12.67±0.58 [a] | 11.33±0.58 [a] | 13.00±0.00 [a] |
| 200 | 13.67±1.16[a] | 15.00±2.65 [a] | 18.33±4.16 [a] | 19.00±5.29 [a] |
| CAZ (30 µg) | 22.67±1.53[b] | 15.00±1.00[a] | 22.67±1.53[a] | 15.00±1.00[a] |
| AUG (30 µg) | 15.67±5.78 [a] | 10.67±0.58 [a] | 15.67±5.78 [a] | 10.67±0.58 [a] |

This test was conducted by the modified Kirby Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020).

The plant extracts and antibiotic discs were allowed to diffuse for about 30 minutes and then the plates were incubated for 18-24 hours at 37ºC. After incubation, the plates were observed for the formation of clear zones around the wells which corresponds with the diameters of the zones of inhibition. These diameters of the zones of inhibition (ZOI) were observed, measured and recorded in millimetres (mm).

**STATISTICAL ANALYSIS**

Data were analysed using the Statistical Package for Social Sciences (SPSS) version 20.0. The inhibition zones were calculated as means ± SEM. The statistical difference of the mean zones of inhibition of the extract and synthetic drugs for individual bacterium was carried out using the one-way analysis of variance (ANOVA) and values were considered significant at $P < 0.05$.

**RESULTS**

**ANTIBACTERIAL EFFICACY OF AQUEOUS AND METHANOLIC EXTRACTS OF GINGER (*ZINGIBEROF FICINALE)***

The antibacterial efficacy of aqueous and methanolic extracts of ginger against MDR *E. coli* and Salmonella *spp.* are presented in Table I.

At 200 mg/kg, the aqueous extract of ginger had the highest zone of inhibition against Salmonella (13.67±1.16) and *E.coli* (15.00±1.00) than that of the antibiotic; Amoxicillin-clavulanate (13.67±5.78 and 15.67±5.78 respectively).

Ceftazidime, on the other hand showed a higher mean zone of inhibition (22.67±1.53), which was significantly different ($P<0.05$) against Salmonella, but a much lower zone of inhibition (15.00±1.00) against the MDR *E.coli*.

Methanolic extracts showed more efficacy than the aqueous extracts. At 200 mg/kg, the zones of inhibition against Salmonella and *E. coli* were 18.33±4.16 and 19.00±5.29 respectively, but they did not differ significantly ($P<0.05$) from those of Ceftazidime.

**ANTIBACTERIAL EFFICACY OF AQUEOUS AND METHANOLIC EXTRACTS OF GARLIC (*ALLIUM SATIVUM*)**

Table II shows the antibacterial efficacy of aqueous and methanolic extracts of garlic against MDR *E. coli* and Salmonella *spp*.

Both extracts also showed concentration-dependent antibacterial efficacy towards both organisms.

Methanolic extracts showed stronger efficacy than the aqueous extracts.

The antibacterial activity of methanolic extracts of garlic at 200 mg/kg, against *E.coli* was greater ($P<0.05$) than against Salmonella *spp*., but was not significantly higher ($P>0.05$) than that of the synthetic antibiotic, Ceftazidime.

Aqueous extracts showed minimal activity against both microorganisms. Garlic showed the highest antibacterial activity than ginger and utazi.

**TABLE II. MEAN CONCENTRATION OF THE DIFFERENT ZONES OF INHIBITION (ZOI) OF AQUEOUS AND METHANOLIC EXTRACTS OF GARLIC AGAINST *SALMONELLA SPP AND ESCHERICHIA COLI***

| Garlic concentration (mg/kg) | Inhibition zones (mm) of Aqueous extracts | | Inhibition zones Methanolic extracts | |
|---|---|---|---|---|
| | ***Salmonella spp.*** | ***Escherichia coli*** | ***Salmonella spp.*** | **E*scherichia coli*** |
| 25 | 8.00±1.00[a] | 10.67±1.15[a] | 8.67±1.16[a] | 5.67±5.13[a] |
| 50 | 10.00±1.00[a] | 14.33±0.58[b] | 10.33±0.58 [a] | 11.33±1.15[a] |
| 100 | 11.67±0.58[a] | 16.33±1.16[b] | 12.00±1.00 [a] | 15.67±2.52 [a] |
| 200 | 13.67±1.16[a] | 24.67±0.58[b] | 14.00±1.00 [a] | 20.33±3.51 [b] |
| CAZ (30 µg) | 22.67±1.53[b] | 15.00±1.00a | 22.67±1.53 [b] | 15.00±1.00 [a] |
| AUG (30 µg) | 15.67±5.78 [a] | 10.67±0.58[a] | 15.67±5.78 [a] | 10.67±0.58 [a] |

