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ANTI-OXIDANT AND ANTI-MICROBIAL EVALUATION OF LYCOPENE ISOLATED FROM WATERMELON

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ABSTRACT : The present study was conducted to isolate lycopene from watermelon and evaluate its antibacterial effects on pathogenic strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The work was also aimed at assessing the isolated lycopene (ILP) antioxidant activity. Agar well diffusion test was utilized to unveil the antibacterial activity of three concentrations (25, 50 and 100mg/ml) of the ILP. Furthermore, DPPH and H_2O_2 scavenging activity assays were employed to uncover the antioxidant effects of ILP. For *S. aureus*, at 100mg/ml ILP, wide inhibition zones were revealed with no significant (*p*Ã0.05) differences with those from the 10µg amoxicillin positive control (AoPC). For *E. coli* at 50mg/ml ILP, high reads of inhibition zones were recorded with no significant (*p*>0.05) differences with those belonged to the 10µg AoPC. In the case of *E. coli*, at 100mg/ml ILP, the inhibition zones showed large diameters with no significant (*p*>0.05) differences with those of 10µg ampicillin positive control (ApPC). Regarding *P. aeruginosa*, at 100mg/ml ILP, wide zones of inhibition were seen with no significant (*p*>0.05) differences with those reads from 5µg ciprofloxacin positive control (CfPC). The scavenging activities of the ILP recorded high reads and were similar to those from the positive control, ascorbic acid (AA). In conclusions, Lycopene exerts high inhibitory activity against the pathogenic bacteria especially when used at 50 or 100mg/ml and they were similar to those from the positive controls. In addition, the recorded high antioxidant activities of lycopene may provide a possibility for using it not only as a replacement for some antibiotic, but also as an antioxidant compound.

Key words : Antibiotic resistance, antioxidant, lycopene, oxidative stress.

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INTRODUCTION

Resistant bacteria are emerging so quickly in the planet, undermining the efficacy of antibiotics which have changed medication and saved millions of lives. After several decades of antibiotic therapy of the first cases, bacterial infections are again a big challenge to human beings and to these drugs. The problem of antibiotic resistance is linked to the massive use and abuse of such products, as well as the absence of new pharmaceutical product development due to decreased economic opportunities and demanding safety standards (Gould and Bal, 2013; Wright *et al*, 2014; Lushniak, 2014; Viswanathan, 2014; Hawthorn *et al*, 2018; Charifson and Trumble, 2019).

The antibiotic history was first built up in 1928 with the uncovering the presence of penicillin by Sir Alexander Fleming. Since then, antibiotics have incorporated and saved millions of lives through modern medicine. The earliest therapy for dangerous diseases in the 1940s was recommended with antibiotics. The bacterial infections of World War II soldiers were treated by Penicillin effectively. Unfortunately, penicillin resistance soon became a major health concern in the 1950s, endangering other developments in the previous decade. The solution was to find, create, and deploy new beta-lactam antibiotics to restore faith in the use of these medicines. During the 1960s, in the United Kingdom and the United States, though, the first outbreak was detected with the methicillin-resistant Staphylococcus aureus (MRSA) (Piddock, 2012 and Ventola, 2015). Regrettably, almost all antibiotics produced have finally been resisted. In 1972, in clinical practices for both *S. aureus* and coagulase-negative staphylococci, vancomycin was incorporated for the therapy of resistant bacteria. Vancomycin resistance was thought that it was so hard to be stimulated and so in the clinical environment. In coagulase negative Staphylococci in 1979 and 1983, nevertheless, cases of vancomycin resistance were observed. In the late 1960s

to the early 1980s, several new drugs were released by the pharmaceutical companies to tackle the issue of resistance; however, the parallel increase in the emergence of resistant bacteria have kept going.

Oxidative stress is another health issue that can be induced due to different factors. Antibiotics can be a major influential factor that enhance the production of reactive oxygen species (ROS) and promote mitochondrial dysfunction (Kalghatgi *et al*, 2013). The oxidative stress can lead to many health conditions that are underlined by the presence of mutation into cancerous cells and apoptosis (Reuter *et al*, 2010). According to these risks induced by the use of antibiotics, finding novel agents, especially of herbal nature, may enhance the way to defeat the problems of antibacterial resistance and oxidative stress. In the present work, we used lycopene to estimate its suitability to be as an alternative to antibiotics by screening its antibacterial actions against pathogenic strains of *S. aureus*, *E. coli* and *P. aeruginosa*. Moreover, the compound was also used for evaluating its antioxidant abilities. Lycopene can be found in watermelon, tomatoes, papaya, guava, and red grapefruits (basically in red-colored fruits and vegetables. The substance is characterized by its lipophilic and unsaturated carotenoid chemical features (Mozos *et al*, 2018).

