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Isolation of Lycopene from Tomato and Study of Its Antimicrobial Activity

Sohan Sunil Dhanawade[1], Aditya Vikas Sakhare[2]

Abstract: ***The extraction of lycopene was done from tomato. The identification of isolated lycopene was done by using various tests like TLC, U.V spectroscopy, IR. TLC analysis reported the Rf value of lycopene to be 0.4098. The U.V analysis results reported the wavelength which was 472.2nm. The IR analysis was done which results were as follows. Cycloalkane observed value was 2922.97, aromatic compounds observed value was found to be 1564.79, alkane observed value was found to be 1020.99. Antimicrobial activity of lycopene was then studied by using gram-positive bacteria (bacillus substills) the antimicrobial activity of lycopene was found to be 25.***

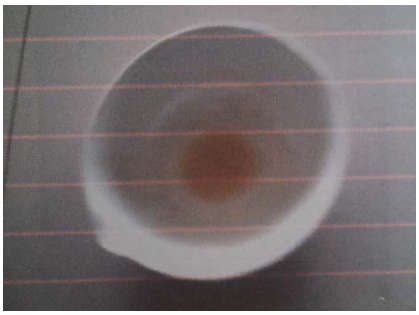
Keywords: Isolation, Lycopene, Tomato, Antibacterial

1. Aims and Objectives

- To explore traditional fruits chemical and therapeutic profile.
- Extraction of lycopene by using different fruit's.
- Extraction of lycopene from different fruit and identification by TLC, UV, spectroscopy, IR.
- To study the Anti-microbial activity of lycopene.

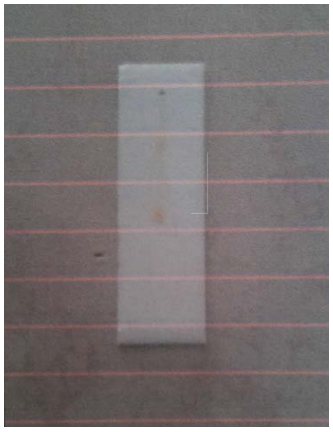
2. Materials and Methods

Isolation of lycopene from tomato:



Fifty grams tomato paste was dehydrated by adding 65 ml methanol. This mixture was immediately shaken vigorously to prevent the formation of hard lumps. After 2hr the thick suspension was filtered, the dark red cake was shaken for another 15 min with 75ml mixture of equal volume of methanol and carbon tetrachloride and separated by filtration. The carben tetrachloride phase was transferred to a separator funnel, added one volume of water and shaken well. After phase separation the carben tetrachloride phase was evaporated and the residue was diluted with about 2ml of benzene. Using a dropper, 1 ml of boiling methanol was added in portion, then crystals of crude lycopene 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.

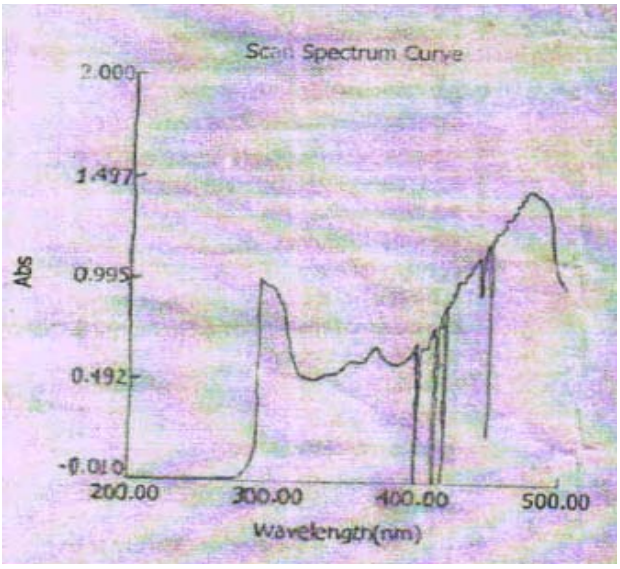
1) Identification test of lycopene from tomato: -
TLC:



- Retardation factor:

Mobile phase – methanol: chloroform – 9.5: 0.5
Formula: retardation factor= distance travelled by solute / distance travelled by solvent
= 2.5 /6.1
= 0.4098

- UV- spectra of lycopene:

