STUDY THE EFFECT OF LYCOPENE EXTRACT AND NANO-LYCOPENE EXTRACTED FROM TOMATO WASTE IN INHIBITING MICROBIAL GROWTH AND AS AN INTIOXIDANT

Abd Al-Hussain Attia Ali Rasheed **Iman Hameed Al-Anbari**
Researcher **Prof.**
Dept. of Food Sci, Coll. of Agric Engin Sci, University of Baghdad.
Abdulhussain.atiya1102a@coagri.uobaghdad.edu.iq **Dr.imanh.alanbari@coagri.uobaghdad.edu.iq**

ABSTRACT

This study was aimed to investigate effectiveness of lycopene and nano-lycopene extracted from tomato waste powder in inhibiting the growth of microorganisms was studied. The study included preparing lycopene extract using a triple solvent mixture (hexane, acetone, and ethanol) in ratios (2:1:1) after evaporating them from the extractor and using it to prepare nano-lycopene by the technique of High-energy mechanical grinding. The surface morphology and roughness of the lycopene nanoparticles was determined, and their dimensions were determined using an atomic force microscope (AFM).The effectiveness of both lycopene and nano-lycopene extract was tested to inhibit gram-positive and negative bacteria, fungi, and yeast using the suitable diffusion method at concentrations of (50, 100, 150, 200, and 250) micrograms/ml. Its antioxidant activity was studied using the DPPH method and compared to lycopene extract and industrial antioxidant.

Key word: HPLC, XRD, carotenoid, chemical additives, free radicals, pigment, retention time.
***Part of Ph.D. dissertation of the 1st author**

دراسة تأثير مستخلص اللايكوبين والنانو لايكوبين المستخلص من مخلفات الطماطة في تثبيط نمو الميكروبات وكمضاد أكسدة

عبد الحسين عطية علي **أيمان حميد الأنباري**
باحث **أستاذ**
قسم علوم الاغذية / كلية علوم الهندسة الزراعية , جامعة بغداد

المستخلص

هدفت الدراسة الى التعرف على فعالية كل من اللايكوبين الطبيعي و النانوي المستخلص من مسحوق مخلفات الطماطة في تثبيط نمو الأحياء المجهرية,وتضمنت الدراسة تحضير مستخلص اللايكوبين الطبيعي بأستخدام المزيج الثلاثي من الهكسان والأسيتون والأيثانول وبالنسب (2: 1: 1) وتجفيفه ثم تحضير اللايكوبين النانوي بأستعمال تقنية الطحن الميكانيكي العالي الطاقة, وتم تحديد التشكل السطحي لجسيمات اللايكوبين النانوية وخشونتها وتحديد أبعادها بأستعمال مجهر القوة الذرية Atomic force microscopy AFM وأختبرت فعالية كل من اللايكوبين المستخلص والنانوي في تثبيط البكتريا الموجبة والسالبة لصبغة كرام والفطريات والخميرة بأستعمال طريقة الأنتشار well diffusion method وبتراكيز (50, 100, 150, 200, 250) مايكروغرام/ مل. وتمت دراسة نشاطه المضاد للأكسدة باستخدام طريقة DPPH ومقارنته بمستخلص اللايكوبين ومضاد الأكسدة الصناعي.

الكلمات المفتاحية: HPLC, XRD , الكاروتينويدات, المضافات الكيميائية,الجذور الحرة, صبغة,وقت الأحتفاظ.
***جزء من أطروحة الدكتوراه للباحث الأول.**