MATERIALS AND METHODS

Watermelon collection and preparation

The fresh red watermelon fruit (*Citrullus lanatus*) was purchased from local market in Al-Diwaniyah City and transported to the laboratory. Then, the watermelons were washed under running stream of water to remove any contaminants on the rind. The external rind was removed by a sterile knife until appearing the red pulp. After that, the seeds were removed from the red pulp. Following that, the seedless red pulps were cut to small pieces and grinded by electrical blender. Finally, the blend was passed through a 500-micron stainless-steel mesh sieve and saved in a clean container at -8^{0}C until use in the extraction process.

Soxhlet apparatus extraction

The extraction of lycopene was performed using a Soxhlet of powdered freeze-dried watermelon (PFDW). In brief details, 5gm of PFDW was placed in a porous cellulose thimble for soxhlet extraction. The flask that contained the solvent was heated at boiling point 77°C. Then, the content was let to evaporate into the condenser of apparatus to be a liquid accumulating into the extraction chamber that contained the sample. The extraction was lasted for 12hrs using 150ml of ethyl acetate. After extraction, the solvents were removed utilizing a vacuum-rotary evaporator which was then ended up by weighing the final products. The crystallization of ILP was carried out by adding suitable anti-solvent (100ml of methanol) to the crude ILP leaving ILP as a precipitant within several minutes. The ILP was stored at -20°C until conducting the remaining analyses. The procedures of the lycopene recovery were performed within 2hrs.

Antibacterial activity

Agar well diffusion test

Agar well diffusion test was utilized to unveil the antibacterial activity of three concentrations (25, 50 and 100mg/ml) of the ILP. Each cell suspension concentration of *S. aureus*, *E. coli*, or *P. aeruginosa* was adjusted to the 0.5 McFarland standard turbidity (Muli *et al*, 2007). Then, 50µL of each suspension was spread on a Mueller-Hinton agar plate separately. Each 5-mm well was filled with 100µl of the each ILP concentration followed by incubating the plates at 37°C for 24hrs. The zones of inhibition were measured using a caliper. AoPC (10µg), ApPC (10µg) and CfPC (5µg) were used and 99% hexane was employed as a negative control. Each experiment was performed in triplicates (Doro *et al*, 2016).

Minimum inhibitory concentration

A micro-dilution method was employed in 96-well micro-titer plates to identify the minimum inhibitory concentration (MIC) of ILP against the tested bacteria according to the method described by Eloff (Eloff, 1998). Briefly, 100µl nutrient broth and a 10µl of 24hr suspension of each bacterium were mixed. The step was followed by adding 50µl ILP and 40µl of sterile water to each well making the final volume of 200µL. The ILP concentrations (0.039, 0.078, 0.156, 0.312, 0.624, 1.248, 2.5, 5, 10 and 20µg/ml) were used. The plates were incubated at 37°C for 24hrs. For color indicated results, 10µl of 125mg/ml MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was used.

In vitro antioxidant activity

DPPH scavenging activity assay

The ILP effects on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was studied using the method described by Shen *et al* (2010). This method depends on reducing DPPH in a methanol solution in the presence of a hydrogen donor represented by an antioxidant due to the formation of the non-radical form; DPPH-HA. Briefly, methanol solution of 0.1mM DPPH was prepared, and 0.5mL of this solution was added to 0.5ml of different concentrations of cyclohexane-based ILP each one alone (12.5, 25, 50, 100, 200, 400 and 800µg/ml). The mixture

was then vortexed thoroughly and left to react at room temperature for 30mins. The reads were performed at 518nm using a UV-VIS spectrophotometer. AA was used as antioxidant positive control. The ILP-based DPPH scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (% inhibition) = {(Abs0 –Abs1)/Abs0) × 100}

Abs0: Absorbance of the control reaction

Abs1: Absorbance of the ILP concentration and reference.

A triplicate based analysis was followed.

H_2O_2 radical scavenging activity

Hydrogen peroxide radical scavenging activity was estimated by the method described by Ruch *et al* (1984). In brief, a 40mM H_2O_2 solution in phosphate buffer (pH 7.4) was prepared. Different ILP concentrations (12.5, 25, 50, 100, 200, 400, 800µg/ml) were added to 0.6ml of the H_2O_2 solution. A wave length at 230nm was used. The phosphate buffer (pH 7.4) alone was used as a blank solution. AA was used as a positive control. The activity was calculated using the following equation:

H_2O_2 radical scavenging activity (%) = [{Ao – A1/Ao}] ×100

Ao: Absorbance of the H_2O_2

A1: Absorbance of the presence of the extract in H_2O_2 solution.

