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Short Communication

Lycopene for Wound Infection: *In-Vitro* Susceptibility of Drug-Resistant Clinical Pathogens

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ABSTRACT

Wound infections caused by drug-resistant pathogens have limited treatment options, consequently the need to screen new antimicrobials. Phytochemicals have been adopted as sources for new antimicrobials. Consequently, the study aimed to estimate the antibacterial activity of lycopene phytochemical against drug-resistant wound pathogens: *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antibiotic susceptibility profiles of all the resistant clinical isolates to commonly administered antibiotics were determined using disc diffusion method. The drug-resistant Gram-negative and Gram-positive bacteria (both groups) isolated from infected wounds were investigated for their in-vitro susceptibility to the lycopene extracted from tomato (*Lycopersicon esculentum*) by agar-well diffusion assay and broth dilution method. Differences of mean zone of inhibitions (ZOIs), minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) values among the drug-resistant wound isolates were considered significant where $P < 0.05$. All isolates were resistant to Augmentin, Gentamicin, Ciprofloxacin and Streptomycin. In-vitro susceptibility to lycopene revealed the mean and standard deviation of the ZOIs for all isolates as 7.6 ± 3.49 mm. The studied lycopene showed bactericidal effects against all the drug-resistant bacteria tested. The MICs and MBCs of the wound isolates ranged from 50 and 125 µg/mL. The difference between the mean ZOIs, MICs/MBCs of both groups were not significant. This study demonstrated that lycopene has antibacterial activity against some drug-resistant wound isolates thus, offers a good alternative to existing treatment options for wound infections.

Keywords: Wound infection, Lycopene, Antibacterial activity, Drug-resistant pathogens, Agar-well diffusion assay.

Introduction

Wound infections caused by drug-resistant bacteria are a serious public health concern with the association in delayed wound healing, increased treatment cost and mortality.[1] Among Gram-positive bacteria, *Staphylococcus aureus* is one of the utmost predominantly isolated bacteria from wound whereas, *Pseudomonas aeruginosa* is mostly prevalent amongst gram-negative bacteria.[2] In Nigeria, the prevalence of wound infection caused by drug-resistant bacteria is quite high as compared to other parts of the world. The prevalence of drug-resistant wound pathogen is reported at 70.1%; with frequently isolated pathogens such as *S. aureus, P. aeruginosa, Proteus mirabilis, Klebsiella pneumoniae,*[3] and *Escherichia coli.*[4] The persistent rise of antimicrobial resistance in recent times impacts on wound infection.[5] Hence, it is imperative to explore new antimicrobials for treatment of wounds infected with drug-resistant bacteria.

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Phytochemicals are characteristic wide groups of organic substance present in plants. On the other hand, lycopene phytochemical is a red carotenoid pigment in nature. It belongs to the class Carotene. Lycopene is fat-soluble and naturally synthesized by plants.[6] Lycopene offers several advantages over conventional antibiotics as an alternative therapy for foodborne infections[7] but not reported for wound infection. As an alternative and complementary option, it has shown a range of antimicrobial properties. The antimicrobial activity of lycopene emanates from its highly lipophilic nature as a strict hydrocarbon which typically incorporates inside the lipid bilayer of cell membranes.[8] The incorporation alters the membranes' integrity (rigidity or permeability), which is critical for the growth of the bacterial cell. Furthermore, phytochemicals such as lycopene have reported antibiotic resistance altering properties among pathogenic bacteria.[9] Studies have evaluated their antibacterial activity against both drug-resistant Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria.

Tomato (*Lycopersicon esculentum*), an important agricultural commodity possesses the highest concentration of lycopene when compared to other sources. Consequently, serving as a significant contributor of carotenoids to human diet but not as alternative therapy for several infections including wound infection. Due to the paucity of research on antibacterial properties of lycopene against drug-resistant bacteria, the present study aim was an investigation of lycopene phytochemical activity against selected clinical strains of drug-resistant bacteria isolated from wound.