Received:13 /12/2023, Accepted:24/4/2024, Published:December 2025

INTRODUCTION

Lycopene is a natural carotenoid pigment produced by plants and microorganisms during photosynthesis to protect them from photo-oxidative activity. It is a plant chemical found mainly in tomatoes and their products, and it is a hydrocarbon compound composed of eight isoprene units. (1,10) Lycopene is one of the carotenoids that does not contain oxygen in its structure and has the chemical formula $C_{40}H_{56}$ The lycopene molecule consists of 13 double bonds, including a central chain that includes 11 conjugated bonds (meaning each double bond is followed by a single bond) called the chromophore, which gives lycopene the distinctive red color of this dye due to Double bonds and their light absorption properties (9) Lycopene is the primary pigment in tomatoes and is one of the most important natural antioxidants because it contains several conjugated double hydrocarbon bonds. Therefore, it is an effective antioxidant in inhibiting and removing free radicals compared to other carotenoids, as it has been proven that lycopene has antioxidant activity superior to beta-carotene and alpha-tocopherol. It protects lymphocytes from the danger of free radicals, which strengthens the immune system. (6,16) Lycopene is an effective compound with a wide range of biological activities, including the inhibitory effect on the growth of multidrug-resistant (MDR) microorganisms such as *Streptococcus pyogenes* (4) Other researchers (10,15) found that lycopene extracted from tomato waste inhibited the growth of gram-positive (G +) and gram-negative (G-) bacteria, *Escherichia coli, Staphylococcus aureus,* by affecting cell membranes and their internal structure, which leads to Changes in the permeability and structure of cell walls, their effect on proteins responsible for cell division, and their effectiveness against fungal growth by stimulating programmed cell death and mitochondrial dysfunction. The term "nano" refers to nanoparticles, which are materials with at least one nano dimension, giving them new physical, chemical, and biological characteristics and properties that differ from the material's natural state, making it suitable for different applications. This difference is attributed to the large surface area of the nanoparticles, which leads to an increase in the speed of their reactions, catalytic activities, and mechanical properties. (2,3) The study conducted by (5,4) demonstrated various properties of lycopene, including its anti-growth activity of the yeast *Candida albicans,* which causes skin infections and poses an increasing threat to the lives of women, especially patients who suffer from immunodeficiency, pregnant women, and the elderly. Lycopene has anti-fungal activity. Such as *Trichosporon beigelii* and *Malassezia furfur.* Other researchers (3,13) found that lycopene extracted with organic solvents such as ether, chloroform, acetone, methanol, and ethanol has inhibitory activity against the microorganisms *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger, Aspergillus flavus, and Candida albicans.* Due to the severe health problems that chemical additives cause to foods, researchers have resorted to extracting microbial growth inhibitors and natural antioxidants from natural sources with effectiveness equal to or greater than the effectiveness of chemical additives. (9,7) Other researchers (10) found that adopting a diet rich in antioxidants such as carotenoids, especially lycopene, flavonoids, and tannins, reduces the chances of developing cancer and cardiovascular diseases due to their influential role in removing types of free radicals. there are many neutral extracts used as antimicrobial. Lycopene has gained attention due to its various biological functions, as it stimulates the accumulation of species Reactive Oxygen (ROS) in bacteria, specifically the free radical hydroxyl (OH), which are highly destructive molecules that attack cell components and contribute to cell death. They are considered a major factor in stimulating programmed cell death and cause damage to cell membranes. And breaking DNA strands (14.11) This study aimed to use nano-lycopene as one of the areas of recent discoveries that include the manufacture and development of various nanomaterials. It has a vital role in our daily lives and in improving agricultural and industrial production, communications and medicine (12) Many studies and extensive research have been conducted to investigate the properties of

nanomaterials and their potential applications for several purposes, such as antimicrobial agents, anti-cancer agents in wound dressings, and water treatment. Recent research has turned to nanotechnology to increase the effectiveness of prepared plant preparations in inhibiting microbial growth (12,27) Other researchers (5) found that nano-lycopene is an antibacterial to antibiotic-resistant bacteria through oral administration of nano-lycopene, which led to increased bioavailability through increased solubility and easy for absorption.

MATERIALS AND METHODS

1 - Lycopene was extracted from dried local tomato waste powder obtained from farms in Karbala Governorate in Iraq, following the method described by (12) and modified by us to obtain the pigment extract from dried tomato waste by taking 1 gram of the dried sample and it was mixed with 10 ml of a solvent mixture (acetone: hexane: ethyl alcohol) in a ratio (1:2:1) and placed on the magnetic stirrer mixer for 2 to 3 hours to ensure optimal extraction, then 1.5 ml of water added to it to separate the hexane layer from the acetone and ethyl alcohol layer and it mixed for another 5 minutes, and the upper layer containing lycopene removed and kept in a closed, opaque bottle. The process repeated until the dye was extracted entirely, and the extracted dye was concentrated in a rotary evaporator at a temperature of 40°C. Then, placed in the oven until the constant weight was obtained.

2 - **Diagnosis of lycopene:** The extracted lycopene was diagnosed using the HPLC technique and the device model 9100 and equipped by the Korean (Yangle) company. The lycopene dye was diagnosed at a wavelength of 472 nanometers. The qualitative detection was carried out using standard lycopene supplied by CGMUP (Hungarian origin). The retention time (Rt) and spectral properties of the extracted lycopene were compared with standard lycopene by dissolving the samples in petroleum ether at a concentration of (20 ppm). 50 microliters were injected into the HPLC device and the retention time(Rt) was calculated.AC18 type separation column was used with dimensions of (250 x 4.6) mm and a size of porous particles of 5µm. The mobile phase consisted of Acetonitrile: Methanol 10:90 and the flow speed was 1 ml per minute. The operation process, calculation of results, and study of the spectroscopic properties of lycopene were carried out using the Clarity Chromatography SW program.

3- **Preparation of nano-lycopene**. A high-energy steel ball mill equipped by the German company Retsch was used at a speed of 400 revolutions per minute for 15 minutes and for intermittent periods to avoid high temperatures and changes in the properties of lycopene. The ground product was then collected in sterile, opaque glass bottles and refrigerated (4±2)°C. XRD , AFM examination were conducted to characterize the nanomaterials in the Optics Department, Laser Division of the Ministry of Science and Technology.

