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Original Research Article

Antibiotic-Potentiating Activity of Garlic and Turmeric Methanolic Extracts against Resistant *Escherichia coli* Isolated from Food and Water Samples

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Abstract: Antibiotic-resistant *E. coli* from food and water sources is a public health concern because it may contribute to the dissemination of resistance outside the clinical settings. This study investigated the antibacterial and antibiotic-potentiating activities of methanolic extracts of garlic (Allium sativum) and turmeric (Curcuma longa) on a resistant isolate of *E. coli*. Food and water samples were collected, cultured and screened for *E. coli*. The isolate was characterized by standard microbiological methods, and its resistance profile was determined by the disc diffusion method. Soxhlet extraction with methanol was used to prepare garlic and turmeric extracts. Antibacterial activity was determined by disc diffusion method with streptomycin and gentamicin alone and in combination with plant extracts. Also, a 96 well plate inhibition assay was performed, and bacterial growth was followed at OD600 for 13 hours. Streptomycin and gentamicin alone gave very weak inhibition zones of about 0–1 mm. Combination treatments, however, increased antibacterial activity. Maximum zone of inhibition was observed in turmeric extract with streptomycin (20 mm) followed by garlic extract with streptomycin (18 mm). The combination of gentamicin with turmeric and garlic gave inhibition zones of 14 mm and 12 mm respectively. In the 96-well assay, the lowest final absorbance was observed for the streptomycin–turmeric combination, indicating stronger growth inhibition. Overall, the results suggest that extracts of garlic and turmeric may enhance antibiotic activity against resistant *E. coli*. Further studies are necessary to confirm the mechanism of this potentiating effect.

Keywords: *E. Coli*, Antibiotic Resistance, Garlic Extract, Turmeric Extract, Streptomycin, Gentamicin, Disc Diffusion Assay, Inhibition Assay, Plant Extracts, Antibiotic Potentiation.

INTRODUCTION

Antimicrobial resistance is one of the major problems of present-day microbiology and public health. It happens when bacteria don't respond correctly to antibiotics that used to work [1]. Therefore, infections are hard to treat. This leads to more treatment failure, longer illness and more opportunities for resistant organisms to spread. The issue isn't restricted to hospitals. Resistant bacteria are also found in the environment, food, water, animals and in the community. Food and water can spread bacteria. Bacteria can enter the human body through improper food handling, contaminated drinking water and processing contamination. One of the most common organisms among these bacteria associated with contamination of food and water is *E. coli* [2, 3]. Many strains of *E. coli* are normal residents of the intestine of man and animals. But some strains can cause intestinal and extraintestinal infections. Also, the presence of *E. coli* in water or food samples is an important index of fecal contamination. The isolation of antibiotic-resistant *E-coli* from food and water has been an increasing concern. These isolates may have resistance traits and may serve as reservoirs to spread resistance genes. Several studies have reported resistant *E. coli* from drinking water and different food sources [4, 5].

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Antibiotics remain the primary treatment option for bacterial infections. Aminoglycoside antibiotics, such as streptomycin and gentamicin, have been utilized in the treatment of numerous bacterial pathogens. These antibiotics mainly interfere with bacterial protein synthesis [6]. However, when bacteria develop resistance, activity can be mitigated. Resistance to aminoglycosides may be due to several mechanisms including enzymatic modification of the drug, decreased uptake of the antibiotic, efflux activity or changes in the bacterial target site [7]. The rise of antibiotic resistance has led to a surge in research into alternative and adjuvant antibacterial strategies. Medicinal plants are one of the important resources of natural bio-actives. Many plants contain secondary metabolites such as phenolics, flavonoids, alkaloids, tannins, terpenoids and sulfur containing compounds. Such compounds may affect bacteria growth by disrupting the cell membrane, inhibiting enzymes, decreasing the formation of biofilm or disrupting the cell metabolism [8]. Plant extracts may not always replace antibiotics but may help improve antibacterial activity when used in combination with selected antibiotics.

