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Studies on Extraction, Isolation and Application of Lycopene

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Abstract: Lycopene is a member of the carotenoid family of chemical substances. Lycopeneis similar to carotenoids is a natural fat soluble pigment. It is principally responsible for the characteristic deep-red color of ripe fruits. It is found in certain plants and micro-organisms, which protect them against the toxic effect. The extraction of lycopene was carried using watermelon. The identification of isolated lycopene observed by using various tests like U.V spectroscopy and TLC. The U.V analysis results reportedhat the lycopene content in watermelon was0.6037 mg/ml. TLC analysis reported the Rf value of lycopene was 0.8857. It is reported that H_2O_2 cause oxidative damage to the DNA and RNA. In this study lycopene employed to protect standard DNA and RNA sample from oxidative damage.It is concluded that lycopene had protective role in oxidative damage of DNA and RNA.
Key Keywords- Lycopene, carotenoids, TLC, spectroscopy, DNA, RNA damage assay.

I. INTRODUCTION

Lycopene is the red pigment compound of watermelon. It received significant attention after a clinical study on human subjects found strong negative correlation between lycopene in blood serum and the occurrence of prostate cancer (Giovannucci*et al*., 1995). Since then, several additional studies on the health benefits of lycopene have found that the regular consumption of a lycopene rich diet can prevent some cancers and cardiovascular diseases (Agarwal and Rao, 2000). Lycopene is the most prevalent carotenoid of the human blood stream and is found in numerous organs such as the prostate, testicles, adrenal gland, pancreas, liver, breast, and skin (Rao and Agarwal, 1999).Most of the studies of lycopene asssay methods have been conducted using tomatoes (Beerh and Sidappa, 1959). However, recently there have been some studies on lycopene assay in watermelon (Fish *et al*., 2002; Perkins-Veazie*et al*., 2001; Davis *et al*., 2003a and 2003b). Lycopene is a highly unsaturated hydrocarbon, $C_{40}H_{56}$, of the carotenoid family (Shashikant*et al* 2011)). Due to its molecular structure, lycopene and other carotenoids react rapidly with oxidizing agents and free radicals. As a result, carotenoids act as natural antioxidants. Lycopene is the most potent antioxidant among carotenoids as it has the highest singlet oxygen quenching rate of all the carotenoids found in biological systems (Di Mascio*et al*., 1989).

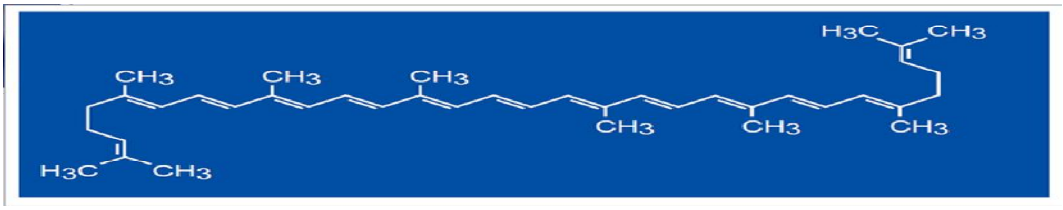

Fig. 1- Lycopene Structure

All aerobic cells generate reactive oxygen species, including superoxide, H_2O_2 and hydroxyl radicals, enzymatically or non-enzymatically. The mitochondrial electron transport chain is the principal site of cellular production of reactive oxygen species.H_2O_2 is one of the primary oxidants in biological systems. It induces damage to the cell membrane and decreases cell viability and reactive oxygen species are also involved in the modification of DNA/RNA bases and the resultant bases such as 2-hydroxyadenine (2- OH-Ade), 8-hydroxyadenine (8-OH-Ade), 5-hydroxycytosine (5-OH-Cyt) and 5- hydroxyuracil (5-OH-Ura) are also found to be promutagenic due to miscoding potential In view of the above mentioned facts and the induction of somatic mutations as a result of DNA adduct formation, oxygen free radicals might be considered as an important class of carcinogens. There is need to find agent which protect DNA/RNA from Reactive oxygen species. (Olinski*et al*., 2002) Due to the increasing popularity of lycopene as one of the important nutraceuticals for use in food and nutritional supplements, scientists are interested in developing lycopene rich products and ingredients by extracting lycopene from tomato, watermelon. So there is need of study which extracts lycopene with less using of organic solvent and also there is need to explore antioxidant potential of lycopene. Protection of DNA/ RNA from oxidative insult by using lycopene is yet to be explored.