4 - The two standard isolates of the bacteria *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, the yeast *Candida albican,* and two isolates of the fungus *Penicillium sp.* and *Aspergillus niger* were obtained from the laboratories of the Environmental Research Center / University of Technology / Baghdad / Iraq for the purpose of studying the effect of lycopene and nano-lycopene prepared at different concentrations (250, 200, 150, 100, 50) µg /ml to study its effect on the microbial community, by activating microbial cells by culturing them in brain and heart infusion broth for 24 hours at a temperature of 37 degrees Celsius with continuous stirring. The activated bacterial cells were grown on Muller Hinton medium prepared by the company (Himedia) from India. It was designed according to the supplier company's instructions and intended to study the effect of antibiotics on bacterial cells. Yeast cells and activated fungi were grown on potato dextrose agar medium prepared by the Dutch company DCM, which was intended to study the antibiotic effect on fungal cells, Then holes with a diameter of 6 mm were made in the medium, with equal dimensions designated for placing the lycopene solution in it, with a volume of 100 microliters for each hole. The dishes were left to settle, and the agar absorbed the lycopene solutions for a quarter of an hour at room temperature, Then the plates were transferred to an incubator at 37 degrees Celsius for 24

hours. The damping diameter around the holes was then measured using a ruler.

5- The method described in (18) was followed to prepare DPPH and measure the antioxidant activity.

RESULTS AND DISCUSSION

The results of extracting lycopene from tomato waste powder indicated that the amount of lycopene amounted to 185.6 mg/100 g of waste powder. The reason for the superiority of this treatment can be attributed to the high polarity of both acetone and ethyl alcohol, as they synergize in the process of penetrating cellulosic tissues and mixing with water, for hexane, which is characterized by low polarity, which helps in dissolving the dye lycopene, which is characterized by low polarity. Figures (1) and (2) shows that the retention time (RT) of the lycopene dye is close to that of the standard compound, and the (RT) for the standard lycopene was (5.04 minutes) with a percentage of (96.4)% and the appearance time was (RT) for the extracted lycopene is 5.09 minutes and a percentage of 85.5%, which confirms the purity of the extracted lycopene .Figure (3) indicates that the X-ray diffraction examination showed that the average crystal size is (12.52) nanometers. We can observe from Figure 4. (A-B) the three-dimensional microscopic images and the graph of the lycopene using the AFM device, where the atomic force microscopic analysis showed a pore-free shape with homogeneity and uniformity on the surface of the sample, and the surface of the film did not show any evident cracking. The granules showed well-separated conical columnar growth; it collects grains all over the surface with some vertical grains merging in a few places and showed an average particle size of (50.42) nm