Garlic (Allium sativum L.) is a commonly used medicinal plant with reported antimicrobial properties. Its antibacterial activity is mainly due to sulfur-containing compounds, especially allicin [9]. When garlic tissue is crushed or damaged, allicin is made. It can react with thiol-containing enzymes and proteins in microbial cells, which may disrupt essential cellular functions [10]. Previous studies have demonstrated that garlic and its bioactive compounds can act against both Gram-positive and Gram-negative bacteria. This makes garlic a potential candidate for testing against resistant bacterial isolates. Another important medicinal plant is Turmeric (Curcuma longa L.). It is commonly used as a spice and in folk medicine. Curcumin is the main bioactive component of turmeric and has been investigated for several biological activities including antibacterial effects [11].

The mechanisms of action of curcumin on bacteria may be different. These include membrane disruption, inhibition of virulence factors, oxidative stress and interference with biofilm formation. Due to these properties, turmeric extract may have beneficial activity against resistant bacteria, and may also support the action of antibiotics. Combining plant extracts with antibiotics is an area of increasing interest. Some natural products might make bacteria more susceptible to antibiotics. This effect can be due to weakening the bacterial cell envelope, reducing resistance mechanisms or increasing antibiotic entry into the cell [12]. A plant extract which has potentiating activity on an antibiotic may produce a larger zone of inhibition or decreased bacterial growth than the antibiotic alone. Such an effect is called antibiotic potentiating activity [13]. This study was hypothesised that garlic and turmeric methanolic extracts could enhance the antibacterial activity of streptomycin and gentamicin against resistant *E. coli*. It was also hypothesised that the extract–antibiotic combinations would cause greater inhibition than either the antibiotic or plant extract alone. The antibacterial properties of turmeric and garlic were expected to decrease bacterial growth and increase the inhibition zone against the resistant isolate in the combination treatment.

Materials and Methods

Fresh garlic bulbs (*Allium sativum* L.) and turmeric rhizomes (*Curcuma longa* L.) were collected for extract preparation due to their reported antibacterial properties [14, 15]. The plant materials were thoroughly washed with distilled water to remove dust and surface impurities. Samples were washed, cut into small pieces and dried in a shaded place at room temperature to complete dryness. The dried samples of garlic and turmeric were ground individually to a fine powder, using a clean grinder. The powder samples were stored in airtight containers before extraction. The solvent used for extraction was methanol. Soxhlet extraction was carried out with 100 g of each dried plant powder separately in a thimble and extracted with 500 mL of methanol. The extraction was done in a Soxhlet apparatus for 6–8 h or until the solvent in the siphon tube was nearly clear. The methanolic extracts were filtered through Whatman filter paper No. 1 after extraction [16]. Filtrates were concentrated on a rotary evaporator at low temperature to remove methanol. The concentrated extracts were dried, weighed and stored in sterile containers at 4 °C for further use. The dried extracts were dissolved in an appropriate solvent to prepare the desired working concentrations before antibacterial testing.

The percentage yield of each extract was calculated by the following formula:
Yield of extract (%) = Weight of dried extract Weight of dried plant powder × 100.

Isolation and Characterization of Bacterial Strains

Selected food and water samples were cultured and bacterial isolates were obtained. Samples were aseptically collected and transported to the laboratory for microbiological analysis under appropriate conditions. Standard culture techniques were used to isolate bacteria from the samples. Samples were inoculated on selective and differential culture media for the isolation of *E. coli* and incubated at 37 °C for 24 h. Suspected *E. coli* colonies were picked on the basis of colony morphology and further purified by subculturing. The purified isolates were identified by gram staining and standard biochemical tests [17].

The antibacterial experiment was conducted with confirmed isolates of *E. coli*. Prior to the main assay, the antibiotic resistance profile of each isolate was determined using the disc diffusion method [18]. Isolates were screened against selected antibiotics to determine their resistance pattern. Based on the resistance pattern, a resistant *E. coli* isolate was selected to be the test organism to evaluate the antibacterial activity of garlic and turmeric extracts alone and in combination with antibiotics.