**TABLE III: MEAN CONCENTRATION OF THE DIFFERENT ZONES OF INHIBITION (ZOI) OF AQUEOUS AND METHANOLIC EXTRACTS OF UTAZI AGAINST *SALMONELLA SPP AND ESCHERICHIA COLI***

| Utazi concentration (mg/kg) | Inhibition zones (mm) of Aqueous extracts | | Inhibition zones (mm) of extracts | |
|---|---|---|---|---|
| | ***Salmonella spp.*** | ***Escherichia coli*** | ***Salmonella spp.*** | **E*scherichia coli*** |
| 25 | 5.67±5.13[a] | 7.00±1.00[a] | 9.67±0.50[a] | 8.33±1.15[a] |
| 50 | 10.67±1.16[a] | 14.67±0.5[b] | 13.67±0.58[b] | 11.00±0.00[b] |
| 100 | 13.33±1.53[a] | 16.33±2.3[b] | 15.00±0.00[b] | 13.00±1.00[a] |
| 200 | 15.33±2.31[a] | 16.67±5.13[a] | 18.33±1.53[a] | 15.67±0.58[a] |
| CAZ (30 µg) | 22.67±1.53[b] | 15.00±1.00[a] | 22.67±1.53[b] | 15.00±1.00[b] |
| AUG (30 µg) | 13.67±5.78 [a] | 15.67±5.78[a] | 15.67±5.78[a] | 10.67±0.58[a] |

**ANTIBACTERIALEFFICACY OF AQUEOUS AND METHANOLIC EXTRACTS OF UTAZI (*GONGRONEMA LATIFOILUM*)**

The antibacterial efficacy of aqueous and methanolic extracts of Utazi against MDR *E. coli* and Salmonella *spp*. are presented in Table III. Again, both extracts showed concentration-dependent antibacterial activity towards both organisms, with methanolic extracts showing stronger antibacterial activity. At 200 mg/kg, the methanolic extract of Utazi had the highest zone of inhibition against Salmonella (18.33±1.53), but Ceftazidime had significantly greater activity (P>0.05) than the methanolic and aqueous extracts of Utazi. Methanolic extracts of Utazi showed more efficacy than the aqueous extracts.

**DISCUSSION**

The present study evaluated the antibacterial activity of methanolic and aqueous extracts of garlic, utazi and ginger against Salmonella and *E.coli*. The results demonstrated that all extracts exhibited some activity against the test organisms, indicating their potential as natural antimicrobial agents. All extracts showed a dose-dependent response, as all extracts had the highest antibacterial activity at 200 mg/kg. This finding is consistent with previous studies, which reported increased antimicrobial activity with increasing concentrations of plant extracts (Safithri *et al.*, 2011; Zakari*et al.*, 2023). The observed antibacterial activity can be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, allicin and saponins that have been reported to have antimicrobial properties by inhibiting enzyme activity and disrupting bacterial membranes (Al Sheikh *et al.*, 2020)

The aqueous and methanolic extract of the tested plants did not show statistically significantly different inhibitory activity against *E.coli* and Salmonella. This is consistent with the findings of Akani & Hakam (2020). Utazi exhibited the least antibacterial activity against the test organisms, while Garlic demonstrated a higher antibacterial activity, compared to Utazi and ginger. This agrees with previous studies by Safithri*et al.* (2011), Akani & Hakam (2020) and Zakari*et al.* (2023). In contrast, methanolic extracts exhibited higher efficacy against both *E.coli* and Salmonella, indicating a broader spectrum of antimicrobial activity. This

may be because the active ingredients are more soluble in methanol than in water, and it is in agreement with previous studies, which reported enhanced antimicrobial activity of methanolic extracts compared to aqueous extracts, due to their ability to solubilize a broader range of bioactive compounds (Cutler & Wilson, 2004). Aqueous extracts may not effectively solubilize non-polar bioactive compounds, such as allicin, a compound responsible for the antimicrobial effect of garlic (Verma *et al*., 2008). All extracts showed greater antibacterial activity against *E. coli* compared to Salmonella, this may be due to variations in cell wall composition and membrane permeability between the two bacteria. Comparative analysis with standard antibiotics (Ceftazidime and Amoxicillin/clavulanate) revealed that the inhibition zones of these antibiotics were higher than those of Utazi's methanol and aqueous extracts. However, the methanolic extracts of garlic and ginger showed comparable inhibition zones to the antibiotic. This suggests that garlic and ginger may be promising alternatives to conventional antibiotics.

## CONCLUSION

This study demonstrates the antibacterial potential of extracts of ginger, garlic and utazi, as natural antimicrobial agents, (especially the methanolic extracts) against Salmonella and MDR *E.coli*. The findings suggest that garlic methanolic extracts may be effective against these organisms, particularly *E.coli*. This work provides a foundation for further research to explore the therapeutic potential of these plant extracts.

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