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Studies on Isolation and Quantification of Lycopene from Tomato and Papaya and its Antioxidant and Antifungal Properties

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Abstract **– Lycopene is a plant carotenoid that imparts the red pigment to the fruits and vegetables like tomatoes, watermelon, papaya, pink guava, apricots, carrot and red bell peppers. With respect to antioxidant property, lycopene has the highest singlet oxygen quenching activity, thus making it one of the best dietary constituent in combating the ill effects caused by reactive oxygen species. In the present study, investigation was made to evaluate the lycopene content in local varieties of tomato and papaya available in North Karnataka region of Indian origin. Studies were also carried out further to find out antioxidant and antifungal properties of the lycopene. The result showed that the Namadhari variety of tomato contains highest amount of lycopene (14.04mg/100g) followed by Rupali and Pusa ruby variety with 12.79mg/100g and 10.92mg/100g respectively and Solo variety of papaya contains 10.29mg/100g lycopene. As far as antioxidant activity of tomato and papaya, is concerned, it was observed that the papaya contains higher activity compared to tomato varieties studied. However, the extract of tomato and papaya when tested for antifungal activity, both did not show any inhibiting activity against the growth of the fungus *Candida albicans*.**

Keywords **– Lycopene, Antioxidant, Antifungal, Tomato, Papaya.**

I. INTRODUCTION

Lycopene is a plant carotenoid that imparts red pigment to the fruits and vegetables like tomatoes, watermelon, papaya, pink guava, apricots, carrot and red bell peppers. It is naturally synthesized by plants (1). This pigment was first discovered by a French botanist Alexis Millardet in 1876 in the tomato fruit (2). It was later named as lycopene by chemist C. A. Schunck (3). It is a tetraterpene (terpenes consisting of eight isoprene units) and have the molecular structure $C_{40}H_{64}$ (4). Lycopene is found in both *cis* (human blood) and *trans* (plants) forms. It is water insoluble, but soluble in organic solvents like chloroform, hexane, benzene, methylene chloride, acetone, and petroleum ether (4, 5).

Among carotenoids, lycopene is of the great interest due to its high antioxidant activity (6). Reactive oxygen species (ROS) are the unstable compounds like singlet oxygen, hydrogen peroxide, superoxide anion, hydroxyl radical, hydroxyl ion, and nitric oxide. The imbalance of excess of oxidants is referred as oxidative stress which leads to chronic diseases such as, cardiovascular disease(7-11), cancer (6, 12-15) diabetes (16, 17) eye diseases and aging associated ailments (18) and skin damage(19, 20). Lycopene due to its potential antioxidant activity, encounters these singlet oxygen or peroxy radicals and transfers energy between these molecules (18).

Lycopene also exerts potent antifungal activity on *C. albicans* by causing significant damage to the cell membranes of the yeast. The antifungal mode of action of lycopene was understood by examining its action against fungal cell membranes by Fluorescence Activated Cell Sorter (FACS) scan analysis and glucose and trehalose-release test (1). The killing property of lycopene was assessed by conducting a killing-curve assay against *S. aureus* and *C. albicans*, and the results showed that lycopene has antimicrobial activity (1). Lycopene due to its strong color and non-toxicity can be used as a food coloring agent and also as a preservative in place of toxic artificial coloring agents like nitrites, monosodium glutamate (MSG) (21-23). Apart from fruits and vegetables, lycopene is also found in photosynthetic bacteria, fungus (*Blakeslea trispora*) and algae (1, 4, 24). Processed tomato products such as pasteurized tomato juice, soup, sauce, and ketchup contain the highest concentrations of bioavailable lycopene. All fruits and vegetables mentioned above form a staple part of day to day diet; hence it calls for a study to assay the lycopene content in the tomato and papaya varieties available in this region.

II. MATERIAL AND METHODS

Samples of tomatoes were selected from local market of Hubli-Dharwad (North Karnataka) region with distinct morphological characteristics depending on its size, shape, color and locules and they were identified by Horticulture Department of University of Agriculture Science, Dharwad (Fig. 1). One variety of papaya was collected from University of Agriculture Science, Dharwad. Pure culture of *Candida albicans* ATCC 10231, NCIM No: 3471 was procured from National Chemical Laboratory (NCL), Pune. Antibiotics, ketoconazole and fluconazole were used as standard.

A) Lycopene Assay:

One mole of Lycopene when dissolved in one liter light petroleum ether and measured in a spectrophotometer at 503 nm in one cm light path gives an absorbance of 17.2×10^4. Hence, a concentration 3.1206 µg lycopene/ml gives unit absorbance.

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B) *Preparation of Extract for Lycopene Assay* (25)

The samples were cleaned and wiped dry. The weight of the whole sample was noted. The sample was blended using blender to form smooth pulp. Homogenizer was used for complete extraction of lycopene from the weighed amount of sample. 10g of this pulp was weighed and homogenized with 20ml of acetone till the tissue becomes colorless. During the process of homogenization ice cold water was used to maintain temperature. The acetone solution was taken in centrifuge tube and was spin for 1 min at 5000 rpm. Supernatant obtained was collected in a brown bottle. The pellet was re-suspended into 20 ml acetone and the homogenization and centrifugation process was repeated for complete extraction of lycopene from the tissue. Pool the acetone extracts. Separating funnel was set up. Acetone extracts were transferred to a separating funnel and 20 ml of petroleum ether was added to the separating funnel; mix the contents of the funnel gently. The solution in the separating funnel was kept undisturbed for a while. 20ml of 5% sodium sulphate (Na_2SO_4) was then added to obtain clear solution. The volume of petroleum ether was reduced by evaporation hence 20ml of more petroleum ether was added to the separating funnel. Majority of the color will be observed in the upper petroleum ether organic layer. Two layers were separated and the lower aqueous phase was re-extracted with additional 20ml of petroleum ether till the aqueous phase was colorless. Petroleum ether extracts were washed with 10ml of distilled water to remove debris. These petroleum ether extracts were collected in brown bottle containing 10g anhydrous sodium sulphate (Na_2SO_4) and was kept for 30 minutes. The extract was filtered to a volumetric flask containing cotton wool.