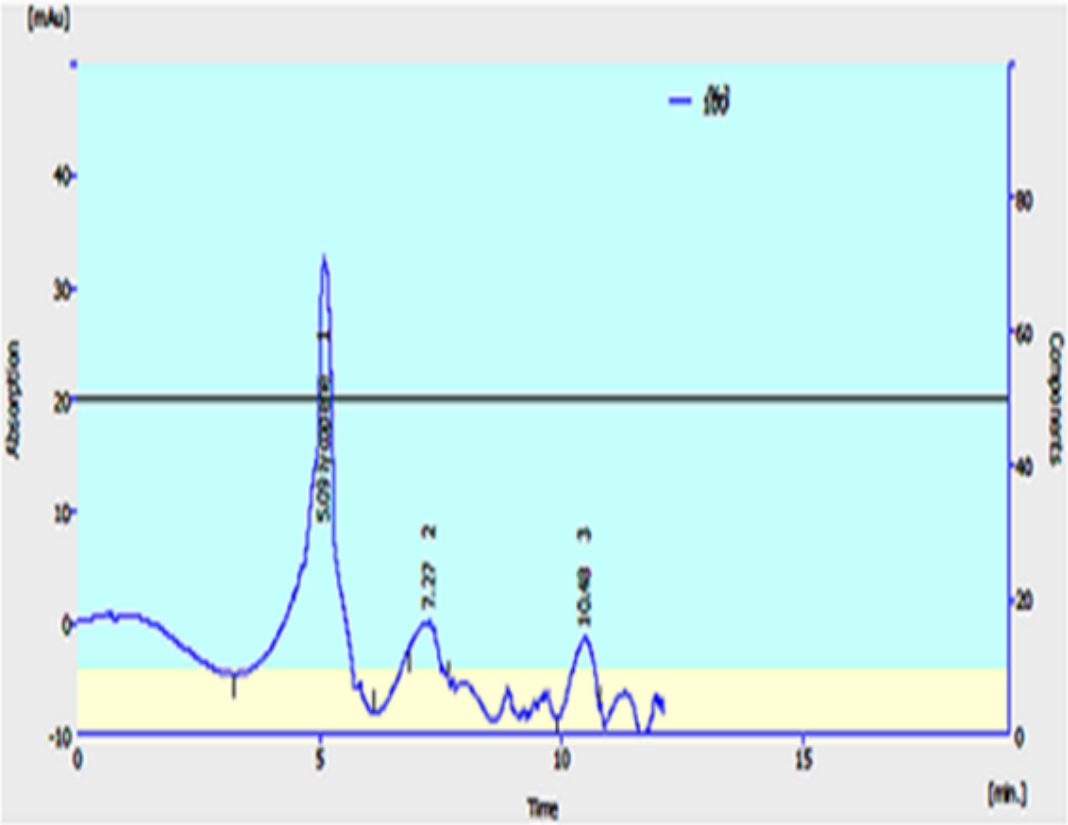


Figure 1. RT for standard lycopene

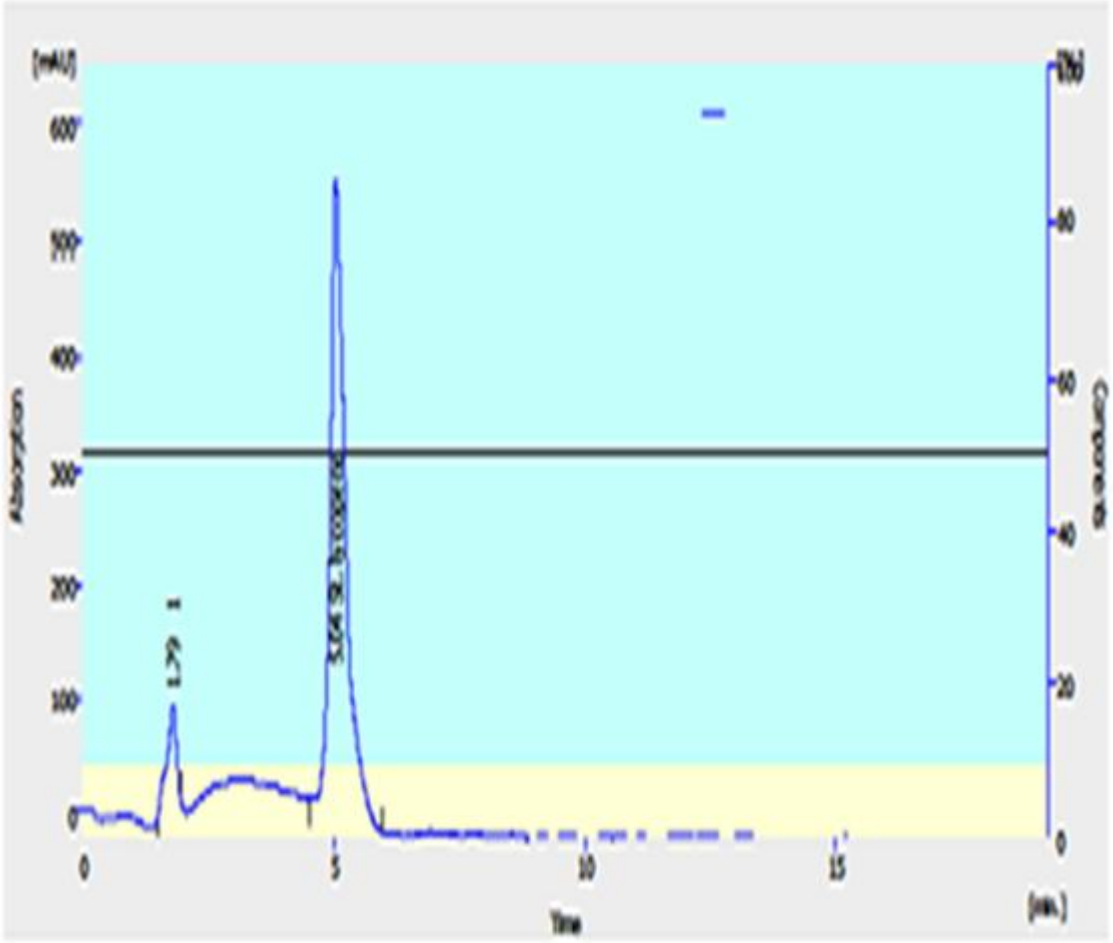


Figure 2. RT (Retention time) of extracted lycopene

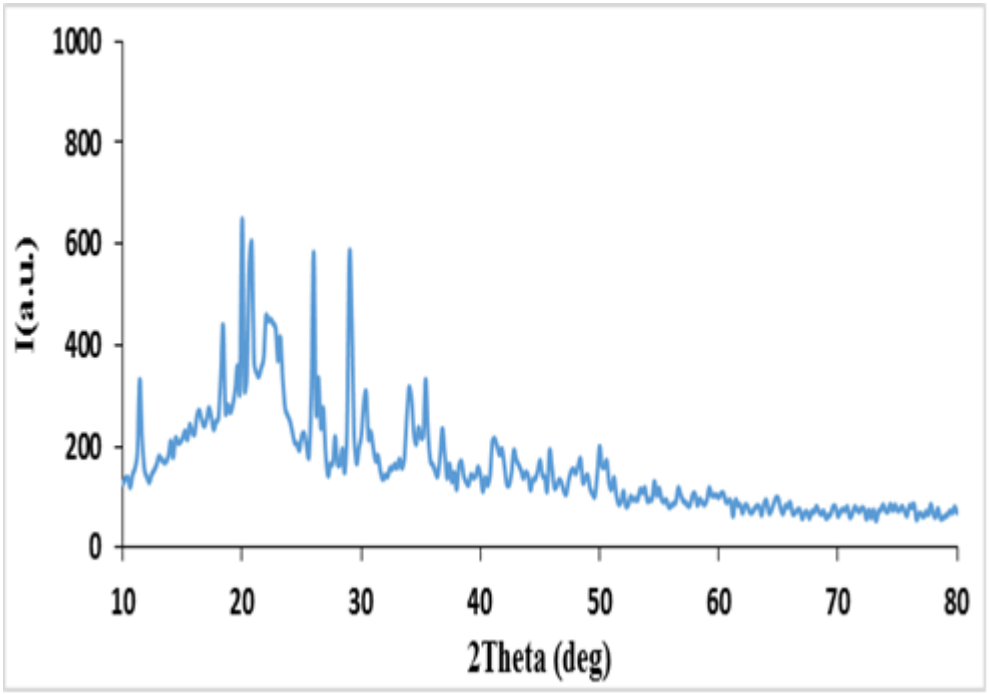
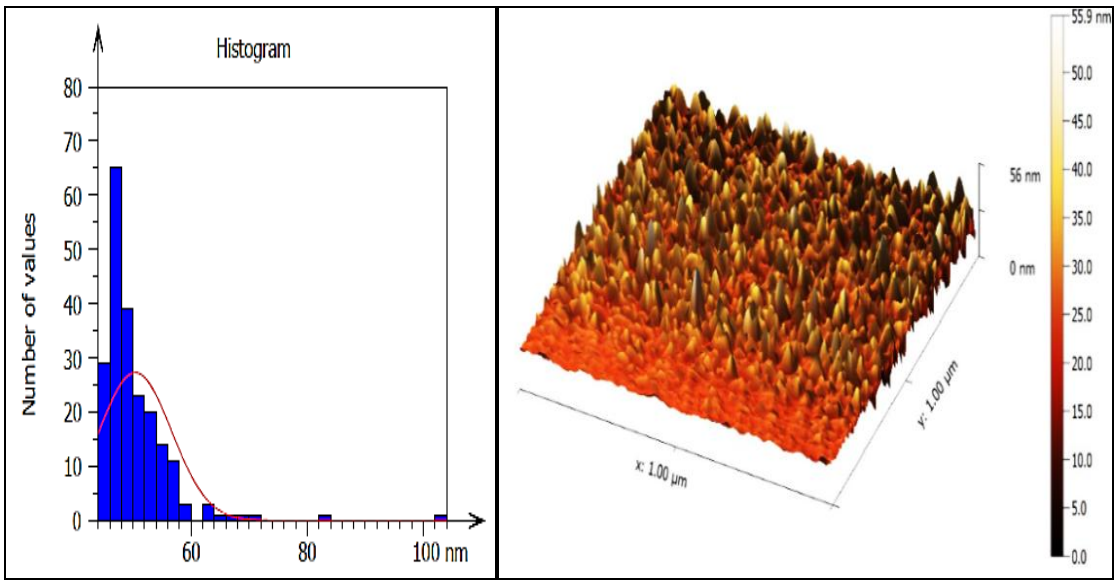


Figure 3. X-ray diffraction of lycopene nano composite



A **B**

Figure 4.(A-B) 3D microscopic image of lycopene and a diagram of the dimensions in the AFM device

Inhibitory activity of lycopene and nano-lycopene on bacterial growth: Table (1) shows that nano-lycopene showed the highest inhibitory activity against bacteria. The average diameter of the inhibition corona for *Escherichia coli* was (21.5 mm) and for *Staphylococcus aureus* (24.2 mm) at a concentration of 250 micrograms/ml. In comparison, the average diameter of the inhibition corona for the extracted lycopene was (12.5 mm and 15.8 mm), respectively, for the same concentration. We notice an increase in the inhibitory activity in both extracted and nano-lycopene with increasing concentrations used, It was observed that the inhibitory effectiveness of the Gram-positive bacteria *Staphylococcus aureus* is more than the inhibitory effectiveness of the Gram-negative *Escherichia coli.*