II. MATERIALSAND METHODS

A. Sample collection

Watermelon was collected from local market of Nanded District. The fruits of watermelon was washed with tap water and cut into pieces. The inner red fleshy material was used for extraction of lycopene. Benzene (Himedia),Silica plates (MERCK), Lycopene sample and methanol, chloroform, Acetone, Hexane. Standard. DNA and RNA, Tris buffer (30mM, pH 7.4), H_2O_2 (30%), $FeCl_3$ (500M), Agarose (1%) in 1X TAE buffer, EtBr (10mg/ml), Gel loading dye (0.25%)bromophenol blue, 0.25% xylene cyanol, 50% glycerol), 50X TAE buffer (Tris base 24.2g, EDTA 18.612g, glacial acetic acid 5.7ml, in a total volume of 100ml, pH (8.0)

B. Extraction

In present study watermelon paste were prepared. 100 gm paste of water melon was weighted separately. Paste was warmedwith 30ml of warmed benzene. The mixture was, stirred well andbenzene layer was decanted. The step was repeated for about 5 times.Then benzene was distilled off and lycopene residue was obtained.(Lilwani and Nair 2015)

C. Analysis of Lycopene Content

The analysis of lycopene was carried out by using standard formula. The optical density of extracted sample was taken at 503nm against benzene (Bhagat*et al* 2012, Bunghez*et al* 2011)
Absorbance (1unit) = 31.206 × abs. at 503 nm / wt. of sample (g)

D. Thin Layer Chromatography

TLC was performed using crude lycopene (obtained by extraction) .Silica plates were prepared by drawing a pencil line 1cm from the bottom of the TLC plate. Samples were spotted using glass spotters. The organic solvent (9.8:0.2, methanol: chloroform) was used. TLC plate was placed in the tank for 5-10 min. The edge of plate was marked to indicate how far the solvent travelled up the plate. TLC plate was dried in hood, the pigments were marked with a pencil and the plate was analyzed. Rf value were calculated by the formula.(Bhagat*et al* 2012) Formula: - RF value = Distance travelled by solute / Distance travelled by solvent

E. Column Chromatography

Packed chromatography column (a 5ml pipette) was used. 1.5 to 2.0 g of neutral Silica as an adsorbent was added. Gathered all organic solution, 10 mL of hexane (the first eluent), 10 mL of 10:90 (% volume) acetone: hexane (the second eluent), a small Erlenmeyer flask, (for collecting the lycopene fraction), and a beaker before start running the column. The beaker was placed under the column. Hexane was added to the column until the liquid wets all of the silica. Then a lycopene was added extract via sterile pipette to the top of the column little of the hexane to rinse the extract vial and add this to the column as well. As soon as the extract entered the silica layer, filled the column almost all the way with hexane, added hexane as necessary to keep the solvent level in your column relatively constant. When the first yellow band starts to drain out of the column, second eluent was added (10:90 volume acetone: hexane) to the top of the Column and the eluent level was kept constant as before. When the lycopene layer (orange-red) begins to leave the column, the orange-red layer was collected into the Erlenmeyer flask. When the band was almost completely off the column, the sample vial was removed and replaced it with the waste beaker. (Butnaria and Butnarium 2016)

F. Estimation of DNA and RNA Damage

The reaction was carried out in tris buffer (pH 7.4) at 37°C. Each reaction mixture was contained 5µl of DNA and RNA in tris buffer and 5µl of lycopene. $FeCl_3$ (5µl) and 10µl of H_2O_2 were added to test samples and incubated at 37°C for 15 minutes for DNA .To the reaction mixture, 0.06 ml of gel loading dye was added and electrophoreses in 1% agarose gel containing 3µg/ml EtBr, at 100V for 15 minutes. Gels were viewed under transilluminating UV light and photograph was taken. (Wang and Shi 2004)

III. RESULTSAND DISCUSSION

A. Extraction of Lycopene from Watermelon

Fig. 2 - Extraction of lycopene from watermelon

Whereas; **a** = Lycopene, **b** = Benzene + watermelon extract, **c** = Watermelon pasteOrange-red colourLycopene has been extracted fromwatermelon.