Statistical analysis

All experiments were performed in triplicates. The data were expressed as the mean ± SEM. IC_{50} values were determined by interpolation. These results were then compared using Analysis of Variance (ANOVA) and *P* values at > or <0.05 was considered. Duncan post-hoc analysis was followed. No significant differences between the ILP concentration and the positive control (bacterial-species column-based comparison) indicate similar potency of antibacterial effectiveness.

RESULTS

Antibacterial activity

For *S. aureus*, at 100mg/ml ILP, wide inhibition zones were revealed with no significant (p>0.05) differences with those from the 10µg AoPC (Table 1 and Fig. 1).

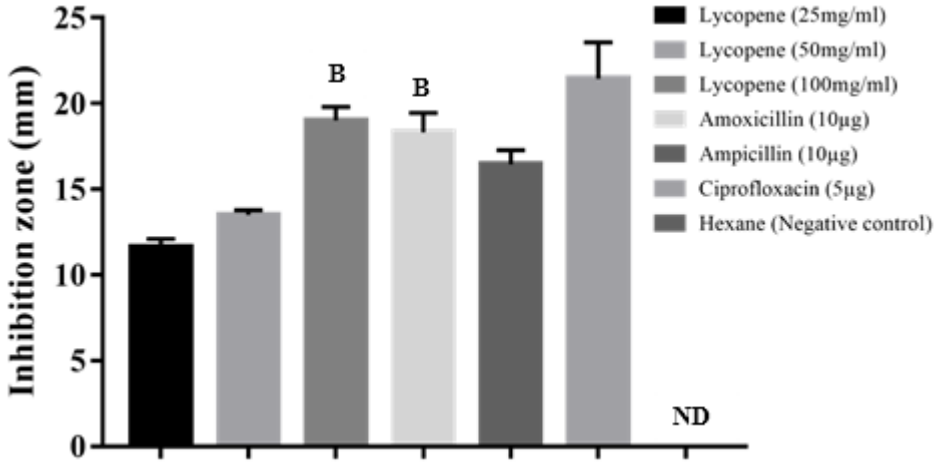

Fig. 1 : Agar well diffusion test (inhibition zones) of lycopene (ILP), antibiotic positive controls, and hexane against *S. aureus*. Similar letters indicate similar potency of the lycopene concentration and the positive control. ND: non detectable (hexane).

Table 1 : Antibacterial activity of the isolated lycopene in cultivating media.

Lycopene and antibiotics		Bacterial species		
		S. aureus	***E. coli***	***P. aeruginosa***
Lycopene	**25mg/ml**	11.62 ± 0.47^{E}	10.87 ± 0.51^{D}	7.5 ± 0.50^{D}
	50mg/ml	13.5 ± 0.28^{D}	12.62 ± 0.23^{C}	11.5 ± 0.28^{B}
	100mg/ml	19 ± 0.81^{B}	15 ± 0.41^{B}	13.75 ± 0.25^{A}
Standard antibiotics	**Amoxicillin 10µg**	18.32 ± 1.12^{B}	12.5 ± 0.98^{C}	0 ± 0^{E}
	Ampicillin 10µg	16.45 ± 0.84^{C}	15.33 ± 1.24^{B}	9.58 ± 1.45^{C}
	Ciprofloxacin 5µg	21.45 ± 2.12^{A}	18.24 ± 0.56^{A}	14.12 ± 2.08^{A}
Negative control	**Hexane**	0 ± 0^{F}	0 ± 0^{E}	0 ± 0^{E}

*Similar letters indicate similar potency of the lycopene concentration and the positive control (bacterial-species column-based comparison).

