



RESEARCH ARTICLE

Lycopene From *Naganishia Albidosimilis*: A Promising Natural Antifungal Against *Echinococcus Granulosus*

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ABSTRACT

Background and Objective: Pathogenic parasites are among the microorganisms that pose a threat to humans, The natural compound lycopene was extracted from the yeast *Naganishia albidosimilis* to evaluate its antimicrobial activity against the pathogenic parasitic *Echinococcus granulosus*, which causes hydatid cysts in human. **Methodology.** *granulosus* was obtained from hydatid cyst samples collected during surgical procedures. After lycopene was extracted and purified, its purity was confirmed using several chemical and physical methods. **Results:** the extracted compound was pure lycopene. Experiments showed that lycopene possesses antimicrobial activity against *E. granulosus*. This effect was confirmed at the molecular level using polymerase chain reaction in real time (RT-PCR), *E. granulosus* expression of vital biological genes had significantly decreased. Lycopene suppresses and development, as evidenced by the considerable decrease in expression of the Cox1 gene in *E. granulosus*. The Cox1 gene is crucial for cell cycle and division. **Conclusion:** Lycopene is a promising for the creation of efficient alternative natural remedies to fight this microbial pathogenicity because of its antioxidant qualities and capacity to interfere with vital metabolic pathways.

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INTRODUCTION

Pathogenic parasites are among the microorganisms that pose a health threat to humans, especially when they transition from naturally occurring organisms in the body to disease-causing agents. On the other hand, parasites like hydatid cysts, caused by the tapeworm *Echinococcus*, are transmitted to humans through contaminated food or water. These parasites develop into dangerous cysts in organs such as the liver and lungs, causing life-threatening symptoms if not treated promptly, pathogenic parasites represent significant health challenges that require awareness and proper treatment to safeguard human health.¹ yeast very important Because of their varied chemical structures and historical significance in drug discovery, natural materials have been used to find and create antimicrobial drugs such as lycopene. where A naturally occurring carotenoid, lycopene is mostly present in tomatoes and tomato-based products. Lycopene has strong antioxidant qualities, according to epidemiologic studies.^{2,3,4} The tomato was where lycopene was initially found by Millardet in 1876, and it is again isolated in pure form by Schunck in 1903, who named it lycopene.⁵ Lycopene is an acyclic carotenoid with 40 carbons and a molecular mass of 536 daltons. One of the carotenoids produced by plants and photosynthetic microbes, it is made up solely of hydrogen and carbon atoms.⁶ Lycopene has outstanding antioxidant qualities since it has 13 double bonds, 11 of which are conjugated. However, lycopene is sensitive to heat and light and vulnerable to oxidants because to the unsaturated bonds in its molecular structure.^{7,8} Lycopene can be produced chemically or derived from natural sources. It is available as a supplement, typically in the form of soft gel, pills, or capsules. For further antioxidant advantages, other nutrients are occasionally added to the mix.⁹ Both bacteria and fungi have been genetically engineered to increase lycopene production. This process known as bioengineering has been a focus of previous research. Studies have shown that producing carotenoids like lycopene, α -carotene, and β -carotene is a relatively simple task in biological organisms through DNA modification. Lycopene and β -carotene are produced commercially by the fungal plant disease *Blakeslea trispora*. Commonly utilized as food additives and dietary supplements are these carotenoids, mainly β -carotene and all-trans-lycopene.¹⁰

Materials and methods

Samples Collection

Specific isolate of *Naganishia albidosimilis* yeast was obtained from the Microbiology Laboratory, College of Science, University of Basra for the purpose of extracting lycopene by activation of sample and cultured samples were incubate at 25°C for five to seven days after being grown directly on media PDA with 250 mg/L chloramphenicol to avoid bacterial contamination. While for the isolation of *Echinococcus* from infected

individuals, that 18 pathological samples were collected. samples were obtained during surgical procedures by the specialist physician of the infected individuals. This cystic is eradicate from located on organs such as the liver or lung and placed in a sterile 100 ml container and transferred to the laboratory within an hour in a cool box at 4 C° to complete the isolation and diagnosis process.