Table 1. Inhibitory activity of lycopene extract and nano-lycopene against bacteria*

Microscopic organism	Concentration µg/ml	The diameter of the inhibition area(mm)	
		Natural Lycopene	Nano Lycopene
Escherichia coli	**50**	**6.0**	**8.9**
ATCC 25922	**100**	**6.8**	**11.5**
	150	**7.9**	**14.2**
	200	**9.2**	**18.8**
	250	**12.5**	**21.5**
Staphylococcus aureus	**50**	**6.5**	**9.6**
ATCC 25923	**µ100**	**7.3**	**12.5**
	150	**9.5**	**15.8**
	200	**11.6**	**19.5**
	250	**15.8**	**24.2**

***Each number represents an average of three replicates**

The variation in the inhibitory efficacy of lycopene is attributed to the cell wall structure's distinct characteristics and outer membranes in these bacterial species. Studies have indicated that the biologically active chemicals derived from plants significantly affect Gram-positive bacteria more than Gram-negative bacteria because Gram-negative bacteria contain a distinctive outer membrane and peptidoglycan layer. It is well known that these microorganisms constitute a global health concern due to their enormous antibiotic resistance.

Inhibitory activity of lycopene and nano-lycopene on the growth of yeast *Candida albicans:* Table (2) shows the inhibitory effect of lycopene and nano-lycopene against *Candida albicans* yeast, as nano-lycopene

showed the highest inhibitory activity against yeast, as the average diameter of inhibition reached 17.8 mm at a concentration of 250 micrograms/ml, superior to the extracted lycopene, which reached 13.5 mm at the same concentration. It was found (15) that lycopene possesses antifungal properties by stimulating apoptosis in both the early and late stages of fungal growth. This mechanism is achieved by raising the levels of reactive oxygen species. (ROS) inside the cells. The demise of fungal cells is mainly attributed to hydroxyl radicals, which leads to a defect in mitochondrial functions and ultimately to cell death. Nanoparticles have an antifungal effect on various fungi and yeasts, such as the pathogenic yeast *Candida albicans.*

Table 2. Inhibitory activity of lycopene and nano-lycopene against yeast*

Microscopic organism	Concentration µg/ml	The diameter of the inhibition area(mm)	
		Natural Lycopene	Nano Lycopene
Candida albicans	**50**	**6.1**	**6.9**
	100	**7.8**	**8.2**
	150	**9.6**	**10.8**
	200	**11.5**	**13.6**
	250	**13.5**	**17.8**

***Each number represents an average of three replicates**

3- The inhibitory effectiveness of lycopene and nano-lycopene on the growth of fungi: Table (3) shows that nano-lycopene showed the highest inhibitory activity against fungi, as the average diameter of the inhibition halo in *Aspergillus niger* reached (19.5) mm and in *Penicillium sp* (21.5) mm at a concentration of 250 micrograms/ml, superior to the extracted lycopene, which reached an average diameter of the inhibition halo is (13.5 and 15.9) mm, respectively, at the same concentration**.** The results showed an increase in the inhibitory effectiveness with increasing concentrations used, and stated that lycopene inhibits the growth of microorganism cells by stimulating programmed cell death, DNA fragmentation and activating the enzymes responsible for programmed cell death, the most important of which is the caspase enzyme (17,18) Other researchers stated that the formation of nanoparticles changes the physical and chemical properties of lycopene, enabling it to target multiple antimicrobial mechanisms effectively. These mechanisms include penetrating cell walls and membranes and inhibiting DNA.(24,23) The antimicrobial activity of nanocomposites can be attributed to their large surface area, which facilitates the attachment of a greater number of ligands to the surface of the nanoparticles and enhances the number of charges, thus increasing the speed of interaction between the nanocomposites and the receptors on the microbial surface(16,21,22) Other researchers indicated that some plant preparations prepared in nano formula demonstrated an inhibitory effect on Gram-positive and negative bacteria and their effectiveness as antioxidants compared to the industrial antioxidant BHT Butylated hydroxyl toluene.(26, 25).