B. Lycopene Content

The extracted lycopene was calculated using standard formula
Absorbance (1unit) = 31.206 × abs. at 503 nm / wt. of sample (g)
Lycopene content was 0.6037mg/ml

C. TLC Analysis

The thin layer chromatography was analysedusing methanol and chloroform as solvent. The Rf value was calculated using formula. TheRf value of lycopene was 0.8857.

D. Column Chromatography

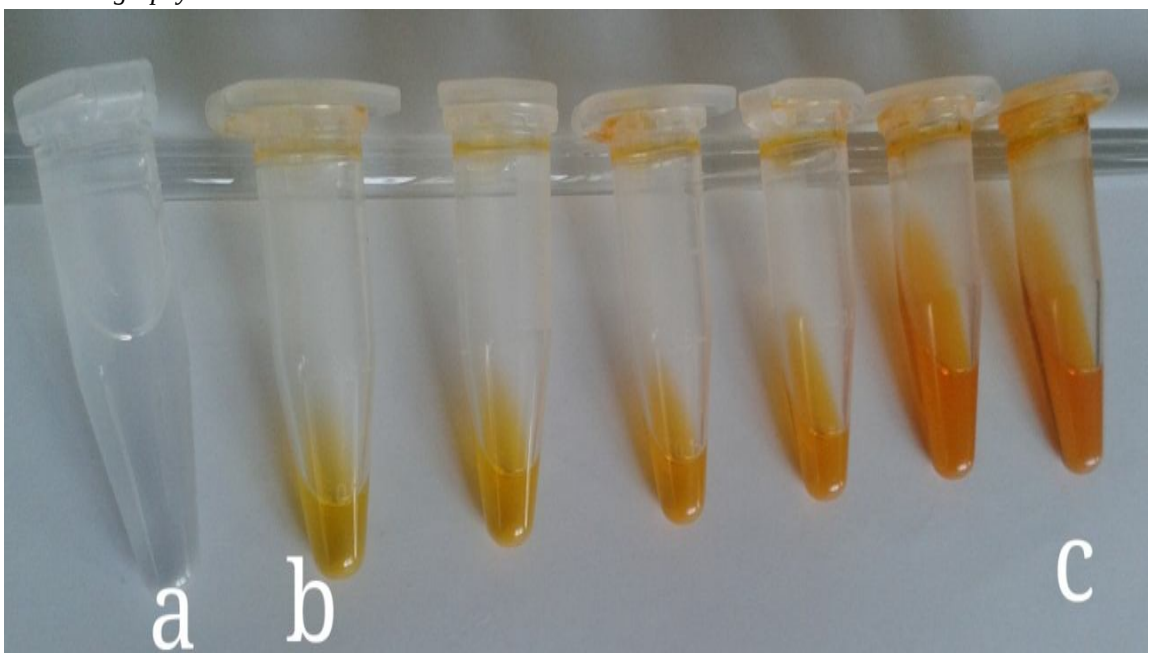

Fig.3 – Fractions of lycopene after column chromatography

Whereas; a = Hexane, b = yellow xanthophylls, **c** = orange- red lycopene

Lycopene, with its 13 double bound, was attracted to silica gel more strongly than beta- carotene and related carotenes, which have 11 to 12 double bond. Therefore, the yellow carotene bond moved down the column faster than the orange-red lycopene band. Yellow xanthophylls pigment trails behind the lycopene band because they contain polar hydroxyl group that was strongly attracted to silica gel.