Morphology identification

Echinococcus granulosus

After transporting the sample to the laboratory, the cystic fluid is taken and placed in a sterile centrifuge at 1500-2000 rpm for 5-10 minutes. The floating portion is then discarded and the sedimentary portion is taken, smeared and the slide examined under a microscope to detect active hydatid cyst.

Lycopene Compound

A. Extraction of the lycopene (L)

isolates strains *Naganishia albidosimilis*, morphologically and molecularly characterized was detect and isolate from soil. Isolate obtained from the Plant Pathology Laboratory at the College of Science, University of Basrah, where they were activated on PDA medium. Eight to ten grams of each isolate were harvested and incubated for 7 days on potato dextrose agar at 25°C. The cells were lysed and harvested by adding n-hexane, then washed with distilled water. The mixture was then placed on a hot plate at 40–50°C to form an oily resin. Propylene glycol was then added to the oily resin in a 3:5 ratio, mixed, and heated to approximately 60°C. An aqueous alkaline solution containing 45% potassium hydroxide was then added in a 2:5 ratio. The resulting mixture was stirred and mixed well, then left at 55–70°C for 1 hour.¹¹

B. Purification of lycopene

Thin layer chromatography

Thin layer chromatography of lycopene was carried out using aluminum thin sheets covered with silica gel (20×20) cm with a thickness of 2 mm using mixture for some of a mobile phases which are:First: A mixture of hexane and acetone in a ratio of 40: 10 ml respectively Second: 70 ml of methanol 10 ml of chloroform Third: 20 ml of methanol and 40 ml of ethanol Fourth: 20 ml of hexane with 40 ml of ethyl acetate Fifth: 70 ml of hexane with 10 ml of ethyl acetate Sixth: 90 ml of petroleum ether with 10 ml of dichloromethane Seventh: 80 ml petroleum ether with 20 ml acetone Eighth: 20 ml of methanol and 40 ml of ethyl acetate Ninth: 1 ml of

toluene and 19 ml of hexane^{12,13,14}. Based on the most efficient mixture according to the experiment, the first mixture was chosen to complete the test and the time taken for the mixture to rise was 30 minutes. The plates were exposed to iodine vapor and after accurately determining the location of the spots, the values of their relative flow coefficients (Rf) were calculated from the following relationship¹⁵:

$$R_f = a/b$$

a = The distance from the starting point to the gravity center of the sample spot.

b = The distance from the starting point to the front of the developing solvent.

Column chromatography (Total purification)

A glass column (30 × 500) mm was used, 3 cm of glass wool was placed at the beginning and end of it, the column was filled with silica gel emulsion (100-200 mm mesh) at a rate of 150 g of silica gel, with a height of 25 cm, and the mobile phase contained a mixture of hexan: acetone at ratio 40:10. The agents lycopene were dissolved 0.5 g in 1 ml of DMSO and gently added to the surface of the silica gel. Then, the mixture was added to the column until the separation process was completed. The flow rate in the column was 1 ml/min. The separated samples were received and collected from the end of the column by means of test tubes at a rate of 1 ml per tube, and then components of each sample were tested by the thin-layer chromatography technique. The TLC was then checked by using iodine vapor. Components with similar paths (Rf are similar) were collected together in one tube¹⁶. Then it was dried in a small glass dish, after which the resulting extract from each isolate was dissolved and then it was sterilized using 0.45 µm filter paper.

Detected using colorimetric chemical reagents Iodine detection:

Carotenoids were detected using a thin-plate chromatography method after spotting the extract obtained from Lycopene that extract from *Naganishia albidosimilis*, then exposing it to iodine vapor. The flow coefficient of each extract was measured. This is a general detection of organic compounds¹⁷.

