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# Extraction, Identification and antimicrobial efficiency of lycopene from Tomato (*solanum lycopersicum*) and Red Guava (*Psidium Guajava)*

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**Abstract**

Lycopene, a plant carotenoid naturally synthesized by fruits and vegetables like tomatoes, watermelon, papaya, red guava, carrot and grapefruit. The diet of carotenoids reduced the risk of degenerative diseases such as prostate, bladder, cervix, breast and digestive tract cancer. Thus, the present study was to extract lycopene from two selected fruits such as tomato and pink guava by using methanol and $CCl_4$ as solvent. Our aim was to investigate antimicrobial efficiency of lycopene extracted from Tomato and Red guava against food born pathogens such as *Escherichia coli, Pseudomonas, Salmonella, Klebsiella* and *Bacillus* using agar well diffusion method. Out of these, Antimicrobial activity of lycopene extracted from Red guava shows highest zone of inhibition as compared to lycopene extracted from tomato. The results of the present study indicate that Red guava extracts possess compounds containing antimicrobial properties that can potentially be useful to control food borne pathogens and Identification of lycopene and its morphological study was done by chemical test and microscopic study.

**Keywords:** Lycopene, Tomato (*Solanum lycopersicum*), Red guava (*Psidium guajava*), Methanol-$CCl_4$, Antimicrobial activity.

## Introduction

Lycopene is a carotenoid responsible for the red pigment in many fruits and vegetables. There are more than 600 naturally occurring carotenoids, of this lycopene is the largest and most abundant [1]. Lycopene is found in blood, the reproductive organs, tomatoes and palm oils. [2,3]. Structurally, it is ($C_{40}H_{56}$) a tetrapene assembled from eight isoprene units, composed entirely of carbon and hydrogen. It is a C40 polyisoprenoid compound containing 13 double bonds (Figure 1) [4]. As a polyene, it undergoes cis-trans isomerization induced by light, thermal energy, and chemical reactions. In human plasma, lycopene is present as an isomeric mixture, with 50% as cis isomers. [5,6,7] Natural food sources of lycopene are tomatoes, watermelon, red guava, pink grapes, papaya and apricots.[8]. Many epidemiological studies showed that lycopene rich diet has beneficial effects on human health and reduces the rate of cancer and heart diseases because of the natural antioxidant property which gives protection against harmful free radical .In addition, lycopene is reported to synergistically inhibit low density lipoprotein (LDL) oxidation in combination with Vitamin E.[9] Various researches show that lycopene can be used for the treatment of prostate cancer.[10,11] Thus, it would seem that lycopene is extremely important in the overall role of cancer prevention in the prostate. Besides anticancer activity, it is also beneficial in cardiovascular diseases, osteoporosis, bone health, male infertility, skin protection, age-related macular degeneration prevention, Alzheimer's disease, amyotrophic lateral sclerosis, asthma caused by exercise, immune stimulation, viral diseases and DNA damages [12]. Thus, if the patients of such type of diseases, the quantity of tomato, pink guava , watermelon and papaya in diet then it is more helpful to fight against diseases[13]. Krinsky and Johnson investigated the antimicrobial activity of lycopene was tested in an agar diffusion test for their growth [15]. Inhibitory effect on five Gram negative and three Gram positive bacterial species and yeasts species [16]. All test organisms were inhibited by lycopene. The antimicrobial activity of tomato were tested against pathogenic aerobic and anaerobic bacteria. Lycopene showed the greater activity, the combination of lycopene with antibiotic is synergistic against Acinetobacter calcoaceticus and leads to in difference against anaerobic bacteria.In our study, we choose Red guava (Psidium guajava L.), tomato (Solanum lycopersicum L.) for extraction of lycopene.The present work is to search antimicrobial activity of lycopene extracted from tomato paste and Red guava using methanol-carbon tetrachloride solvent against five food borne pathogens i.e. *Escherichia coli*, *Pseudomonas*, *Salmonella ,Klebsiella and Bacillus* using agar well diffusion method. Identification of lycopene and its morphological study was done by chemical test and microscopic study.