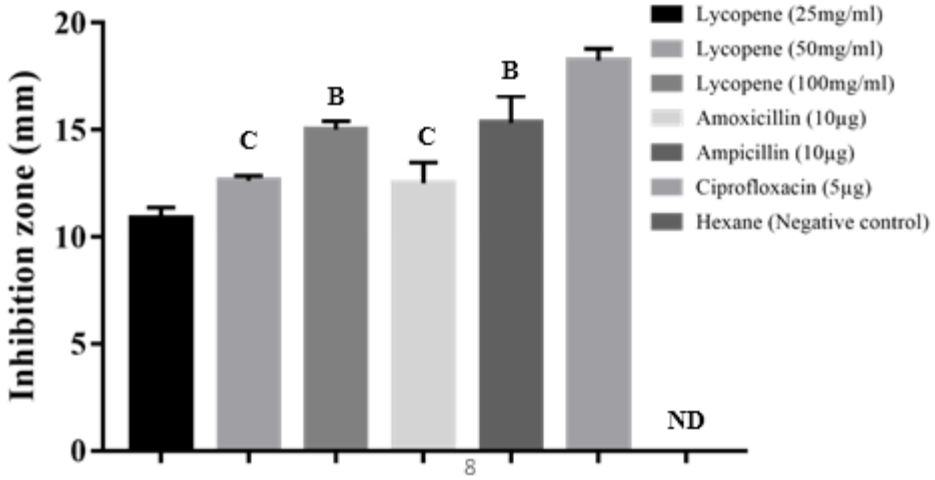

Fig. 2 : Agar well diffusion test (inhibition zones) of lycopene (ILP), antibiotic positive controls, and hexane against *E. coli*. Similar letters indicate similar potency of the lycopene concentration and the positive control. ND: non detectable (hexane).

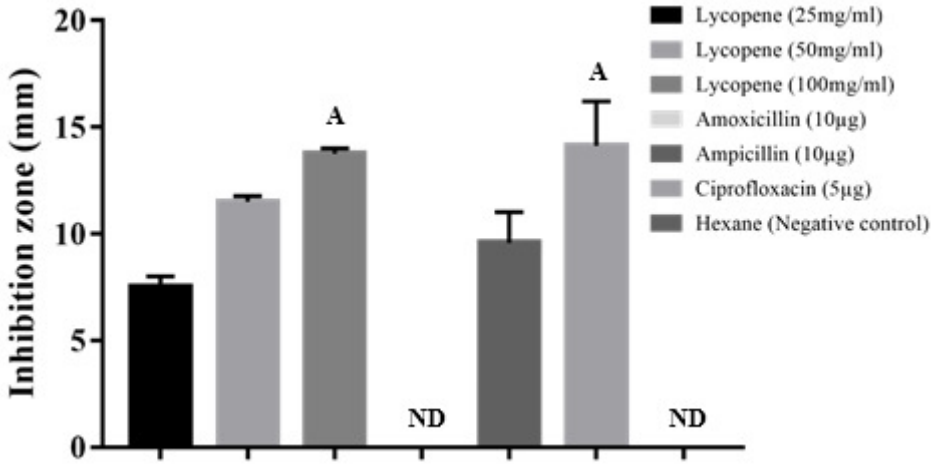

Fig. 3 : Agar well diffusion test (inhibition zones) of lycopene (ILP), antibiotic positive controls and hexane against *P. aeruginosa*. Similar letters indicate similar potency of the lycopene concentration and the positive control. ND: non detectable (amoxicillin and hexane).

Table 2 : The MIC of the ILP against bacterial species.

Bacterial species	MIC value (µg/ml)
S. aureus	0.624
E. coli	2.5
P. aeruginosa	5

Table 3 : IC_{50} of the isolated lycopene and standard drug (Ascorbic acid).

Substance	DPPH assay	H_2O_2 assay
Lycopene	7.989	9.115
Ascorbic acid	22.65	28.811

For *E. coli*, at 50mg/ml ILP, high reads of inhibition zones were recorded with no significant (p>0.05) differences with those belonged to the 10µg AoPC. In the case of *E. coli*, at 100mg/ml ILP, the inhibition zones showed large diameters with no significant (p>0.05) differences with those of 10µg ApPC (Table 1 and Fig. 2).

Regarding *P. aeruginosa*, at 100mg/ml ILP, wide zones of inhibition were seen with no significant (p>0.05) differences with those reads from 5µg CfPC (Table 1 and Fig. 3).

The MIC of the ILP for each bacteria is shown in Table 2.

The scavenging activities of the ILP recorded high reads and were similar to those from the positive control, AA, Fig. 4A (DPPH) and B (H_2O_2).

The IC_{50} of the ILP is displayed in Table 3.