Salkowski reaction:

One ml of chloroform was mixed with one ml of the lycopene extract of *Naganishia albidosimilis*, and then one drop of concentrated sulfuric acid was added. Two layers, red to yellow in color, formed, indicating the presence of carotenoids¹⁸.

Vanillin/Sulfuric acid detection:

It was prepared by mixing 0.4 g of vanillin with 10 ml of sulfuric acid (H₂SO₄), then adding 1 ml of the reagent to 1 ml of *Naganishia albidosimilis* extracts. The detection is considered positive when a dark red color appears at the bottom of the tube. Another detection method is used, using a TLC plate, staining with the extract, and then developing with the reagent instead of iodine¹⁹.

Detected using UV absorbance spectroscopy and F.T.I.R (Fourier transforms infra- red)

These spectra were recorded in the ultraviolet and visible region using UV-1800 Spectrophotometer, Shimadzu, Japan in the Marine Science Center, University of Basrah in the region between 200-900 nm. A 1 cm path length quartz cell was used at laboratory temperature to measure of Lycopene and the concentrations used were 0.1 mg/10 ml hexane^{20, 21}. Infrared spectra were recorded using potassium bromide discs (KBr discs) in the region between 500-4000 cm⁻¹ was used to measure the IR- spectrum of lycopene by FTIR- 8400S Fourier Transform Infra-Red Spectrophotometer, Shimadzu, Japan, in the Chemistry Department, College of Science, University of Basrah²².

Gene expression

The extracts of lycopene and the drug of albendazole were added separately to RPMI 1640 to tubes have hydatid cyst fluid containing the protoscoleces and incubated 37°C for 72 h and Then To extract RNA from parasite cells, solutions were first prepared by adding β-mercaptoethanol to the lysis solution, ethanol to the wash solution, and 95% ethanol to the DNase inactivation solution. A DNase I solution was also prepared. The extraction process began by transferring the cells to a centrifuge tube and spun to remove the supernatant. Grinding beads and the lysis solution were then added to grind the cell walls and homogenize the sample until smooth. The mixture was then heated, and dilution solution and ethanol were added before being transferred to a separation column and spun to separate the RNA. The DNase I solution was then added to remove any residual DNA, and the column was washed with the wash solution. Finally, the purified RNA was extracted using nuclease-free water and stored at -70°C according to the SV Total RNA protocol and then to convert RNA to cDNA, the GoScript™ Reverse Transcription System was used the process began by mixing 6–9 µL of RNA with 1 µL each of Random Primers and OligodT Primers, and the volume was then topped up to 11 µL with nuclease-free water. The mixture was then heated at 70°C for 5 min and immediately cooled on ice. A master mix was then prepared consisting of 4 µL GoScript™ 5x Reaction buffer, 2 µL MgCl₂, and 1 µL each of PCR Nucleotide Mix, Recombinant RNasin Ribonuclease Inhibitor, and

GoScript™ Reverse Transcriptase. The two mixtures were mixed to a final volume of 20 µL. The mixture was placed in a polymerase chain reaction (PCR) machine to synthesize cDNA using a three-step thermal program: primer stabilization at 25°C for 5 min, cDNA synthesis at 42°C for 60 min, enzyme inactivation at 70°C for 15 min, and then cooling at 4°C. The gene expression level of the target gene was measured using Real-time PCR was used to measure gene expression. The *Cox1* and *Actin II* genes were amplified using specific primers: *Cox1* forward: (AGGGGCTGGTGTGTTGGTTGGA), Reverse: (TGAAACACCAGCCAAATGCAGAGA) and *Actin II* forward: (CGAGCAGGAAATGATAACG), Reverse (GAACAGGGCTTCAGGACA). A master mix (Go Taq® qPCR Master Mix) was used to perform the reaction, which contained 10 µL of the master mix, 1 µL of forward and reverse primers (at 20x concentrations each), and 8 µL of cDNA, for a total reaction volume of 20 µL. The amplification program was run on a PCR machine in several steps: an initial denaturation stage at 95°C for 2 minutes, followed by 40 cycles of denaturation (95°C for 15 seconds) and annealing (58°C). for 1 minute), and extension (95°C for 15 minutes), with the addition of a melting curve at the end at 60-95°C. The obtained qPCR data were analyzed using the ΔCT method as the CT values for each gene (CLB1 and Cox1) as well as for the house keeping gene (ACT1 and Actin II). The following steps were performed to obtain the expression of each gene²³:

$\Delta CT = CT \text{ target gene} - CT \text{ HK}$.