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Materials and Methods

Bacterial isolates and antibiotic susceptibility testing

The clinical bacteria isolated from wound (*E. coli*, *S. aureus*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa*) were collected from the Microbiology Laboratory of Edo State Hospital, Benin City, Nigeria. The bacterial isolates were inoculated on fresh nutrient agar and incubated at 37°C for 24 hours after which they were identified using morphological and biochemical tests. The disc diffusion method was used in this study for commercial antibiotics. The antibiotics used were: Augmentin (10 µg), Gentamicin (30 µg), Ciprofloxacin (30 µg), and Streptomycin (30 µg). The test inoculum was inoculated onto Mueller Hinton agar plates with sterile cotton swabs after preparation to 0.5 McFarland standards. Antibiotics were aseptically placed on the surface of the inoculated agar plates and incubated at 37°C for 18 hours. The Zones of inhibition were measured and recorded in millimeters, according to the Clinical and Laboratory Standard Institute (CLSI) guideline.[10]

Lycopene

The tomato lycopene was extracted using acetone extraction method of Simran and Vrinda.[11] One hundred grams (100 g) of tomato powder were subjected to extraction with 1 L of acetone (1:10) by means of shaker for 30 minutes. The extraction was performed at room temperature with light protection by using a dark amber coloured flask covering the vessel with aluminum foil. The extract was filtered and supernatant solvent was formerly evaporated using a rotary evaporator. The concentrated lycopene was assayed for purity by UV-Vis spectroscopy at 472 nm (maximum of absorption for lycopene).[12] The concentrated lycopene was further subjected to sterility test by inoculation on blood and potato dextrose agar. The inoculated blood agar plate was incubated for 18 - 24 hours at 37°C while the potato dextrose agar was incubated for 5 days at 25°C. The concentrated extract was stored at 4°C till further analysis.

Antibacterial activity assay

The lycopene extract was prepared into different concentrations: 25 µL, 50 µL, 75 µL and 100 µL and tested against each bacterial isolate. The zone of inhibition (ZOI) diameter in millimeter (mm) was measured for the different concentration. Inoculum was prepared by direct colony suspension of 24 hours broth culture of bacteria isolates diluted with a small volume of sterile distilled water in plain test tubes and emulsified with a vortex after which the mixture was centrifuged at 4000 rpm for 5 minutes and the suspension adjusted to a concentration of 10^8 CFU/ml to match the McFarland standard using a spectrophotometer at 600 nm.

The antibacterial activity of the lycopene was evaluated by means of agar-well diffusion assay with different concentration of the prepared extract dispensed on Muller-Hinton agar. Approximately 0.01 mL of the prepared inoculum was inoculated unto agar plate using spread plate method. Antimicrobial activity was estimated by measuring the diameter of the zone of inhibition (ZOI) round the wells and recorded in millimeter (mm). Determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of the extracted lycopene was done using agar broth dilution method with 100 µL of Mueller Hinton (Sigma-Aldrich) broth and serially diluted with 10 µL of bacterial inoculums (10^6 CFU/mL). Lycopene was diluted in concentrations (10 µg/ml to 500 µg/ml) using Muller Hinton broth. All the tubes were incubated at 37^0C for 24 hours. Tubes containing Mueller Hinton Broth and 100 µl of inoculum served as the negative control. The MICs of each extract were detected after 18 hours of incubation at 37°C. The MICs were determined at the concentration of lycopene which absolutely inhibited the bacterial growth. Whereas, MBC was calculated as the lowest concentration of lycopene (the broth dilution of MIC) which killed the bacterial culture within 18 – 24 hours. The assay was done independently in triplicates.

Statistical analysis

Statistical Package for Social Science (SPSS 21.0) was used for data analysis in this study. The mean values for the ZOIs and MICs of tested lycopene against drug-resistant clinical isolates of wound and the standard deviation values were calculated. Fisher exact test was used to determine differences of mean ZOI values among the drug-resistant wound isolates. The result was considered significant where $P < 0.05$.