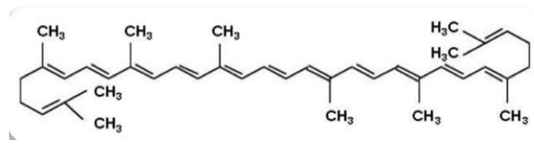


**Figure 1: Structure of Lycopene**

- Formula: $C_{40}H_{56}$
- Molecular weight: 536.873

## Methodology

**SAMPLE:**

Tomato ***(Solanum lycopersicum)*** and Red Guava ***(Psidium guajava )*** was collected from local market of Nagpur city,Maharashtra. These were purchased from the local fruits and vegetable shop, ready for consumption and were transported to the laboratory, clean washed and stored in refrigerator.

**Figure 2: Fruit Tomato *(Solanum lycopersicum*)**

**FRUIT TOMATO**

Botanical Name - Lycopersicum esculentum Miller.
Common Name - Tomato
Family – Solanaceae
Parts Used - Fruits

Tomato, *(Solanum lycopersicum* L.) is one of the most important vegetables, contains a variety of phytochemicals such as lycopene, carotene, vitamin C, quercetin glycosides, naringenin chalcone and chlorogenic acid and have good health protective effects.

**FRUIT GUAVA**
**Botanical Name - Psidium guajava**
**Common Name – Guava**
**Family – Myrtaceae**
**Parts Used – Fruits**

**Figure No.3 Red guava *(Psidium guajava)***

Red Guava *(Psidium guajava)* used for different purposes: hepatoprotection, antioxidant, anti-inflammatory, antispasmodic, anti-cancer, antimicrobial, anti-hyperglycemic, analgesic, endothelial progenitor cells, anti-stomachache and anti-diarrhea.

**Test organisms:**
A total of 5 species (food borne pathogens) were used as test organisms in this study. These organisms were isolated from the soil suspension and the subcultures were maintained on Nutrient Agar slants and were kept at 4°C. The Nutrient Agar slants cultures were periodically transferred onto fresh Nutrient Agar slants.

**Lycopene Extraction Method**

***Methanol extraction method***
50 gm of paste 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 carbon tetra chloride phase was transferred to a separatory funnel, added 1 vol of water with proper shaking. After phase separation, the carbon tetrachloride phase was evaporated and the residue was diluted with about 2ml of benzene. Using a dropper, 1ml of boiling methanol was added in portion, then crystal of crude lycopene was appeared immediately and the crystallization was done by keeping the liquid at room temperature and ice bath, respectively. The crystals were washed 10 times using benzene and boiling methanol. [14] (Figure 4).

**Crystallization method of lycopene**
The solvent is removed completely in vacuum leaving a dark oily residue which is diluted with 2ml of benzene and evaporated again to remove carbon tetrachloride completely. The partly crystalline dark residue is transferred with 1 ml benzene to a 25ml of flask. The flask is immersed in hot water. Boiling methanol added in portion using dropper to benzene solution with stirring until 1 ml methanol has been introduced. The crystal of crude lycopene begins to appear. The crystallization is completed by keeping the liquid at room temperature and then in ice water after 1-2 hrs. The crystals are collected on small funnel and washed with 2ml boiling methanol. The crystals were washed 10 times using benzene and boiling methanol. Long, red lycopene prisms were collected (Figure 5) and observed under the microscope (Figure 6). for another 15 min with 75 ml mixture of equal volume of methanol and carbon tetrachloride and separated by filtration. The carbon tetra chloride phase was transferred to a separatory funnel, added 1 vol of water with proper shaking. After phase separation, the carbon tetrachloride phase was evaporated and the residue was diluted with about 2ml of benzene. Using a dropper, 1ml of boiling methanol was added in portion, then crystal of crude lycopene were appeared immediately and the crystallization was done by keeping the liquid at room temperature and ice bath, respectively. The crystals were washed 10 times using benzene and boiling methanol. [14] (Figure 4)

**Figure 4: (A) Extraction of lycopene from Tomato (B)Extraction of lycopene from Guava**

**Figure 5: (A)Lycopene crystals of Tomato (B) Lycopene crystals of Guava**

**Identification**

**Physical properties:** An ample amount of lycopene was dissolved in the various solvents like water, ethane, acetone, chloroform, benzene, conc. $H_2SO_4$, and hexane.

