EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 9
353-356
Year: 2016

ANTIBACTERIAL PROPERTY OF LYCOPENE EXTRACTED FROM LYCOPERSICON ESCULENTUM AGAINST PSEUDOMONAS AERUGINOSA

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Article Received on 02/07/2016 **Article Revised on 23/07/2016** **Article Accepted on 14/08/2016**

ABSTRACT
Tomato, Lycopersicon esculentum 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. The present work is to search antimicrobial activity of lycopene extracted from tomato paste using different solvent against, Pseudomonas aeruginosa, a gram negative bacteria. By agar dilution test it was found that most of the extracts executed good antimicrobial activity against the tested micro-organism.

KEYWORDS: lycopene, tomato, extraction, antibacterial property.

INTRODUCTION

Lycopersicon esculentum, tomato is a rich source of wide variety of antioxidants including vitamin E, ascorbic acid, carotenoids, flavonoids and phenolic compounds. Lycopene is the major antioxidant of tomato. Antioxidants give protection against harmful free radicals and reduce rate of cancer and heart disease[1].

It is also one of the most abundant non-vitamin analogues present in human blood from food consumption. Lycopene is one of about 600 naturally occurring carotenoids and is responsible for the red colour in fruits (Cámara et al., 2013; Roh et al., 2013)[2].

Carotenoids are natural antioxidants which protect the cells of the body from the harmful effects of oxidation due to free radicals. Lycopene is one of the popular pigments highly accepted by food industry as a food additive and also for its health benefits [3,4].

The lycopene molecule is a 40 carbon acyclic carotenoid, with a molecular mass of 536 Daltons. It consists of only hydrogen and carbon atoms and is one of the carotenoids synthesized by plants and photosynthetic microorganisms. Lycopene has 13 double bonds, of which 11 are conjugated, resulting in excellent antioxidant Properties, which is an important feature in the carotenoids responsible for their attractive colors because it forms the light absorbing chromophore. However, the unsaturated bonds in its molecular structure make lycopene susceptible to oxidants, Sensitive to light and heat.

The biochemistry of lycopene is unique as it has no pro-vitamin A activity, in contrast to other carotenoids such as alpha-carotene and beta-carotene. Because of its non-polarity, lycopene is lipophilic, insoluble in water, and can be dissolved only in organic solvents and oils.

The human body can not produce lycopene so it must be obtain from food sources lycopene to provide protection against different types of cancer.[5]

The number of infection which are caused by multi drug resistant gram positive and gram negative becteria are life threatening for human being. Infection caused by these organisms pose a serious challenge to the scientific community and need for a effective therapy has need for novel antimicrobial agents. Consumption of tomatoes and tomato-based food products reduce the risk of cancer (oral cavity, pharynx, esophagus, stomach, rectum, colon, urinary bladder, prostate and breast) in humans[6-8].

MATERIAL AND METHODS

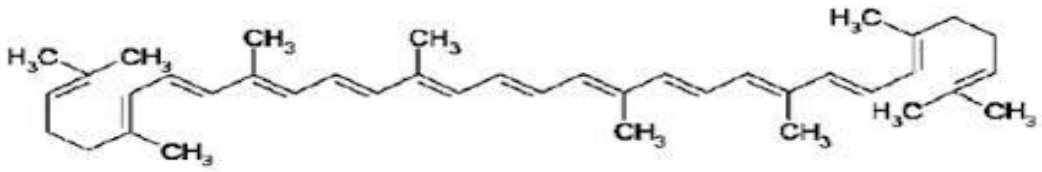
Extration of lycopene using different methods- Different extraction and quantization methods for lycopene are recorded in the literatures [9-12].

(1) BENZENE EXTRATION METHOD- In a 250 ml beaker 50 gm of tomato paste was taken. Paste was warm and about 20 ml of warm benzene was added to it. Stir and decant the benzene. This has been done about 5 times. Then benzene was distil off and residue of lycopene was obtained which was recrystallized by ether and weighed. [2]

2) METHANOL EXTRATION METHOD- 50 gm of paste was dehydrated by adding 65 ml methanol. Mixture was immediately shaken vigrously 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 sepration, 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 crystalization was done by keeping the liquid at room temprature and ice bath, respectively. The crystals were washed 10 times using benzene and boiling methanol.[5]

3) COLOUMN CHROMATOGRAPHY METHOD- To 30 gm tamato paste was added 50 ml acetone 4-5 min for the removing all water and water soluble substance. The dehydrated tomato paste was taken in a beaker and added 1:1 mixture of dichloro methane and ether and stirrer again 4-5 min. After stirring small cubes of anhydrous magnesium sulphate was slowly added for removing water.The mixture was filtered and taken into a small beaker, solvent was evaporated at room temperature and dried First β-carotene was removed and then Lycopene was separated.