Table 3. Inhibitory activity of extracted lycopene and nano-lycopene against fungi*

Microscopic organism	Concentration µg/ml	The diameter of the inhibition area(mm)	
		Natural Lycopene	Nano Lycopene
Aspergillus niger	**50**	**6.3**	**7.5**
	100	**7.2**	**9.7**
	150	**9.1**	**12.8**
	200	**11.4**	**15.9**
	250	**13.5**	**19.5**
Penicillium sp.	**50**	**6.6**	**7.7**
	100	**7.5**	**9.9**
	150	**9.4**	**13.8**
	200	**11.8**	**17.9**
	250	**15.9**	**21.5**

***Each number represents an average of three replicates**

4- Antioxidant activity of nano-lycopene

Table (4) shows that nano-lycopene is significantly superior at the level of significance ($p \leq 0.05$) in suppressing free radicals compared to natural lycopene and BHT, and that the higher the concentration, the greater the antioxidant effectiveness as the highest percentage of inhibition reached 82.9% at the concentration of 250 micrograms/ml, where the percentage of free radical suppression for them reached 57.1% and 51.3%, respectively, and this It agrees with. who stated that nanomaterials are characterized by higher effectiveness than their effectiveness in their natural state.(28,26) The superiority of nano-lycopene over extracted lycopene is due to the qualities and properties it possesses that enable nanocomposites to interact in different ways compared to their interactions when they are in their normal size due to their reduced size, increased surface area of nanoparticles, and the spread of surface charges, which allows them to have a greater increase in the number of atoms and molecules participating in the interactions (19,20,27).

Table 4. Effect of lycopene concentration and nano-lycopene on antioxidant activity in DPPH assay

concentration µg/ml	% Inhibition				
Treatment	**50**	**100**	**150**	**200**	**250**
* L_0	14.7	24.7	35.6	46.6	57.1
** L_N	21.1	31.7	54.1	62.2	82.9
BHT	10.9	21	31.7	41	51.3
LSD	4.61 *	5.92 *	5.88 *	6.01 *	7.57 *

L_0 represents extracted lycopene, L_N represents nano sized lycopene

Conclusions

Tomato industry waste can be utilized in the production of dried nano-lycopene by mechanical grinding using steel balls, which produced nano-lycopene particles that showed distinct effectiveness as microbial inhibitory compounds antioxidants at low concentrations, which encourages their use as an effective and safe alternative to preservatives whose use has been associated with many health risks and negative effects.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

REFERENCES

1. .Imran, M., F. Ghorat, I., Ul-Haq, H., Ur-Rehman, F., Aslam, M., Heydari, M. A., Shariati, E., kuskhanova, Z., Yessimbekov, M.Thiruvengadam, and M. H. Hashempur, .2020. Lycopene as a natural antioxidant used to prevent human health disorders Antioxidants, 9(8): 706-710. doi: 10.3390/antiox9080706

2. Al-Anbari, I. H., S. R., Khairi, and L. K. Hassan,.2021. Study the physicochemical, microbiological and sensory characteristics of soft cheese incorporated with Lupine (*Lupinus albus L.*) powder in different proportion. In IOP Conference Series: Earth and Environmental Science 761(1):21-24 doi 10.1088/1755-1315/761/1/012124.

3. Al-Anbari, I. H., A. M. Dakhel, and A. Adnan, .2019. The effect of adding local orange peel powder to microbial inhibition and oxidative reaction within edible film component. Plant Arch, 19 (1): 1006-1012. doi.org/10.1111/j.1365-2672.2006.03124

4. Alboresi, A., L., Dall'Osto, A., Aprile, P., Carillo, E., Roncaglia, L., Cattivelli, and R. Bassi, 2011. Reactive oxygen species and transcript analysis upon excess light treatment in wild-type Arabidopsis thaliana vs a photosensitive mutant lacking zeaxanthin and lutein. BMC Plant Biology, 11(2):1-22 doi.org/10.1186/1471-2229-11-62

5. Alshahrani, M. Y., E. H., Ibrahim, M., Asiri, M., Kilany, A. G., Alkhathami, M. N., Alshahrani and H. C. Chandramoorthy, .2022. Lycopene augments and enhances anti-oxidant/antibacterial efficiency of ethanolic leaf extract of (*Helianthu annuus*) over multidrug-resistant bacterial isolates. Journal of King Saud University Science, 34(7):102-250 doi.org/10.1016/j.jksus.2022.102250

6. Al-Shebli, W. C. H. and I. H., Al-Anbari, 2023. Studying the antioxidant activity of moringa leaf extracts (*Moringa oleifera Lam.*).

In IOP Conference Series: Earth and Environmental Science,1262(6):2-9 doi 10.1088/1755-1315/1262/6/062009.

7. Al-Taweel, S. K., I. H. Al-Anbari, and M. Al-Hamdani,. 2022. ntioxidant identification, antimicrobial activity of stevia rebaudiana bertoni leaves extracton flavored milk. International Journal of Agricultural and Statistical Sciences , 18(2):547-556 doi.org/10.5336/pharmsci.2020-79419

8. Altemimi, A., N. Lakhssassi, A. Baharlouei, D. G. Watson, and D. A. Lightfoot, 2017. Phytochemicals: Extraction, isolation, and identification. Plants, 6(4), 42.