Antibacterial Activity Assay

The antibacterial activity of garlic and turmeric methanolic extracts were evaluated against selected resistant *E. coli* isolate by using disc diffusion method. Mueller–Hinton agar plates were prepared and solidified under aseptic conditions [19]. A suspension of fresh bacteria was prepared and standardized to a standard turbidity. Sterile cotton swabs were used to spread the suspensions evenly over the surface of Mueller–Hinton agar plates.

Sterile paper discs were placed on the surface of the inoculated agar. The discs were impregnated with garlic extract, turmeric extract and selected antibiotics. The antibacterial activity of the test isolate was compared to the standard antibiotics, streptomycin and gentamicin. To observe the enhancement of antibiotic activity by the extracts, the extracts were applied in combination with the antibiotic discs for combination testing [20]. The plates were incubated at 37 C for 24 h. The antibacterial effect was determined after incubation by measuring the clear zones around the disks. The inhibition zone was measured in millimeters using a ruler or a digital caliper. The greater the inhibition zones, the greater the antibacterial activity; the smaller or absent the zones, the lower the sensitivity or the greater the resistance. The activity of extract alone, antibiotic alone and extract–antibiotic combinations was compared. An increase in the zone of inhibition in the combination group was taken as evidence of possible antibiotic-potentiating activity of the plant extract. All the tests were done in triplicate to ensure the reliability of the results.

Inhibition Assay Using 96-Well Plate

Inhibition assay was carried out in sterile 96-well microtiter plate to study the growth pattern of the selected resistant *E. coli* isolate in presence of streptomycin and plant extracts. A fresh bacterial culture was prepared and standardized for turbidity before use. Mueller–Hinton broth was added to each well, followed by the bacterial inoculum and the test treatments.

The test antibiotic used for the inhibition assay was streptomycin. Comparison of bacterial culture treated with streptomycin alone with untreated bacterial control Also tested were garlic extract and turmeric extract in combination with streptomycin to determine whether the extracts were able to enhance the inhibitory effect of the antibiotic. After the plates were prepared it was incubated at 37°C. The bacterial growth was measured by absorbance at 600 nm with a microplate reader. This wavelength is usually reported as OD600. This wavelength is usually used to measure bacteria turbidity and growth. Absorbance readings were taken over a 13-hour period to examine changes in bacterial growth as a function of time. The absorbance values of bacteria without treatment, bacteria treated with streptomycin and extract-streptomycin combination groups were compared. A lower absorbance value indicated less bacterial growth and a higher absorbance value indicated more bacterial growth. The assay was performed in triplicate and the mean absorbance values were used for analysis.

RESULT AND DISCUSSION

Antibacterial Activity of Garlic and Turmeric Extracts

The antibacterial activity of the methanolic extracts of garlic (Allium sativum) and turmeric (Curcuma longa) against the selected resistant *E. coli* isolate was determined using the disc diffusion method. This isolate was previously isolated from food and water samples and identified as *E. coli*. Also, before the antibacterial assay its antibiotic resistance profile was determined. The results showed that the extracts of garlic and turmeric had little antibacterial activity against the resistant *E. coli* isolate. Reduced activity was also observed with streptomycin and gentamicin alone, in agreement with the resistant nature of the isolate. However, the plant extracts, in combination with the antibiotics, showed an enhanced antibacterial response.

When compared to streptomycin alone, the turmeric extract–streptomycin combination displayed a wider inhibitory zone. Combining gentamicin with turmeric extract also showed a comparable effect. Although the activity of garlic extract was lower than that of turmeric extract, the combinations of garlic and antibiotics still shown some improvement over the extract alone.

In general, the combination treatments outperformed the individual plant extracts or antibiotics in terms of inhibition. This finding implies that extracts from garlic and turmeric may have antibiotic-potentiating properties against resistant *E. coli*. Under the tested conditions, turmeric may enhance the antibacterial action of streptomycin and gentamicin more effectively than garlic, as the effect was more pronounced in the turmeric extract combination (Table 1).