**Chemical test:** Identification test were performed using colour chemical Reactions.

A. **Reaction with conc. $H_2SO_4$**: In order to identify the lycopene, a few crystals of Extracted Lycopene were dissolved in concentrated sulfuric Acid, imparting an indigo blue color to the solution.

**B. Reaction with chloroform:** In this test, by adding a solution of antimony Trichloride in chloroform to a solution of lycopene in Chloroform, an intense unstable blue colour appeared.
These tests proved the presence of lycopene in the extract.

**Microscopic method:** Transfer 1 drop of lycopene containing solution onto a microscope slide and spread evenly. Examine the slide under a microscope equipped with a calibrated ocular micrometer. Long, red lycopene prisms were observed under the microscope (Figure 6).

**Antibacterial activity**

Prepared Muller Hinton Agar sterilized it by autoclaving and cool it up to $45^0$C then dispensed the media into each of the Petri dish and allow it to solidify. Transferred 1 ml of 24 hrs bacterial broth culture onto Solidified plate labelled as one for red guava and one for tomato and spread it with the help of sterile glass rod. After seeding with bacterial culture make the well at the centre of the media with the help of cork and borer. Then transferred lycopene containing solution to each of the well. Incubate the plates at $37^0$C for 24 hrs and observed plates for zone of inhibition, compared with the control and measured their diameter.

## Results and Discussions

**Extraction of lycopene:** Extraction of lycopene from tomato and red guava was done by methanol extraction method. The result shows that product was obtained. The concentrated product was dried in the hot air oven.

**Physical properties:**

**Table 1: Solubility of Lycopene**

| Solvent | Solubility |
|---|---|
| Water | Insoluble |
| Ethane | Soluble |
| Benzene | Soluble |
| Acetone | Insoluble |
| Ethanol | Insoluble |
| Conc.$H_2SO_4$ | Soluble |
| chloroform | Soluble |
| Hexane | Soluble |

The maximum solubility of lycopene was found to be in acid and nonpolar solvents such as ethane, benzene, chloroform, hexane and insoluble in polar solvents such as water, acetone, and ethanol.

**Chemical test:**

Crystals of Lycopene + **Conc. $H_2SO_4$** = Blue colour

Crystals of lycopene In chloroform + solution of antimony trichloride in chloroform = Blue colour

In both the test, Intense blue colour indicates (positive test) there was lycopene in extracted solution from tomato and red guava respectively.

**Microscopic method:**

Long, red lycopene prisms were observed under the microscope (Figure 6).

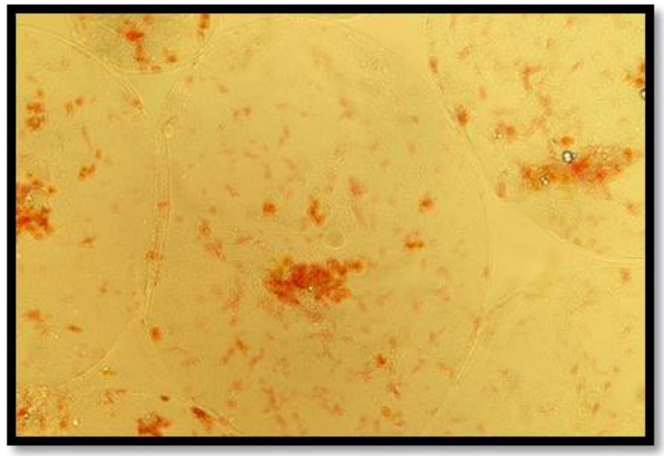

**Figure 6: Microscopic observation of lycopene**

**Antimicrobial activity**

Antimicrobial activity of lycopene was tested against *E. coli, Pseudomonas, Samonella, klebsiella* and *Bacillus* by well diffusion method.

In this study (Table 2) we found that lycopene extracted from guava (Figure 8) were highly effective against Klebsiella, Bacillus and then E.coli, Pseudomonas, Salmonella as compared to lycopene extracted from Tomato (Figure 7) as there was very less zone of inhibition in *E.coli, Pseudomonas, Klebsiella, Bacillus* and no zone in *Salmonella* at all. Thus, Red guava was found that most executed good antimicrobial activity against the tested micro-organism.