Results and Discussion

Estimation of the effect of lycopene extract on drug-resistant clinical isolates

In the present research, the activity of lycopene against drug-resistant bacterial strains of 5 species was tested at different concentration (25 µL, 50 µL, 75 µL and 100 µL). This phytochemical demonstrated activity against all 5 drug-resistant bacteria isolated from wound. The drug-resistant bacteria had resistance zone of inhibition (ZOI) for Augmentin ranged from ≤ 35 mm, for Streptomycin ranged from ≤ 19 mm, for Gentamicin ranged from ≤ 19 mm, for Ciprofloxacin ranged from ≤ 21mm. An evidently stronger antibacterial activity of lycopene against Gram-negative than Gram-positive bacteria was observed. The mean ZOIs for Gram-positive bacteria were between 0.0 and 5.3 mm, while for Gram-negative ranged from 0.0 to 17.2 mm. The difference between the mean ZOIs of both groups were not significant (p = 0.059). The highest lycopene concentration activity in the agar well-diffusion method against *S. aureus* (mean ZOI 5.3 mm) was 75 µl (Table 1). Simultaneously, lycopene activity was found to increase consecutively with each concentration against *P. aeruginosa* (mean ZOI 3.1 – 9.0 mm), *K. pneumoniae* (mean ZOI 0.0 – 7.2 mm), *E. coli* (mean ZOI 5.0 - 10.1 mm) and *P. mirabilis* (mean ZOI 0.0 - 17.2 mm).

In this paper, the activity of lycopene against 5 drug-resistant bacteria isolated from wound was presented. These bacteria were resistant to four commonly used antibiotics for the treatment of wound infection. To the best of our knowledge, this is the first work from Nigeria in which the effect of lycopene (extracted from tomato) on such drug-resistant wound bacteria has been studied. Moreover, this is the first research on lycopene, in which the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were tested against drug-resistant wound pathogens.

In our study, the zone of inhibition (ZOI) for the drug-resistant bacteria: *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *P. mirabilis* were highest with 7.2, 10.1, 9.0 and 17.2 mm respectively at the concentration of 100 µl. Unlike, *S. aureus* which had the highest ZOI at 75 µL concentration. Contrary, Kavitha *et al*., 2017 [13] examined lycopene isolated from tomato at the concentration 25 to 100 µL tested against food pathogens with varied ZOIs. Thus, the used concentrations were similar to our study. Nevertheless, in our study the purity and concentration of the original lycopene extract were of the standard rule. Unfortunately, in the above-mentioned article, the methodology for extraction of the active compound was different from ours with the purity and concentration of the extract not tested. These authors tested the activity of methanol extract of lycopene against *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus*. The zones of growth inhibitions for the above bacteria were 4.0–19.0 mm in the concentration of 25 to 100 µl of the methanol extract (non-purified, non-concentrated lycopene extract), and the result did not indicate the test bacteria were drug- resistant. The ZOIs were slightly high. In our study, mean ZOIs amounted to 7.6 mm for all the drug-resistant bacteria using purified concentrated lycopene.

In 2014, the antibacterial activity of lycopene was reported by Natividad and Rafel[14] using disc diffusion method. The carotenoid was extracted from tomato (*Solanum Lycopersicum* L.) and tested against two human pathogens (*E. coli* and *S. aureus)*. The extract showed no ZOIs for *E. coli*; however presented an antibacterial activity against *S. aureus* with the highest ZOI against *S. aureus* (7.50 mm) resistant to Streptomycin (34.67 mm). Somewhat similar to our results, *S. aureus* had ZOI of 5.3 mm resistant to Streptomycin (18.00 mm). But differ in the case of *E. coli* which had highest ZOI of 10.1 mm resistant to Augmentin (8.00 mm), Streptomycin (0.0 mm), Gentamicin (10.00 mm) and Ciprofloxacin (6.00 mm). This difference could have been attributed to the difference in the methodology employed in antibacterial assay. Earlier in 2012, Unnisa *et al*.,[15] reported the

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antimicrobial activity of aqueous and ethanolic extract of tomato against wound isolates: *S. aureus*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* with ZOI of 8.0-10.0 mm, 4.0 - 6.0 mm, 6.0 - 8.0 mm and 4.0 – 5.0 mm respectively. These isolates also showed resistance to Streptomycin ≤ 22 mm, Ciprofloxacin ≤ 30 mm, Gentamicin ≤ 24 mm. Due to the low antibacterial activity of both extracts of tomato, MIC was not conducted. Similar to our study, is the ZOI for *P. aeruginosa* and *E .coli*; still our MIC and MBC results further demonstrated the antibacterial activity of lycopene.