9. Binsuwaidan, R., A. A., Sultan, W. A., Negm, N. G., Attallah, M. J., Alqahtani, I. A., Hussein, M. A., Shaldam, S. A. El-Sherbeni, and E., Elekhnawy,. 2022. Bilosomes as nanoplatform for oral delivery and modulated in vivo antimicrobial activity of lycopene. Pharmaceuticals ,15(9):1042-1043. doi.org/10.3390/ph15091043

10. Black, H.S., F., Boehm, R. Edge, and T. G., Truscott,. 2020. The benefits and risks of certain dietary carotenoids that exhibit both anti-and pro-oxidative mechanisms—A comprehensive review. Antioxidants, 9(3): 263-264.

11. Brand-Williams, W., M. E. Cuvelier, and C. L. W. T., Berset,. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28(1):25-30 https://doi.org/10.1016/S0023-6438(95)80008-5

12. Choi, H. and D. G. Lee,. 2015. Lycopene induces apoptosis in *Candida albicans* through reactive oxygen species production and mitochondrialdysfunction. Biochimie 11(5): 108-115. doi.org/10.1016/j.biochi.2015.05.009

13. Cousins B. G.; H. E. Allison; P .J. Doherty;C. Edwards; M. J. Garvey; D. S. Martin and R. L. Williams. 2007. Effects of a nanoparticulate silica substrate on cell attachment of *Candida albicans.* Journal of Applied Microbiology.102(3):757-765

14. Deraz, S. F. 2018. Antimicrobial and preservative effects of natural compounds on meat quality. Journal of Food Protection, 81(7), 1073–1081. doi 10.3390/antiox9030264

15. El-Desoky, N. I., N. M. Hashem, and S. A. Abdelnour, 2021. Influence of vitamin E and selenium supplementation on growth performance and antioxidant status of lambs. Animals, 11(3), 664

16. Hassan, F., M. Imran, and S. Mahmood, 2020. Camel milk cheese: processing, quality and safety aspects. Journal of Food Science and Technology, 57, 2911–2920.

17. Hussein, J. L., A. A. Ahmed and D. J. Raheem,. 2023. Study of antibacterial, antioxidant activity and biochemical parameters of different honey samples. Iraqi Journal of Science, 64 (5): 2189-2201. doi: 10.24996/ijs.2023.64.5.8.

18. Jasim, Q. A., and T. S. AI-Obaidi,. 2022. Substitution of animal protein by different ation of dried rumen meal in common carp Cyprinus carpio DIETS. Iraqi Journal of Market Research and Consumer Protection, 14(1):65-74. doi.org/10.28936/jmracpc14.1

19. Khalid, N. T., R. K. Shaymaa, and H., Luma Khairy,. 2021. Effect of incorporated soft cheese with wheat germ extracts quality and on shelf life. Indian J. Ecol, 48(13):244-248. doi:10.21608/ejfs.2023.203837.1162.

20. Khan, I., Saeed, K., and I. Khan, 2019. Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry, 12(7), 908–931.

21. Mahmed, A.. M., and B. H. Mohammed,. 2020. Chemical determination of heart of palm type kedraaweey, (*Phoenix dactylifera* L) identification of active compounds in its water extract and application in the manufacture of ice cream. Biochemical and Cellular Archives,20(1):20-21 doi: 10.35124/bca.2020.20.1.2021

22. Mozos, I., D. Stoian, C. T. Luca, and M. G. Barbu, 2020. Lycopene and oxidative stress: An updated review. Antioxidants, 9(3), 236.

23. Nahla, T. K., S. U., Wisam, and N. M. Tariq, 2018. Antioxidant activities of Beetroot (*Beta vulgaris L.*) extracts. Pakistan Journal of Nutrition, 17(10):500-505. doi:10.3923/pjn.2018.500.505.

24. Qing, Y., et al. 2018. Potential antibacterial mechanism of silver nanoparticles and synergy with antibiotics. International Journal of Nanomedicine, 13, 3311–3324.

25. Rasheed, A. A. H. A. A. and I. H., Al-Anbari,. 2023. Study of the antioxidant activity of lycopene and lycopene nanoparticles extracted from tomato waste powder. In IOP Conference Series: Earth and Environmental Science, 1262 (6):2-3 doi10.1088/17551315/1262/6/062003.

26. Rawat, S., A. Siddiqui, and R., Singh,. 2023. Effect of different processing and preservation techniques on lycopene: A mini review. Research Journal of Pharmacy and Technology, 16(5):2537-2542. doi.org/10.52711/09740x.2023.0041.

27. Tarko, T., A. Duda-Chodak, and D. Semik-Szczurak, 2022. Antioxidant potential of selected plant extracts in food applications. Antioxidants, 11(2), 345.

28. Thompson, K. A., M. R., Marshall, C. A., Sims, C. I., Wei, S. A., Sargent, and J. W. Scott, 2000. Cultivar, maturity and heat treatment on lycopene content in tomatoes. Journal of Food Science, 65(5):791-795. https://doi.org/10.1111/j.1365-2621.2000.tb13588.x