

Enhancement of Antibacterial Property of Ciprofloxacin and Isoniazid against *Pseudomonas Aeruginosa* and *Mycobacterium Smegmatis* in Combination with Extracted Lycopene from *Lycopersicon Esculentum*

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Abstract

Tomato is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components. Tomato contains a variety of phytochemicals such as lycopene, carotene, vitamin-C, quercetin glycosides, and chlorogenic acid and has good health protective effects. The present work is to search antimicrobial activity of lycopene extracted from tomato in combination with ciprofloxacin and isoniazid. The antimicrobial property was evaluated using agar dilution and diffusion method using bacterial cultures *Pseudomonas aeruginosa* and *M.smegmatis*. It was found that extracted lycopene executed good antimicrobial activity against *Pseudomonas aeruginosa* and *M.smegmatis* in combination with ciprofloxacin and isoniazid. An increase in the zone of inhibition was observed by increasing the concentration of lycopene and decreasing the concentration of the drug in combination.

Keywords: Lycopene; Tomato; Extraction; Antibacterial properties

Introduction

The main source of lycopene is tomatoes because tomato products have high concentrations of lycopene, but it is also present in gas, watermelon, apricots, red, grapes, pink grapefruit, pink guava, papaya, peaches, berry and cranberries. It is also one of the most abundant non-vitamin analogues present in human blood from food consumption [1]. Lycopene is one of about 600 naturally occurring carotenoids and is responsible for the red colour in fruits [2]. Carotenoids are natural antioxidants which protect the cells of the body from the harmful effects of oxidation due to free radicals [3]. Lycopene has 13 double bonds, of which 11 are conjugated, resulting in excellent antioxidant properties. The presence of unsaturated bonds in its molecular structure makes lycopene susceptible to oxidants, sensitive to light and heat. Because of its non-polarity, Lycopene is lipophilic, insoluble in water, and can be dissolved

only in organic solvents and oils [4]. It is the most abundant carotenoid in tomatoes, followed by beta-carotene, gamma carotene and other minor carotenoids. The human body cannot produce lycopene so it must be obtained from food sources [5].

Materials and Methods

Extraction of lycopene from different method

Benzene extraction method: 50 gm of tomato paste was taken in 250 mL beaker. It was warmed and added about 20 ml of warm benzene to it. This has been done about 5 times. Benzene was distilled off and lycopene residue was obtained. Residue was recrystallized by ether and weighed [6].

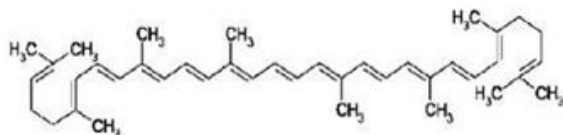
Methanol extraction method: 50 gm paste of tomato was taken and was dehydrated by adding 65 ml methanol. Mixture was immediately shaken vigorously to prevent the formation of hard lumps. After 2 hr, the thick suspension was filtered. The end cake was shaken for another 15 min with 75 ml mixture of equal volume of methanol and carbon tetrachloride and separated by filtration. The CCl₄ phase was transferred to a separatory funnel added 1 vol of water and shake well. After phase separation, the CCl₄ phase was evaporated and the residue was diluted with about 2 ml of benzene.

Using a dropper, 1 ml of boiling methanol was added in portion, then crystal of crude lycopene were appeared immediately and the crystallization was completed by keeping the liquid at room temperature and ice bath, respectively. The crystals were washed 10 times using benzene and boiling methanol [7].

Column chromatography method: To 30 gm of tomato paste was added 50 ml acetone and dehydrated for 4-5 min for the removing all water and water soluble substance. The dehydrated tomato paste was taken in beaker and added 1:1 mixture of di chloro methane and ether and stirring was done for 4-5 min. After stirring added slowly small cubes of anhydrous MgSO₄ for removing the water. The mixture was filtered and taken into a small beaker and solvent was evaporated at room temperature.

Then Lycopene was separated from column firstly by removing the β -carotene and then Lycopene [8,9].

Evaluation and characterization of lycopene



Identification of lycopene

Thin Layer Chromatography (TLC) analysis

Sample details: lycopene

Adsorbent: Precoated silica gel

Solvent system: Pet ether; acetone (40:10)

Sample preparation: Lycopene (1 mg) was dissolved in n hexane (1 ml) and this solution was applied on the TLC plate with the aid of capillary tube.