$Exp = 2^{-\Delta CT}$

Fold change (FC) = $Exp. \text{ target genes} / Exp. \text{ HK}$

Statistical analysis

Using SPSS to assess the difference between lycopene's anti-parasite and anti-yeast actions, $p \leq 0.05$ was deemed statistically significant.

Results

The study included eighteen patients with surgically confirmed hydatid cyst diseases where the site of infection was in the liver which was diagnosed by a specialist physician. The hydatid cysts were taken during the surgical operations (Figure 1). The percentage of viability was calculated by counting live and dead protoscoleces in microscopic by using Stain Eosin where Viable protoscoleces stained green with row of hooklets (black arrow) and calcareous bodies (blue arrow), and non-viable protoscoleces stained red.

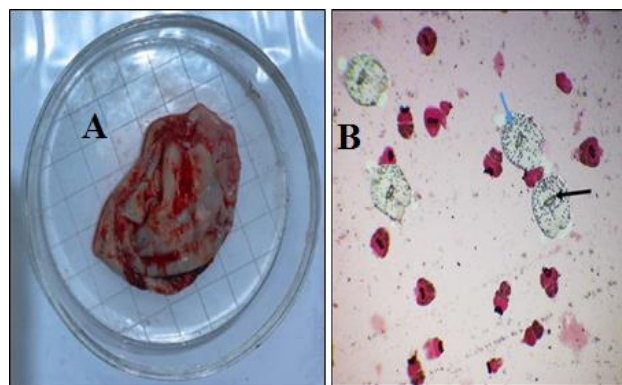


Figure 1. A: Surgically excised hydatid cysts from humans. B: Protoscoleces of *Echinococcus granulosus* under microscopic magnification: 10x.

Lycopene

Three tests were used for detection of lycopene, which included Iodine detection, Salkowski reaction and Vanillin/Sulfuric acid detection. All tests showed positive result (Table 1). In the column chromatography for purification, samples were collected from the column at a flow rate of 1 ml per minute. The colors of the agents were brown for lycopene (Figure 2). Each sample was tested by TLC and checked by iodine vapor. All samples with similar RF were collected together in one tube. The RF result recorded 0.5 for lycopene

Table 1. The colorimetric chemical reagents for detection of lycopene

Test	Extract from isolation (<i>Naganishia albidosimilis</i>)
Iodine	+
Salkowski	+
Vanilline/ Sulfuric acid	+



Figure 2. Lycopene extract and Flow coefficients in thin-layer chromatography.

The results of UV absorption spectra for lycopene compound showed strong absorption for lycopene at wavelengths 481.5, 447 nm (Figure 3 and Table 2). These absorption bands are related to $\pi-\pi^*$ transitions for the resulting from a long chain of conjugated single and double bonds ($C=C-C=C-C=C$) along the main carbon chain. Lycopene specifically contains 11 conjugated double bonds.