Table 1: Antibacterial activity of garlic and turmeric extracts with streptomycin and gentamicin against resistant *E. coli*

Treatment	*E. coli* inhibition zone (mm)
Garlic extracts alone	Weak activity
Turmeric extract alone	Moderate activity
Streptomycin alone	Reduced activity (~0-1 mm)
Gentamicin alone	Reduced activity (~0-1 mm)
Garlic extract + streptomycin	Increased activity (18 mm)
Garlic extract + gentamicin	Increased activity (12 mm)
Turmeric extract + streptomycin	Higher increased activity (20mm)
Turmeric extract + gentamicin	Higher increased activity (14 mm)

Values should be presented as mean from three independent replicates after final measurement.

Antibacterial Activity of Garlic and Turmeric Extracts with Antibiotics

The antibacterial activity of garlic and turmeric methanolic extracts was evaluated against the selected resistant *E. coli* isolate using the disc diffusion method. The results are shown in Figure 1. Panel a shows the activity of streptomycin alone and in combination with the plant extracts, while Panel B shows the activity of gentamicin alone and in combination with the plant extracts. The garlic extract alone showed weak antibacterial activity while the turmeric extract alone showed moderate activity against the resistant *E. coli* isolate. The isolate showed decreased sensitivity to streptomycin and gentamicin alone as they produced very small inhibition zones of about 0–1 mm. When plant extracts were combined with antibiotics, antibacterial activity was significantly enhanced. The combination of garlic extract and streptomycin has produced 18 mm inhibition zone, whereas the combination of turmeric extract and streptomycin has produced slightly larger zone of 20 mm. Garlic extract with gentamicin showed 12 mm inhibition zone and turmeric extract with gentamicin showed 14 mm inhibition.