Detection: Saturated Iodine Chamber.

Procedure: The lycopene was subjected on to the precoated and activated Silica gel TLC plates. (Plates were kept in oven for 1 hr at 70°C) The mobile phase is pet- ether:acetone ratios. After the TLC run and spraying the detecting agent reddish sports of lycopene were identified visually. Rf value was calculated. Rf-value=0.35 (Figure 1).

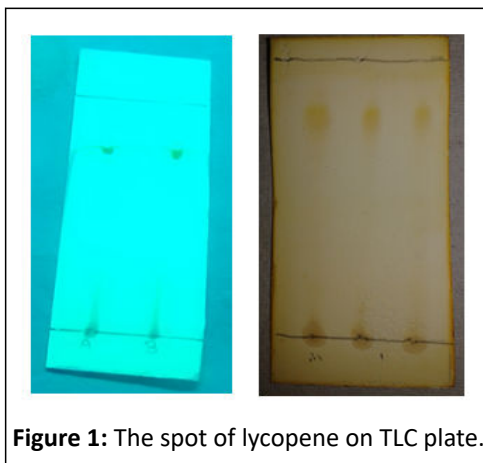


Figure 1: The spot of lycopene on TLC plate.

Ultra violet-spectrophotometer analysis: The 0.01% w/v solution of lycopene in methanol was prepared and λ_{\max} was determined (Figure 2).

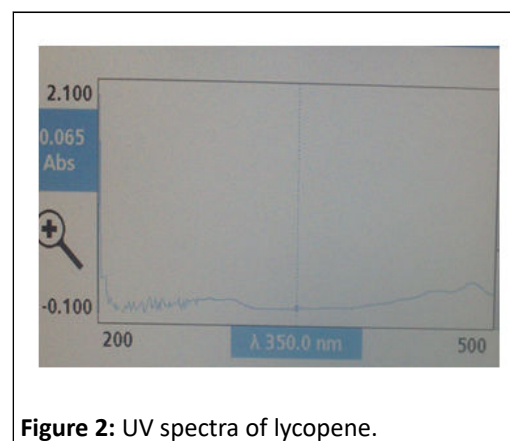


Figure 2: UV spectra of lycopene.

λ_{\max} =475; Absorbance=2.100

Antibacterial activity: Antibacterial activity was studied against two Bacteria *Pseudomonas aeruginosa* (MTCC-3541) and *M.smegma is*. Antibacterial activity of lycopene against *Pseudomonas aeruginosa* (MTCC-3541) (Figures 3 and 4).

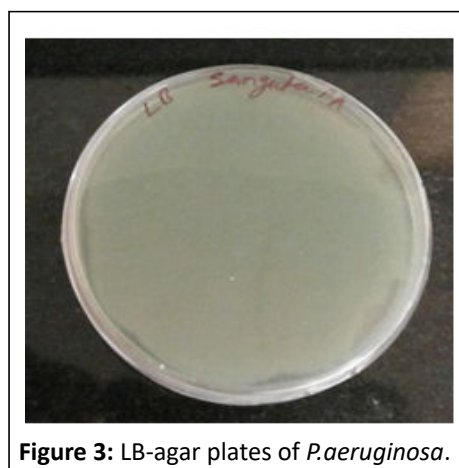


Figure 3: LB-agar plates of *P.aeruginosa*.



Figure 4: Antibacterial activity of lycopene against *P.aeruginosa*.

Antibacterial activity of lycopene against *M.Smegmatis*.

Synergistic effect of lycopene with ciprofloxacin and INH against *P.aeruginosa* and *M. smegmatis*.

By disc diffusion method: Test organism: Gram-negative bacteria *P.aeruginosa* and *M. smegmatis*.

Preparation of sub-culture: One day prior to these testing, inoculations of above bacterial cultures were made in the nutrient agar.

Preparation of base layer medium (nutrient ager): It was prepared by dissolving definite volumes of nutrient LB- agar 2 gm, in 25 ml of distilled water and sterilized by autoclaving at for 20 min.

Sterlization of equipments: Petri dishes, pipette, cork borer sterilized by dry heat sterilized at 160°C for 1 hr in hot air oven.