E. Estimation of DNA damage assay

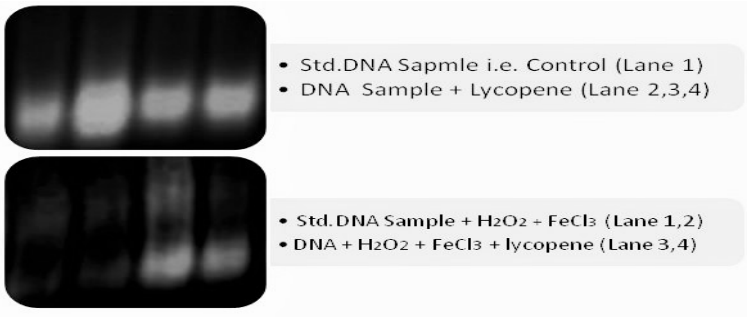

Fig.4 – DNA damage assay

The lycopene antioxidant property was found to be effective against free radical of H_2O_2 that damage DNA. As per observation, first band contain standard DNA as a control. Band (in second figure) first and second band contain DNA, H_2O_2 and $FeCl_3$ in that shows damage DNA due to hydrogen peroxide which has very low frequency compared to control sample. Band third and fourth contain DNA, $FeCl_3$, H_2O_2 and lycopene. (Fig.4)

F. Estimation of RNA damage

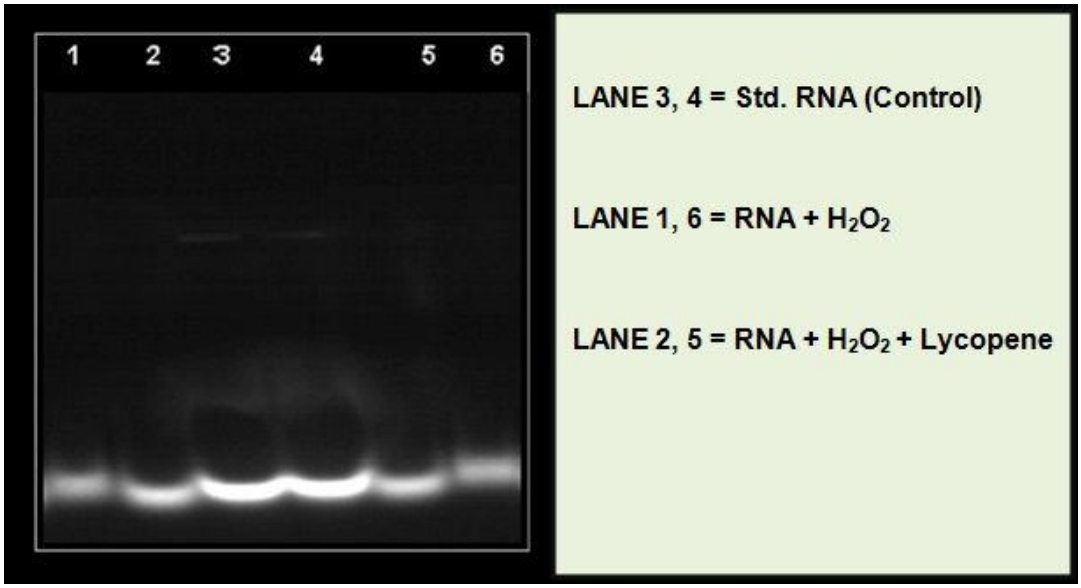

Fig.5 – RNA damage assay

As per observation, third and fourth band contain standard RNA as a control. Band first and sixth contain RNA and H_2O_2 in that shows damaged RNA due to hydrogen peroxide which has very low frequency compared to control sample. Band second and fifth contain RNA, H_2O_2 and lycopene having more intensity compare to damage one. (Fig.5)

IV. CONCLUSION

The following conclusions were drawn from the results of the study. Lycopene was extracted from watermelonby liquid - liquid extraction method using organic solvent benzene. Tomato extract was purified by using column chromatography; silica gel was used as stationary phase. The yield of lycopene was achieved maximum up to 1.3 mg/ml with minimum use of organic solvent i.e benzene only. 2. In DNA and RNA damage assay studied the free radicals that are generated from H_2O_2 cause damage the DNA and RNA. Lycopene protect the DNA and RNA from oxidativebecause lycopene has a great ability for radical scavenging.