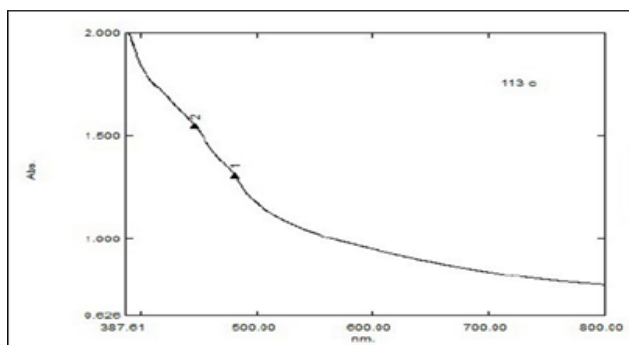


Figure 3. UV absorption spectrum of lycopene compounds

Table 2. UV absorption spectrum wavelength (nm) for lycopene compounds

Compound	$\pi-\pi^*$ Wavelength (nm)
lycopene	481.5, 447

$\pi-\pi^* = \text{for } C=C-C=C-C=C$

FTIR spectroscopy was used to characterize the structure of lycopene compounds. The results of FTIR spectra and their assignments were shown in (Figure 4 and Table 3).

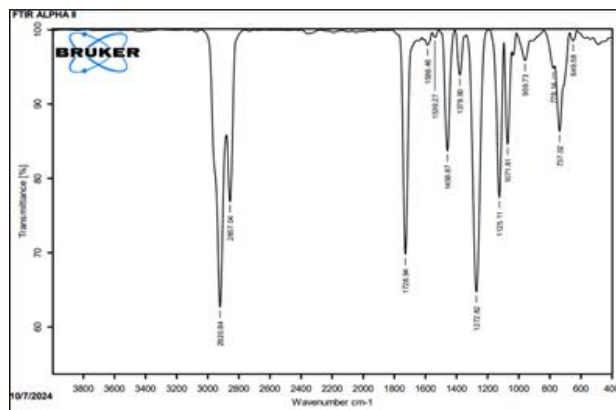


Figure 4. Fourier transforms infra- red for lycopene

Table 3. The functional groups of purified lycopene detected by FT-IR

Wave number (cm ⁻¹)	Assignment
2920.84	C-H, Strong
2857.04	C-H, Strong
1586.46	C=C, Strong
1539.27	C=C, Strong
1458.87	C-H, Strong
1378.90	C-H, Strong
1272.82	C-C Strong
959.73	C-C Strong
1071.81	C-C Strong
1125.11	C=C, Strong
1539.27	C-H, Strong
1458.87	C-H, Strong
778.14	C=C, Strong
737.02	C=C, Strong
649.58	C=C, Strong

The results of lycopene treatment led to decreased expression of *Cox1* gene compared to the control, with all fold change values well below 1. The highest concentration 0.5 showed a less severe decrease (0.099-fold change), but it was still a very significant decrease (about 9.9% of the control level). the greatest decrease in *Cox1* expression occurred at the lycopene concentration of 0.5 (719.574-fold change). The results showed that treatment with albendazole showed a decreased expression of *Cox1* gene. The concentration of 0.5 showed the largest decrease among albendazole concentrations of 0.022-fold change (approximately 2.2% of the control level), as the concentration of 0.5 had the lowest expression of 160.452 (Table 4 and Figure 5). Although the results of the statistical analysis showed significant differences at $P \leq 0.05$.

Table 4. The *Cox 1* expression level in *Echinococcus granulosus* with different treatment

Treatment Con.	<i>Cox1</i> expression	Fold change
L 0.5	719.5743745	0.09944206
Albendazole 0.5	160.4522266	0.022173802
Control	7236.116902	1