2. Evaluation and Characterization of lycopene



(I) Physical and chemical properties of Lycopene

- Molecular formula $C_{40}H_{56}$
- Molecular weight 536.89 Da
- Melting point 172–175˚C
- Crystal form Long red needles from a mixture of carbon disulphide and ethanol
- Powder form Dark reddish-brown
- Solubility Soluble in chloroform, hexane, benzene, carbon disulphide, acetone,
- Insoluble Insoluble in water, ethanol, methanol
- Sensitivity Light, oxygen, high temperature, acids

II. Identification of Lycopene,

(a) Thin Layer Chromatography (TLC) Analysis

Sample Details: lycopene

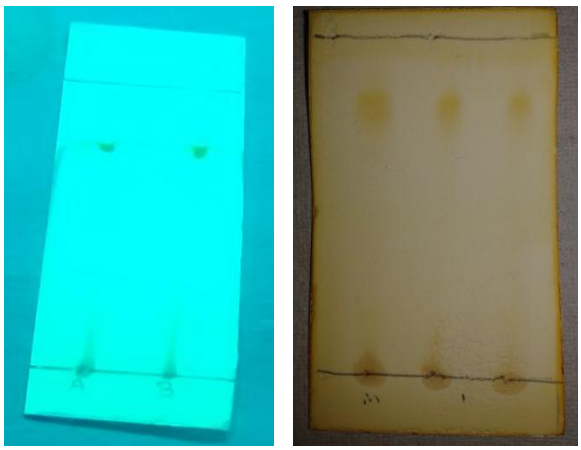
Adsorbent : Precoated Silica gel

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.

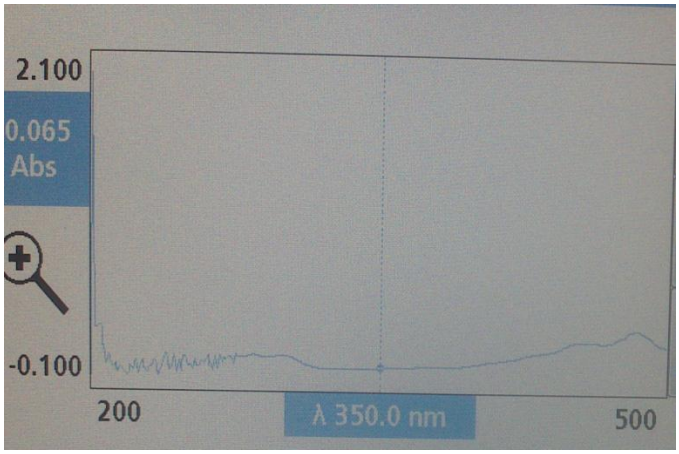
Detection: Saturated Iodine Chamber.

Procedure: The lycopene was subjected on to the precoated and activated Silica gel TLC plates. (Plates were kept in oven for 1hr at 70°C). The mobile phase is pet- ether: acetone ratios. After the TLC run and spraying the detecting agent redish spots of lycopene were identified visually. R_f value was calculated. R_f-value =0.40



b. Ultra Violet- Spectrophotometer Analysis

The 0.01% w/v solution of lycopene in methanol was prepared and λmax was determined.[13]



λmax	Absorbance
475	2.100

III. Antibacterial activity

Antibacterial activity was studied against Gram negative bacteria pseudomonas aeruginosa (MTCC-3541)

Procedure: The in vitro antibacterial activity of the methanol benzene and acetone extracts of lycopene was carried out by agar dilution method.

In first plate we took LB[1.5gm] +agar[0.5gm] in 25ml distill water. After autoclave spreading of Pseudomonas aeruginosa (MTCC-3541)was done and results were noted down next day (fig.a).

In second plate we took the LB[1.5gm] agar+[0.5gm] in 25ml distill water and + lycopene(2.5ml). After autoclave, spreading of bacteria pseudomonas aeruginosa (MTCC-3541), results were noted down next day(fig.b).

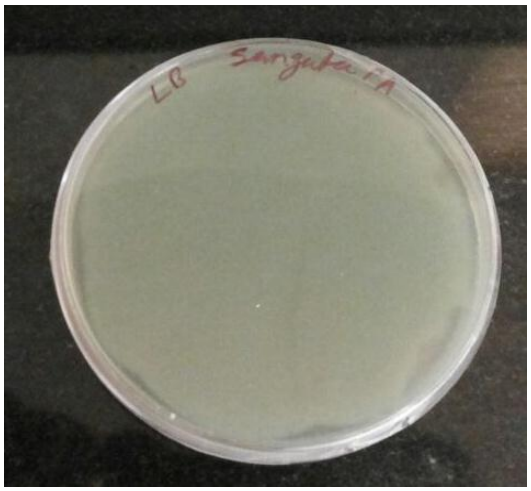
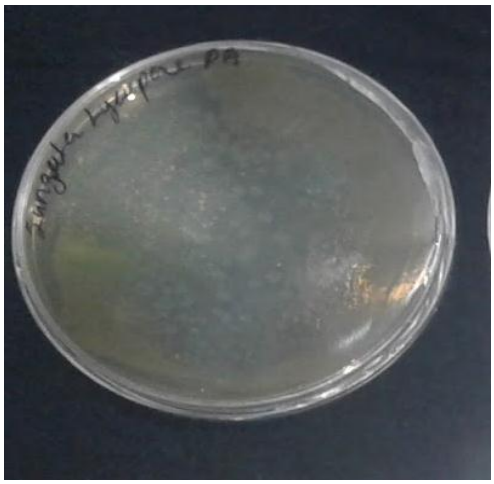


Fig.(a) LB-agar plates of P.aeruginosa



Fig(b) Antibacterial activity of lycopene against P. aeruginosa-

DISCUSSION AND CONCLUTION

Lycopene was extracted from tomato and identified by UV and TLC. The benzene test initially helps us to identify lycopene in residue. A simple liquid- liquid extraction method was employed to extract lycopene in minimum organic solvent. Crystals were purified by recrystallization from ether. Acetone –petroleum ether extraction resulted in higher crude lycopene yield than hexane extraction. The study showed that extracted lycopene by different methods showed antimicrobial activity against P. aeruginosa

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