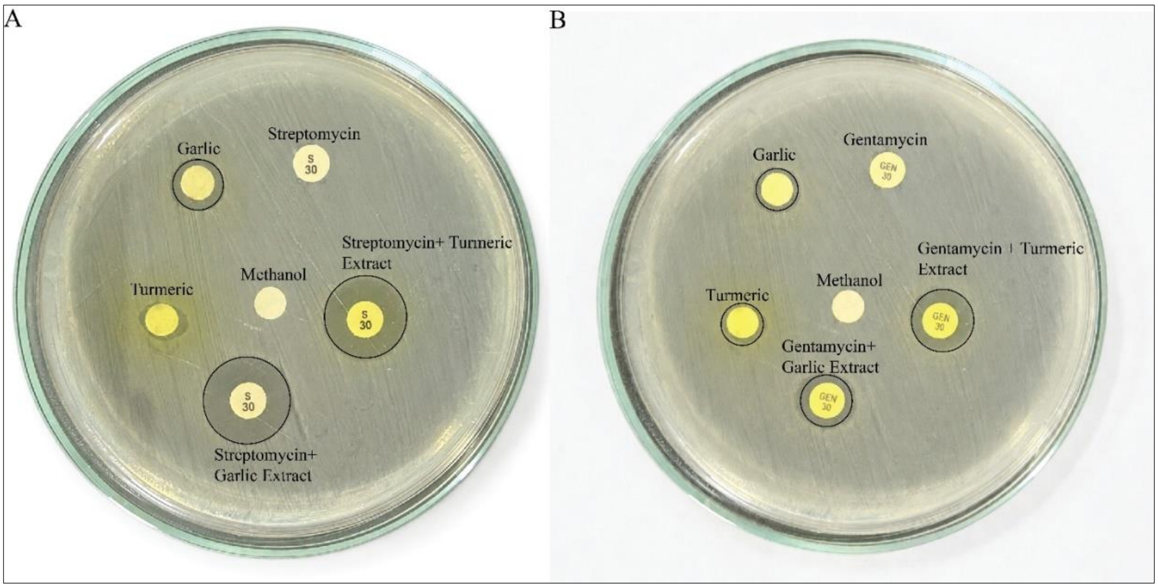

Figure 1: Antibacterial activity of extracts of garlic and turmeric with antibiotics against resistant *E. coli*. (A) Disc diffusion assay showing the activity of streptomycin alone and in combination with garlic and turmeric extract. (B) Disc diffusion assay showing activity of gentamicin alone and in combination with garlic and turmeric extracts. The larger inhibition zones in the combination treatments are indicative of improved antibacterial activity than antibiotics alone.

The highest antibacterial activity was exhibited by turmeric extract-streptomycin combination with inhibition zone of 20 mm in all treatments. For both antibiotics, the combinations of turmeric extracts were more active than those of garlic extracts. The results obtained indicate that the extracts of garlic and turmeric may enhance the antibacterial activity of streptomycin and gentamicin against resistant *E. coli*. The effect was stronger with turmeric extract, particularly when combined with streptomycin (Figure 1).

Inhibition Assay Using 96-Well Plate

The growth inhibitory pattern of the resistant *E. coli* isolate was studied by using a 96-well plate assay for 13 hours. Bacterial growth was followed by measuring absorbance at OD600. The outcome is depicted in Figure 2.

The bacterial control without treatment showed the highest growth during the assay. The absorbance increased from about 0.93 at 0 hour to 3.32 at 13 hours with the maximum value around 12.5 hours of about 3.42. This confirmed active bacterial growth without treatment.

The streptomycin treated group showed also a different increase in absorbance at 0 hour to 13 hours, from 0.55 to 2.71. Though this value was lower than the untreated bacterial control, the growth pattern showed that streptomycin alone was not able to completely inhibit the resistant *E. coli* isolate. Plant extract treated groups showed lower absorbance than the untreated bacterial control. The absorbance of turmeric extract treated bacteria was about 1.74 at 13 hours, while that of garlic extract treated group was about 1.48. These values indicate that both extracts reduced bacterial growth compared with the untreated control.

The strongest inhibition was observed in the combination treatment groups. The streptomycin–turmeric extract combination showed the lowest final absorbance among the treated groups, increasing only to about 1.14 at 13 hours. This represented about a 65.7% reduction in absorbance compared with the untreated bacterial control and about a 57.9% reduction compared with streptomycin alone. The streptomycin–garlic extract combination also reduced bacterial growth, with a final absorbance of about 1.43 at 13 hours. This was about 56.9% lower than the untreated bacterial control and about 47.2% lower than streptomycin alone.