Minimum inhibitory concentration and Minimum bactericidal concentration of the extracted lycopene

Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for Gram-positive bacteria was 75 µg/ml, while for Gram-negative, it varied between 50 to 125 µg/ml. The MICs/MBCs of both groups had no significant difference ($p = 0.071$). The studied lycopene showed bactericidal effects on all the drug-resistant wound bacteria tested. The obtained values of the MICs and MBCs are presented in Table 2. Additionally, the agar broth dilution assay was employed in our study to determine the MIC and MBC of lycopene with concentration ranging from 10 to 500 µg/mL. The results for the MIC and MBC ranged from 50 and 125 µg/mL. Azadeh *et. al*., 2016 [16] demonstrated the MIC and MBC of *Lycopene oleoresin* (tomato skin) against *P. aeruginosa* (ISIRI 275) and *E. coli* (PTCC 1533) of food pathogens using serial micro-dilution method. The MIC and MBC ranged from 156.25 ppm to 2500 ppm. The antimicrobial activity was demonstrated by hexane:ethanol:acetone method for lycopene extraction from tomato skin, and their results were not reported according to Clinical Laboratory Standard Institute guideline. Contrary, in our study, our MIC and MBC results were reported according to Clinical Laboratory Standard Institute guideline (µg/ml); besides the organisms in the above mentioned article were from different sources.

In the case of wound infection caused by Gram-positive bacteria, *S. aureus* as a normal flora of the skin have the highest recovery rate from wound.[2] The antimicrobial activity against both Gram-negative and Gram-positive bacteria observed in our study conquered the structural differences that exist in their cell wall. The possible mechanisms of antibacterial activity of phytochemicals includes: inhibition of cell wall formation, inhibition of nucleic acid synthesis and membrane disruption.[17]

Table 1: Antibacterial activity of Lycopene against drug-resistant clinical pathogens

Bacteria	Antibacterial activity of Lycopene in terms of Zone of inhibition (ZOI) in mm			
	25 µL	50 µL	75 µL	100 µL
Staphylococcus aureus	0.0 ± 0.0	0.0 ± 0.0	5.3 ± 1.1	0.0 ± 0.0
Escherichia coli	5.0 ± 0.87	6.1 ± 1.68	8.0 ± 1.32	10.1 ± 0.87
Pseudomonas aeruginosa	3.1 ± 0.60	6.1 ± 3.79	7.0 ± 2.71	9.0 ± 2.48
Proteus mirabilis	0.0 ± 0.0	9.3 ± 0.58	0.0 ± 0.0	17.2 ± 0.68
Klebsiella pneumoniae	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	7.2 ± 2.00

Values are mean of three replicates ± standard deviation.

Table 2: Minimum inhibitory concentration (MIC) of lycopene for drug-resistant clinical isolates

Bacteria	MIC of Lycopene (µg/mL)
Staphylococcus aureus	75[a]
Escherichia coli	50/65[a]
Pseudomonas aeruginosa	125[a]
Proteus mirabilis	85[a]
Klebsiella pneumoniae	95[a]

[a]MIC including the MBC

Conclusion

This study showed the *in vitro* susceptibility of drug-resistant wound bacterial pathogens of clinical importance to lycopene. There were no significant differences in the susceptibility levels between drug-resistant Gram positive and Gram negative bacterial wound isolates to lycopene. Consequently, our result suggest that lycopene is a potential antibacterial agent against drug-resistant wound pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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