Preparation of test solution: Isolated lycopene (50 mg) were dissolved in n-Hexane to give a 50 µg/ml of these stock solution was pipette out with the help of micropipette and used for testing.

Table 1: Zone of inhibition of lycopene against *P.aeruginosa*.

Name of bacteria	Plant extract+drug	Conc of lycopene (µl)	Conc of drug (µl)	Zone of inhibition (mm)
<i>P.aeruginosa</i>	Lycopene + Ciprofloxacin	50	50	9
		75	25	13
		66.5	33.5	16

Method of testing

0.1 ml bacteria (*P.aeruginosa*) inoculata was added into different petri dish having sterilized nutrient agar media.

Immediately the cups were prepared with help of cork. In each cup then we added (0.1 ml) of test and std drug solution. Finally it was kept for diffusion in a deep freezer for 2 hr and placed in incubator at 37°C for 48 hrs. After incubation measured the zone of inhibition (Tables 1 and 2) (Figures 5 and 6).



Figure 5: Synergistic effect of lycopene with cipro against *P.aeruginosa*.

Synergistic effect of lycopene with isoniazid against *M. Smegmatis*

Table 2: Zone of inhibition of lycopene with isoniazid against *Mycobacterium smegmatis*.

Name of bacteria	Plant extract+drug	Conc of lycopene (µl)	Conc of drug (µl)	Zone of inhibition (mm)
<i>M. smegmatis</i>	Lycopene+INH	50	50	8
		75	25	18
		66.5	33.5	20

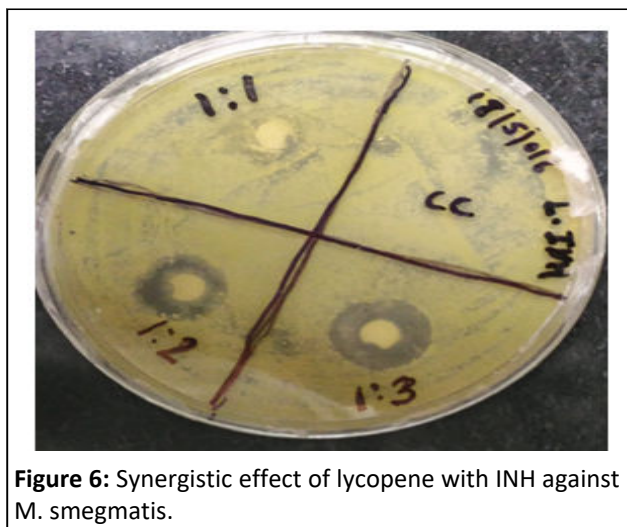


Figure 6: Synergistic effect of lycopene with INH against *M. smegmatis*.

Results

Synergistic effect of lycopene with ciprofloxacin against *P.aeruginosa*

In the present study the antibacterial effect of lycopene with ciprofloxacin on *P.aeruginosa* Table 1. It indicates that zone of inhibition increases as the concentration of lycopene increased. Lycopene has shown an antibacterial activity against gram negative bacteria with zone of inhibition ranged from (9 mm-16 mm).

Synergistic effect of lycopene with Antituberculosis drug (INH) against *M. smegmatis*

The antibacterial effect of lycopene in combination with Isoniazid against *M.smegmatis* is shown in (Table 2). It indicates that zone of inhibition increases as the concentration of lycopene increased. Lycopene has shown an antibacterial activity against *M.smegmatis* with zone of inhibition ranged from (8 mm-20 mm). The combination of Isoniazid with lycopene was effective against *M.smegmatis*, which shows the synergism between Lycopene and drug.

Discussion

The extraction and isolation of lycopene has been carried out using different methods and the yields of lycopene showed to

be high in natural sources of lycopene. A simple liquid- liquid extraction method was employed to extract lycopene in minimum organic solvent. Crystals were purified by recrystallization from ether. The study showed that extracted lycopene by different showed enhanced zone of inhibition with ciprofloxacin and isoniazid against *P. aeruginosa* and *M. smegmatis*.

Conclusion

The experimental report clearly showed that by increasing the concentration of lycopene which has, strong antioxidant and antibacterial property the dosage of synthetic drugs viz ciprofloxacin and isoniazid is reduced, if used in combination. This approach will reduce the cost and increase the effectiveness of the drug. A very detailed and comprehensive study is required to develop this type of combinatorial drug to benefit the society.

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