P ≤ 0.05

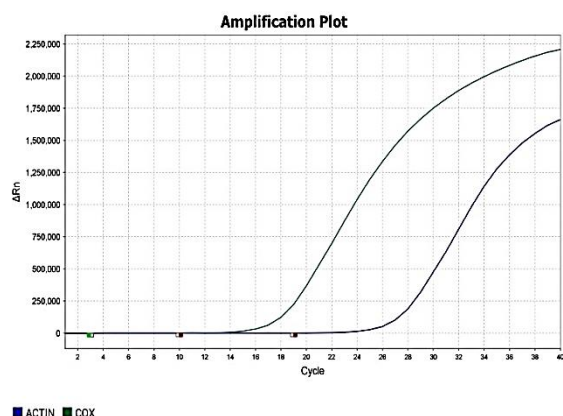


Figure 5. *Cox1*, *Actin* gene amplification scheme by real-time PCR

Discussion

Humans and animals are infected with *Echinococcus granulosus* through the consumption of tainted water and food. This worm can affect both human and animal health, affecting normal animal growth. In severe cases, the disease can lead to the death of infected animals. It may also be a result of the harmful effects of medications and drugs used to treat humans and animals.²⁴ Some chemical treatments have proven effective in treating *Echinococcus granulosus*, but they are associated with toxic effects on humans and animals, in addition to the parasite's resistance to these treatments. Therefore, it is necessary to find alternative treatments for this disease, as numerous studies have indicated the presence of natural compounds with an effective impact on the disease.²⁵

This study relied on the method Ausich and Sanders. (1999), for extracting lycopene with some modifications.¹⁰ The lycopene compound was purified using pure solvents such as hexane and acetone. To verify the purity of the lycopene several tests were performed including TLC, as shown in (Figure 2). A single spot containing no additional carotenoids on TLC was identified as lycopene. The purity of the extract was further tested by conducting a structural analysis. The UV spectrum is shown in (Figure 3), with maximum wavelengths of 447 and 481.5 nm (Table 2). These are the maximum wavelengths of pure lycopene reported in scientific references.²⁶ The purity of the lycopene was

confirmed using FT-IR spectra (Figure 4 and Table 3), which showed that all spectral data indicated that the extract was pure lycopene. Because of its antioxidant properties, lycopene has antibacterial properties. Lycopene is unique among common pigments widely considered to be used in food processing such as flavoring food, in addition to its health benefits. In this current study lycopene extract exhibited an antimicrobial effect against certain microorganisms.²⁷ Particularly the parasite *Echinococcus granulosus*. The results demonstrated that lycopene had effect on it.

The results of the current study demonstrated an inhibitory effect of lycopene, causing decrease in the biological activity of the parasite after treatment with lycopene (Table 4 and Figure 5). This lethal effect of lycopene can be explained by its known mechanisms as an antioxidant and apoptosis inducer in microorganisms in general. It is likely that lycopene induces oxidative stress in the parasite cells affecting the integrity of the cell membrane and the integrity of mitochondria which are vital factors for the survival of the parasite.²⁸ These results are consistent with previous studies that demonstrated the effectiveness of some other plant compounds rich in antioxidants against the parasite *Echinococcus*, confirming the potential of using natural compounds as therapeutic alternatives.²⁹ These results indicate that lycopene has promising potential as a parasiticide paving the way for further studies to evaluate its efficacy in animal models. This biological effect of lycopene was also confirmed at the molecular level through gene expression analysis (Table 4). The study revealed a decrease in the expression of the *Cox1* gene (cytochrome c oxidase subunit 1) a key gene involved in cellular respiration and energy production in the parasite's mitochondria.³⁰ Inhibition of this gene clearly indicates that lycopene interferes with the parasite's vital metabolic pathways leading to mitochondrial dysfunction thereby impeding its growth and causing its death. This finding strengthens the scientific understanding of lycopene's mechanism of action as a potential antiparasitic although the precise mechanism of lycopene's action is currently unclear and requires further studies.

Conclusion

Lycopene a natural compound extracted from some yeast species. This is the first study of its kind in extracting lycopene from yeast. It was used as a natural antibiotic to treat pathogenic species such as parasite (*Echinococcus granulosus*). The results were confirmed through biological experiments and through the results of real-time PCR and how the compound was able to decrease gene expression of the most important vital genes in *Cox 1* gene in the parasite as they showed decrease in expression when treated with lycopene.

Declarations

Funding

Non-Funding

Conflicts of interest

There is no conflict of interest.

Data availability

The data that support the findings of this study are available from (1165) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Basrah health department.

Ethics approval

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of (1165 in Nov. 2022).

Use of AI and AI-assisted technologies in the writing process

The declaration does not apply to the use of basic tools, such as those used for checking grammar, spelling, and references. If there is nothing to disclose, no statement is required.

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