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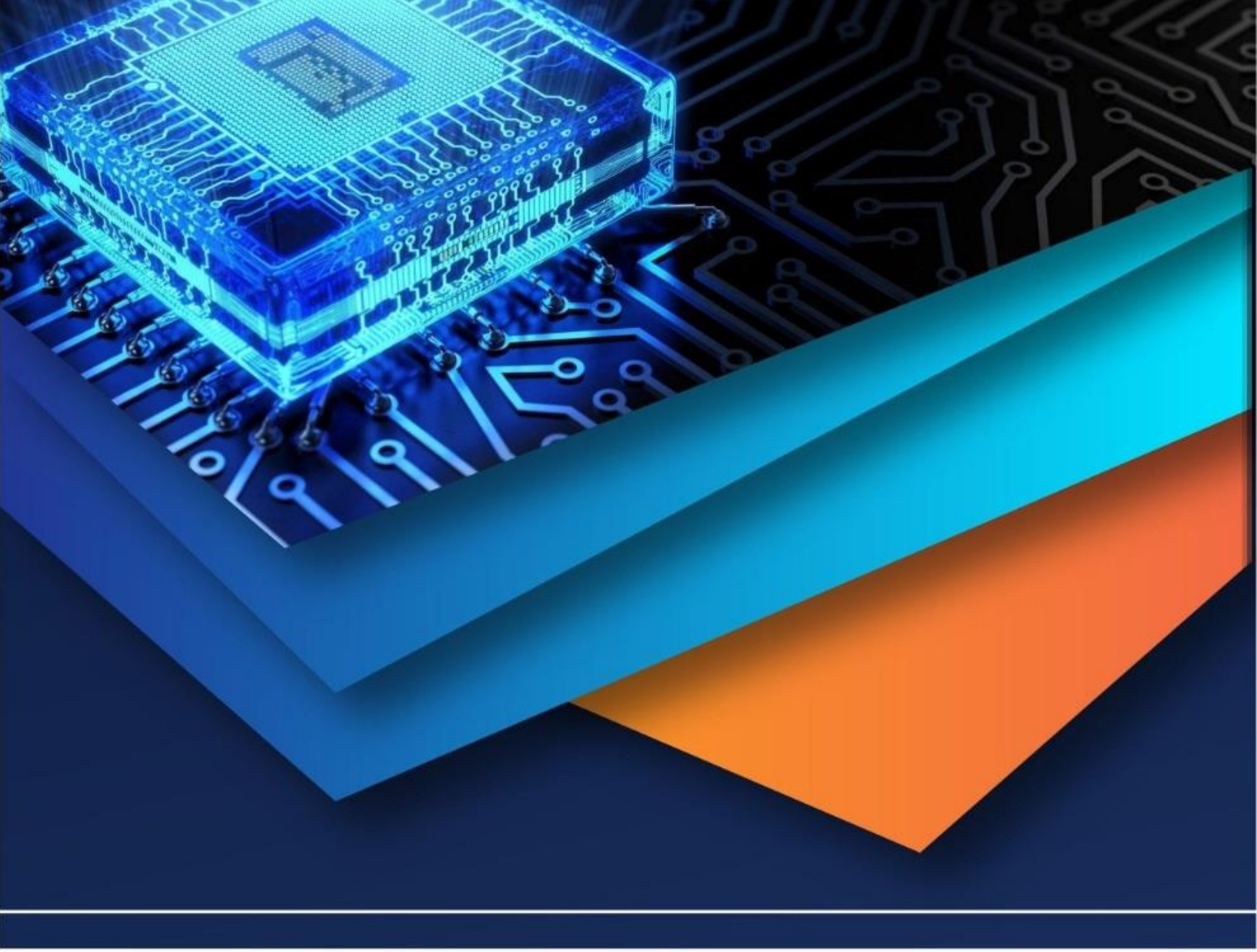

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