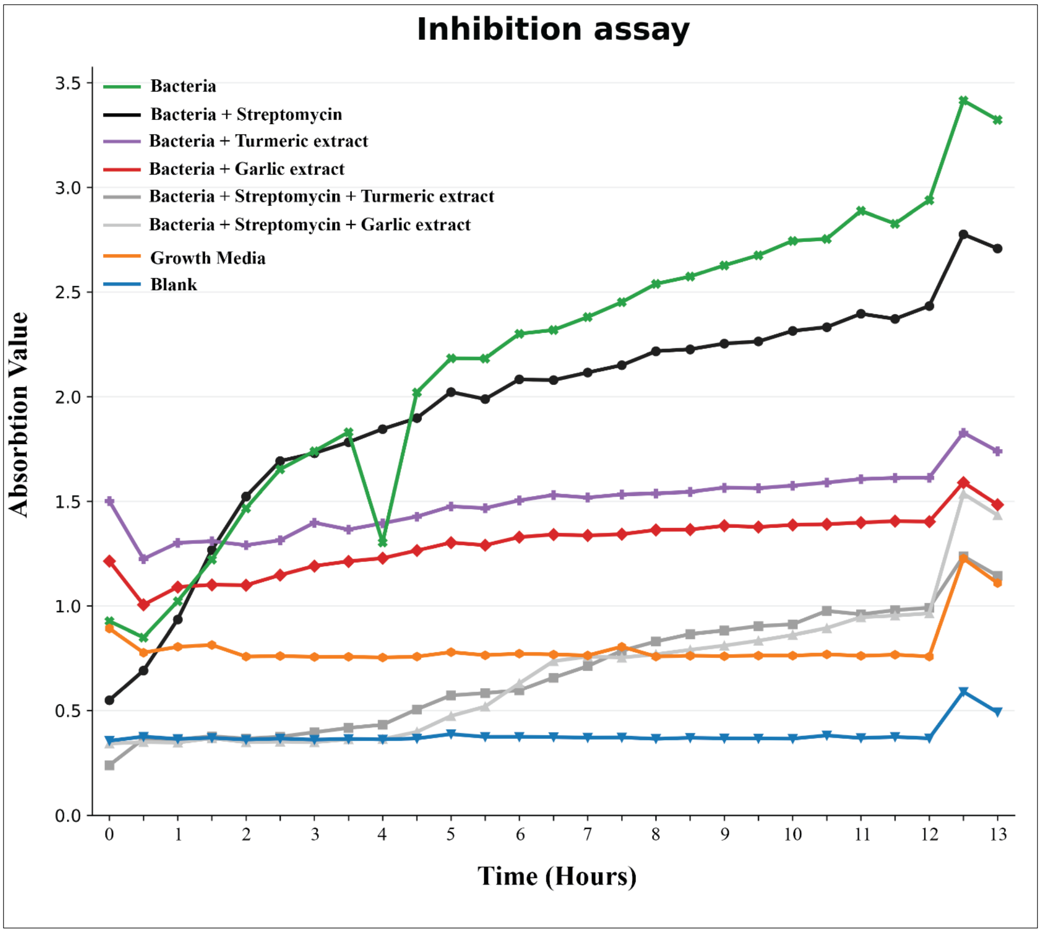

Figure 2: Growth inhibition assay of resistant *E. coli* using a 96-well plate Absorbance was measured at OD600 for 13 hours. The untreated bacterial control showed the highest growth, reaching an absorbance of approximately 3.32 at 13 hours. Streptomycin alone showed partial inhibition, with a final absorbance of about 2.71. The streptomycin–turmeric extract combination showed the strongest inhibitory effect, with the lowest final absorbance of about 1.14.

The growth media and blank controls remained comparatively low throughout the assay, with final absorbance values of about 1.11 and 0.49, respectively. Overall, the inhibition assay showed that streptomycin alone had limited activity against the resistant *E. coli* isolate. However, its activity improved when combined with plant extracts, especially turmeric extract.

DISCUSSION

The presence of antibiotic-resistant *E. coli* from food and water sources is a problem as it may be a pathway for community level spread of resistance [21, 22]. In the present study, garlic and turmeric extracts were tested as potential adjuvants to enhance the activity of selected antibiotics against resistant *E. coli*. The main purpose was to observe whether the combinations of plant extract and antibiotics could give better inhibition than antibiotics alone. Antibacterial activity was first screened by disc diffusion method using streptomycin and gentamicin. Also, a 96-well plate inhibition assay was performed and bacterial growth was followed for 13 h by measuring absorbance at OD600. The disc diffusion result indicated that streptomycin and gentamicin showed very low inhibition zones (around 0–1 mm). However, combination treatment enhanced the antibacterial response. Turmeric extract plus streptomycin showed the highest inhibition with a 20 mm zone followed by garlic extract plus streptomycin with an 18 mm zone. The same pattern was observed in the 96-well assay. The final absorbance of the combination of streptomycin and turmeric was the lowest, which indicated more growth inhibition than streptomycin alone.

These findings are consistent with previous reports that medicinal plant extracts could enhance the efficacy of antibiotics against resistant bacteria. It has been reported that organosulfur compounds present in garlic have antibacterial, antibiofilm and anti-quorum sensing properties [23]. Another study showed that Allium sativum extract inhibited a broad spectrum of bacteria, including multidrug-resistant strains, and the activity could be combined with conventional antibiotics [24].

