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Extraction, Identification and Anti-inflammatory Activity of Lycopene

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ABSTRACT

Lycopene is a carotenoid responsible for the red pigment of many fruits and vegetables. There are more than 600 naturally occurring carotenoids, of these lycopene is the largest and most abundant. Tomatoes and its food products are the most important source of lycopene. The other sources of lycopene are grapefruit, flamingoes, apricot, watermelon, rosehips, pink guava. The intake of carotenoids reduced the risks of degenerative diseases such as prostate, bladder, cervix, breast, and digestive tract cancer. It is a nonpolar, conjugated, lipophilic antioxidant. It has attracted attention due to its biological and physicochemical properties. The lycopene was extracted with methanol and ethyl acetate method. The isolated lycopene was identified done by various Physical test, chemical test, TLC, and colorimetric method. The molecular properties of lycopene was done by molinspiration and chemsketch. The *in-vitro* anti-inflammatory activity of lycopene was studied.

INTRODUCTION

Lycopene is a red carotenoid pigment, C40H56 found in blood, the reproductive organs, tomatoes and palm oils.[1,2] *Lycopersicon esculentum* is considered as one of the best resources of lycopene production, which has an intense red color. It is the most abundant carotenoid in *L. esculentum*, accounting for approximately 85% of the total carotenoids present.[3] Structurally, it is a tetrapene assembled from eight isoprene units, composed entirely of carbon and hydrogen. It is a C40 polyisoprenoid compound containing 13 double bonds.[4] As a polyene, it undergoes cis-trans isomerization induced by light, thermal energy, and chemical reactions.

Figure 1: Ripened tomato

Figure 2: Lycopene powder

In human plasma, lycopene is present as an isomeric mixture, with 50% as cis isomers.[5,6,7] Mango (*Mangifera indica* L), papaya (*Carica papaya*), Blackberry, Watermelon, grapefruit, red pepper, rosehip, and tomato.[8] It is a pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and its products. It is a natural source of antioxidant which has attracted the attention due to its biological and physicochemical properties.

In this study, tomato paste was prepared from fully ripened tomatoes and was dehydrated with methanol, and then lycopene was extracted with methanol-carbon tetrachloride mixture. Pure lycopene was obtained by twice crystallization of the crude product from benzene through the addition of boiling methanol.[9]

It is the main source of the natural antioxidant component which gives protection against harmful free radicals and reduces the rate of cancer and heart diseases. It protects the body by neutralizing the negative effects of oxidants. In the synthesis of vitamin, A lycopene plays an important role and act as an intermediate for carotenoids like β carotene and β cryptoxent in, which influences in development. Regular intake of lycopene-containing food reduces the risk of body tumor especially prostate cancer. Studies have shown that the antioxidants vitamin E, selenium, and lycopene all reduce LDL cholesterol and cardiovascular diseases.[10] 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.

Vegetables and fruits like red tomatoes, red-fleshed watermelon, red guavas and red grapefruit are the main sources of lycopene. It is non-toxic in nature. Average daily intake levels of lycopene range from 0.70 to 25.20 mg/day. Therapeutic dose of lycopene ranges from 6-60 mg daily. Dried tomatoes contain as much as 50 mg of lycopene per 2.2 lbs. Red-fleshed watermelon yields almost 13,000 mcg of lycopene ina 1/4melon wedge. Other red tinged fruits such as guavas and red grapefruit also contain lycopene in the small amount about 1700 mcg in a half of grapefruit.[1]

Structure:

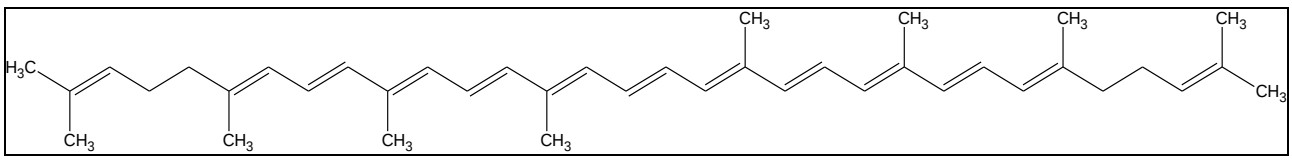

Figure 3: Transform of lycopene structure

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

- Colour: Lycopene is a natural constituent of red fruits and vegetables and of certain algae and fungi.[11,12]