DISCUSSION

Due to the continuous high increases in the emergence of the antibiotic-resistant bacteria and because the side effects that these antibiotics can induce to the human and animal body systems, researchers around the world keep studying a huge number of compounds especially those of herbal nature to use them as alternatives to antibiotics. Moreover, discovering candidate drugs with multiple purposes such as fighting bacterial microorganisms and scavenging ROS generated by oxidative stress. Here, the presence work obtained

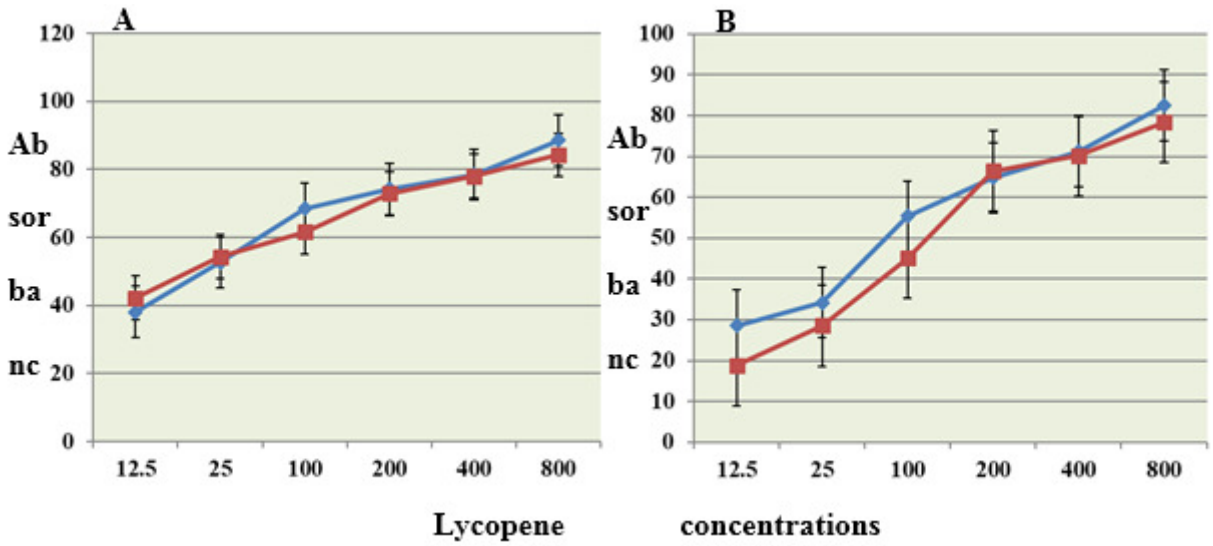

Fig. 4 : *In vitro* free radical scavenging activities of lycopene (ILP). **A.** DPPH. **B.** H_2O_2.

successful results on both directions when lycopene was used.

The bacterial species tested in the current investigation were previously isolated from different infections of human and animals. ILP showed a major antibacterial effect against these bacteria especially at 50 and 100mg/ml concentrations. This was confirmed due to the same degree of antibacterial effectiveness induced by the standard antibiotics used in the current work. It was recommended that according to the presence of lycopene as a main constituent of tomato peels pharmaceutical and food industries can use these peels to fight against different health issues such as antibacterial resistance (Szabo *et al*, 2019). It was mentioned that lycopene may have important antibacterial activity against periodontal infections (Gupta *et al*, 2015). The actions of lycopene against bacterial infections could be direct and indirect.

Lycopene might directly cause death in bacterial species via probable disruption effects of bacterial electron transfer chain, especially, when some compounds may provide their effects using the same scenario. On such event, Jeon *et al* (2019) found that 2-aminoimidazoles induce destruction in the mycobacterial electron transport chain. This is can be true for lycopene that has its quenching activity of free radicles by transferring the energy from these active radicles to lycopene that forms an energy-rich triplet state (Wetz *et al*, 2004; Islamian and Mehrali, 2015). To this point, the indirect antibacterial effect can be maintained by scavenging these free radicles generated in the affected tissues due to bacterial infections (Novaes *et al*, 2019).

The present experimental study showed high capability of ILP in scavenging free radicles such as DPPH and H_2O_2. When the high energy molecule of lycopene is formed, it traps the free radicles leading to destroying the oxidative components. For that, lycopene may help to prevent oxidative-based damages of cellular molecules such as lipids, proteins, and DNA (Stahl and Sies, 2003). It was recommended that daily consumption of tomato may increase the uptake of lycopene and decrease the Fe^{2+}-based DNA damage (Bohm *et al*, 2001; Stahl and Sies, 2002).

CONCLUSION

Lycopene exerts high inhibitory activity against the pathogenic bacteria especially when used at 50 or 100mg/ml, and they were similar to those from the positive controls. In addition, the recorded high antioxidant activities of lycopene may provide a possibility for using it not only as a replacement for some antibiotic but also as an antioxidant compound.

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