The antibacterial activity of allicin and its related thiosulfate derivatives has also been associated with their ability to react with microbial proteins containing thiol groups and interfere with essential cellular functions [25]. These reports support the weak but useful antibacterial effect seen with garlic extract in the present study. The greater effect of turmeric found in this study is also consistent with previous findings. Turmeric's primary bioactive component, curcumin, has been shown to exhibit broad antibacterial activity and potential synergy with antibiotics against drug-sensitive and multidrug-resistant bacteria [26]. Tyagi *et al*., showed that curcumin damages the bacterial membrane, which may explain the inhibitory effect of the bacterial growth [27]. Other studies have also shown that curcumin can potentiate the activity of antibiotics against *E. coli* and other Gram-negative organisms when used in conjunction with selected antibiotics [28, 29]. Kali *et al*., [30], also reported an increase in the susceptibility of Gram-negative clinical isolates to aminoglycosides, including gentamicin, by curcumin. These findings are in agreement with the present result where turmeric extract improved the activity of both streptomycin and gentamicin. The limited activity of streptomycin and gentamicin alone may be due to aminoglycoside resistance mechanisms. Aminoglycoside resistance may result from decreased drug uptake, enzymatic modification of the antibiotic, or alteration of the target site [31, 32].

The small inhibition zones produced by streptomycin and gentamicin alone was expected as the chosen *E. coli* isolate was already resistant. However, the increased activity in the combination groups suggests that garlic and turmeric extracts may help to overcome some of this decreased sensitivity. Natural products can also synergize with antibiotics by disrupting the bacterial membrane, disturbing resistance pathways, or allowing antibiotics to enter bacterial cells [33]. The strength of the study is that the test organism was not a laboratory reference strain, but a resistant *E. coli* isolate from food and water samples from real life. This increases the relevance of the finding to environmental and food-borne antibiotic resistance. Another strong point is the use of both disc diffusion and 96-well inhibition assays. The disc diffusion method showed the visible zone of inhibition. The 96 well assay gave the growth data over time.

This study has also some limitations. One selected resistant isolate was used for main experiment. The active compounds in the garlic and turmeric extracts were not quantified. The exact mechanism of antibiotic potentiation was also not examined. Future work should include more resistant isolates of *E. coli* from different sources. MIC, MBC, checkerboard assay and fractional inhibitory concentration index should also be done to confirm that the interaction is really synergistic. Further phytochemical studies might help in identifying the active compounds responsible for the observed activity. Also molecular analysis of resistance genes would enhance the interpretation of the antibiotic response. To conclude, the study suggests that the methanolic extracts of garlic and turmeric have the ability to enhance the antibacterial activity of streptomycin and gentamicin against resistant *E. coli*. The effect was more pronounced with turmeric extract, especially in combination with streptomycin. These results suggest the possibility of using plant extracts as antibiotic potentiators, but for practical application further confirmatory studies are required.

CONCLUSION

The present study demonstrated that the methanolic extracts of garlic and turmeric can potentiate the antibacterial activity of streptomycin and gentamicin against a resistant *E. coli* isolate recovered from food and water samples. The antibiotics alone demonstrated very low activity, confirming the low sensitivity of the selected isolate. However, the combination of plant extracts and antibiotics showed larger inhibition zones in the disc diffusion assay and lower bacterial growth in the 96-well inhibition assay. The highest effect was shown by combination of turmeric extract with streptomycin followed by garlic extract with streptomycin among the combinations tested. The results suggest that garlic and turmeric extracts may have antibiotic-potentiating activity against resistant *E. coli*. The results also justify the potential use of plant extracts as supportive agents in antibacterial research. However, further studies with more bacterial isolates, MIC and MBC testing, checkerboard assay, phytochemical analysis and molecular detection of resistance genes are needed to confirm the mechanism and strength of the observed activity.

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