- Source: Ripen red tomatoes provide one of the best sources of lycopene, dried red tomatoes may contain as much as 50mg of lycopene per 2.2lb. Red-fleshed watermelon yields almost 13,000 mcg of lycopene in a ¼ Melon wedge. Other red tinged fruits such as guavas and red grapefruit also contain lycopene in the small amount about 1,700 mcg in a half a grapefruit.[1]

MATERIALS AND METHODS

- Methanol Extraction Method

50 grams tomato paste was dehydrated by adding 75 ml methanol. This mixture was immediately shaken vigorously to prevent the formation of hard lumps. After 2 hr, the thick suspension was filtered; the dark red cake was shaken for another 15 min with the 75 ml mixture of the equal volume of methanol and carbon tetrachloride and separated by filtration. The carbon tetrachloride phase was separated using funnel; added 1 volume of water and shaked well. 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 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 ten times using benzene and boiling methanol.[10,9]

- Ethyl acetate extraction method

The extract is produced by crushing tomatoes into crude tomato juice that is then separated into serum and pulp. The tomato pulp is then extracted with ethyl acetate by vigorous shaking at 3 hrs. The final product is obtained after solvent removal by evaporation under vacuum at 40-60° C.[13]

PHYSICAL PROPERTIES

The excess amount of lycopene was dissolved in the various solvents like water, ethane, acetone, chloroform, benzene, con HCL, con H_2SO_4, ammonia, and hexane

CHEMICAL TEST

- Shinoda's test

The alcoholic solution of flavones or flavonol when treated with metallic magnesium and hydrochloric acid gives an orange, red or violet color

- Reaction with vanillin and hydrochloric acid

The sample treated with vanillin solution in hydrochloric acid gives the red color

- Reaction with alkali solution

The sample treated with alkali solution gives yellow or orange color.

- Reaction with ferric chloride

Sample treated with ferric chloride solution give green or violet color.[14,15]

ANTI-INFLAMMATORY PROPERTY

❖ Protein denaturation method

Phosphate saline buffer was prepared by mixing of 1.7 g of disodium hydrogen phosphate, 1.36 g of potassium hydrogen phosphate and 7.02 g of sodium chloride are dissolved in 1000 ml distilled water.

Take 0.2 ml albumin and 2 to 8 ml of phosphate buffer saline for the PH 6.4. Take 2 ml of varying concentration of sample is added to above solution, incubated at 37◦c ± 2◦c for 15 minutes. Sealed with the cotton plug and incubated at 70^0c for 5 minutes. Then it is kept cool for few minutes.

Control is also performed with albumin, phosphate saline buffer and distilled water. Aspirin is used as the standard concentration in 100 mg/ml. [16,17,18]

Percentage inhibition= absorbance of control – absorbance of sample÷Absorbance of control ×100

RESULTS AND DISCUSSION

EXTRACTION OF LYCOPENE

Extraction of lycopene from tomato was done by methanol extraction and ethyl acetate extraction method. The result shows that maximum product was obtained by methanol extraction method. 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
Concentrated Sulphuric acid	Soluble
Acetone	Insoluble
Chloroform	soluble
Ethanol	Insoluble
Hexane	Soluble
Concentrated Hydrochloric acid	Soluble
Ammonia	Insoluble

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

CHEMICAL TEST

- Shinoda `s test: The test indicates the presence of flavonoids.
- Reaction with vanillin and hydrochloric acid: The test indicates the presence of flavonoids.
- Reaction with alkali solution: The test indicates the presence of flavonoids.

- Reaction with ferric chloride: The test indicates the presence of flavonoids.

Table 2: Anti-Inflammatory Activity by Protein denaturation method

Concentration (µg/ml)	Absorbance (nm)	Percentage Inhibition (%)
100	1.25	23.7
200	1.21	26.21
300	1.20	26.82%
400	1.13	31.09
500	1.1	32.31
Aspirin (1000 µg/ml)	1.64	100
Control	1.64	100

Anti-inflammatory activity of lycopene was performed by using albumin and phosphate buffer. The maximum percentage inhibition is shown in 500µg/ml.

CONCLUSION

In conclusion, a simple, convenient, inexpensive extraction method was followed for the isolation of lycopene from tomato. The extracted lycopene was identified by the physical and chemical test. Anti-inflammatory activity of lycopene was performed by using albumin and phosphate buffer. The maximum percentage inhibition is shown in 500 µg/ml. From the present study, it can be concluded that lycopene derivatives show anti-inflammatory activity.

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