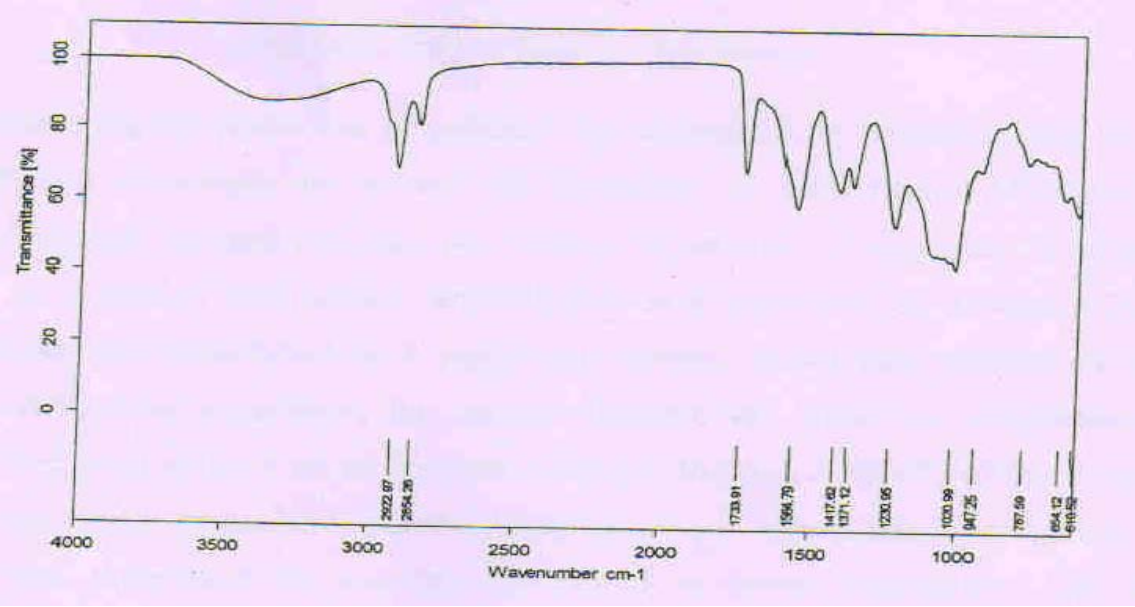


Scan spectrum curve of lycopene tomato

UV- Spectrum is shown in fig the maximum wavelength is 472.2nm, which is nearer the maximum wavelength of pure lycopene reported in the literatures.

- Structure of lycopene: -

- IR- spectra of lycopene:



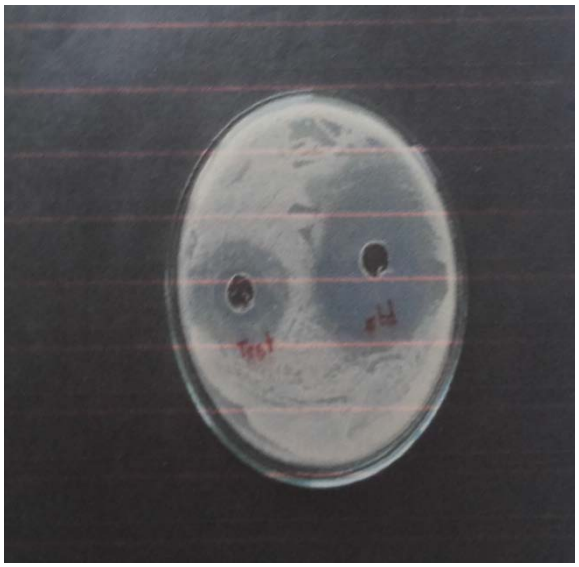
- The presence of peak at 2922.97 in the graph shows the structure –C-H- is present in the sample.
- The presence of peak at 1564.79 in the graph shows the structure –C=C- is present in the sample.
- The presence of peak at 1020.99 in the graph shows the structure –C-C- is present in the sample.

2) Antimicrobial Activity

Gram- Positive bacteria- Bacillus Subtilis

- **Preparation of sub-cultre:** one day prior to these testing, inoculations of above bacterial cultures were made in the nutrient agar.
- **Preparation of base layer medium (nutrient agar) :** It was prepared by dissolving definite volumes of nutrient agar 1.68gm, in 30 ml of distilled water and sterilized by autoclaving at 15 p.s.i for 20 min.
- Sterilization of equipments: petri dishes, pipette, cork borer, test tubes were sterilized by dry heat sterilization at 160°C for 1hr in hot air oven.
- **Preparation of test solution:** isolated lycopene (50mg) were dissolved in chloroform to give a 50µg/ml of these stock solution was pipette out with the help of micropipette and used for testing.
- **Method of testing:** Firstly we prepared sterilized nutrient agar medium and poured into the petridish (25ml). After that near about (0.1ml) bacterial innoculam was added (bacillus sustillis) into different petridish.
- Immediately the cups were prepared with help of cork borer (2cups). In each cup then we added (0.1ml) of test and std drug solution. Finally it was kept for diffusion in a deep freezer for 2hrs. It was removed and placed in incubator at 37°C for 48hrs. After incubation, the zone of inhibition was measured in mm and is reported. Ciprofloxacin was used a standard drug.

Zone of inhibition of lycopene against Bacillus Substilis: -



Antibacterial activity

Organism	Zone of inhibition in mm	
	Standard	Test
Bacillus Substilis	40	25

3. Result and discussion:

Lycopene is a natural source of antioxidants. The methanol extract of tomato studied for antibacterial activity by cup plate method. The antibacterial activity against Bacillus Substilis show better results. The growth of bacillus substilis was inhibited about 25mm i.e. zone of inhibition. The isolated lycopene were analysed by using following

- TLC showed following results

Compound	Rf value
Lycopene	0.4098

- UV- Analysis of lycopene

Compound	Maximum wavelength
Lycopene	472.2mm

- IR analysis of lycopene

Functional group	Observed range	Standard range
Cycloalkane (-C-Hstr)	2922.97	2700-3300
Aromatic (C=C str.)	1564.79	1450-1600
Alkane (-C-C-)	1020.99	800-1200

Above results indicates that pure lycopene were separated successfully.

Antimicrobial activity of lycopene against Bacillus substilis:

Organism	Zone of inhibition in mm	
	Standard	Test
Bacillus Substilis	40	25

4. Summary and Conclusion

The antibacterial activities of lycopene were examined against bacillus substillis. Antimicrobial activity was investigated by disc and agar well diffusion method. The lycopene showed effective inhibition against only the bacillus substilis. Therefore the lycopene can be considered to be the promising source of antimicrobial compounds.