**Table 2: Antimicrobial activity of lycopene (Zone of inhibition) extracted from Tomato and red guava by well diffusion method.**

| Organism | Zone of Inhibition (Tomato) | Zone of Inhibition (Guava) |
|---|---|---|
| *E. Coli* | 15 | 17 |
| *Pseudomonas* | 12 | 16 |
| *Salmonella* | No zone | 15 |
| *Klebsiella* | 18 | 22 |
| *Bacillus* | 17 | 26 |

**Antimicrobial activity of lycopene extracted from tomato**

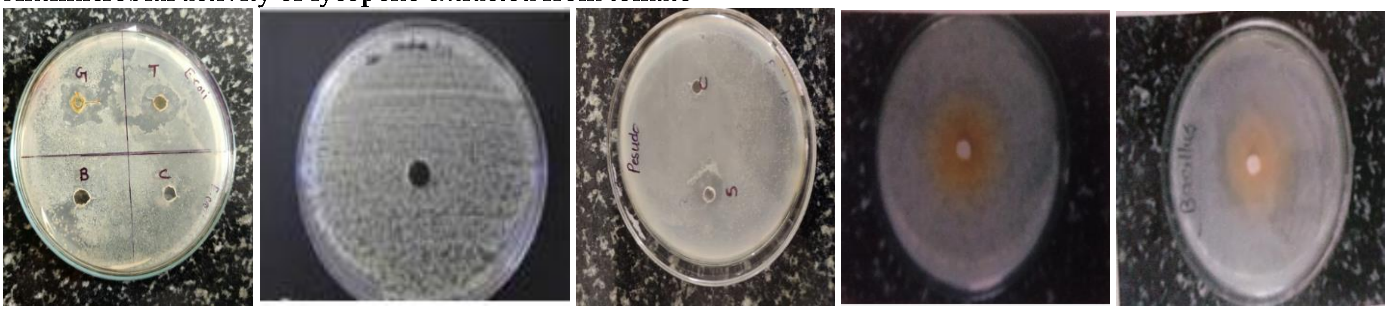

**Figure 7:** Zone of inhibition of lycopene from tomato against *E. coli, Pseudomonas, Klebsiella, Bacillus* and no zone in *Salmonella*.

**Antimicrobial activity of lycopene extracted from Guava**

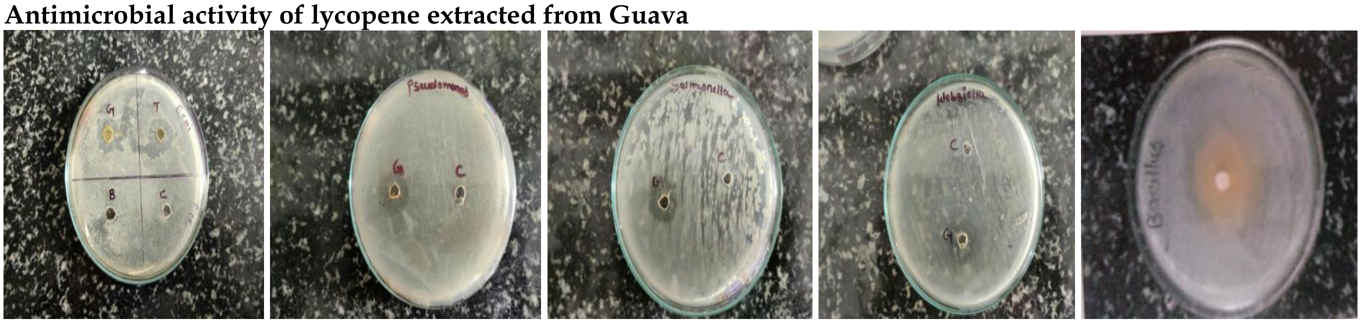

**Figure 8 :** Zone of inhibition of lycopene from Guava against *E.coli, Pseudomonas, Salmonella, Klebsiella* and *Bacillus*.

## Conclusion

In conclusion, simple, convenient, inexpensive extraction method was applied for the isolation of lycopene from tomato and red guava. The extracted lycopene was identified by the physical, chemical test and microscopic study. Anti-microbial activity of lycopene was performed by using agar well diffusion method. From the present study, it can be concluded that lycopene extracted from red guava shows good anti-microbial activity as compared to tomato.

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**Conflicts of interest:** The authors stated that no conflicts of interest.

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