C) Lycopene Estimation using UV Visible Spectrophotometer (25)

Absorbance was measured at 503 nm by using petroleum ether as blank and the readings were noted (1ml of lycopene solution was diluted ten times with petroleum ether).

$$\text{Absorbance (I unit)} = \frac{(31.206 \times \text{Absorbance at 503nm})}{(\text{weight of sample in grams})}$$

D) Antioxidant assay:

Potassium permanganate assay ($KMnO_4$) (26)

The assay is based on the redox reactions between the antioxidant sample and the potassium permanganate in the sulfuric acid media that is leading to the discoloration of the potassium permanganate.

Ten clean and dry test tubes of 50 ml were arranged in test tube stand. 0.01 M KMnO4 solutions was prepared. Dilutions of $KMnO_4$ (0.01M) were prepared in series ranging from 0, 0.2, 0.4 till 2ml. 3.5ml of H_2SO_4 (2M) was added and was followed by 20ml of distilled water addition. The unknown was prepared by using previously extracted petroleum ether lycopene solutions that were diluted in 1:10 ratio. 2ml of this unknown was mixed with 1.5ml $KMnO_4$, 3.5ml H_2SO_4 and 20ml distilled water. The contents of tubes were mixed and immediately the absorbance was measured at 535nm. Ascorbic acid of 0.01M was used as standard.

E) Antifungal Activity (4)

The procured pure culture of *Candida albicans* was sub cultured. Sabouraud's medium was prepared and autoclaved at 120°C for 15 minutes. The slants were prepared under aseptic conditions. With the help of sterile nicrome wire loop the pure culture of *Candida albicans* was streaked on slants. These slants were incubated at 37°C for 48hours.

F) Preparation of Extract for Antibiotic Assay:

Tomato samples were blended using blender and were refrigerated at -20°C for storage purpose. The previously stored tomato samples were brought to room temperature. 10g of each tomato sample was weighed and was mixed with 10ml of DMSO (dimethyl sulfoxide). The mixture was homogenized using homogenizer to obtain clear solution. 1g of standard antibiotic was dissolved in 10ml distilled water to give the concentration of 0.1g/ml. Solutions of antibiotics of subsequent concentrations (5, 10, 20, 40, 80, 100 µg/ml) were prepared in distilled water.

Sabouraud's agar medium was prepared and autoclaved at 120°C for 15 minutes. Petri plates were sterilized by wiping it with alcohol and then autoclaving at 120°C for 15 minutes. Approximately 20ml of sterile Sabourauds's media was poured to each Petri plates to give thickness of 3-4 mm. This was performed under aseptic conditions. The agar was allowed to solidify. 200µl of overnight activated candida broth was spread on solidified medium with help of glass spreader. Well of 1.5-2mm diameter were made using sterile borer. Precaution was taken that well did not touch the bottom of plate. Plates were marked with different concentration ranging from 5, 10, 20, 40, 80, 100 µg/ml. 40µl samples and antibiotics of above prepared dilutions were poured to each well as per the markings made on plate. Fluconazole and Ketoconazole were used as a standard. The plates were incubated for 37°C for 72 hours.

III. Results and Discussion

The varieties of tomatoes were identified as Rupali, Pusa ruby and Namadhari based on the fruit shape, size, color and locules at Horticulture Department of University of Agriculture Science, Dharwad. The papaya variety selected was Solo (fig. 1).

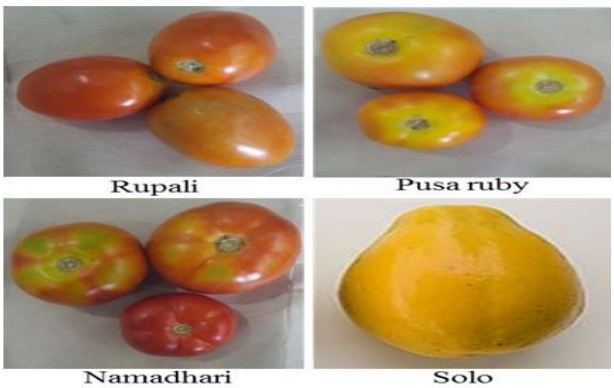

Fig 1: Varieties of tomato and papaya obtained from the local market

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Tomato and papaya are the most prominent source of lycopene and also easily available (17, 24, 27). Hence an attempt was made to evaluate the lycopene content of tomatoes and papaya variety available in Hubli-Dharwad region. In this study lycopene content was found to be highest among Namadhari followed by Rupali and Pusa ruby variety of tomato whereas Solo variety of papaya had least content of lycopene (table 1). Our results are in accordance with reports of other workers indicating tomato has highest nutritional content of lycopene in it (22, 28).

Table 1: Lycopene contents in different varieties of tomatoes and papaya

Sl. No.	Sample	Lycopene (mg/100g)
1.	Namadhari (tomato)	14.04
2.	Rupali (tomato)	12.79
3.	Pusa ruby (tomato)	10.92
4.	Solo (papaya)	10.29

The highest antioxidant activity was seen in Solo variety of papaya. The tomato varieties Namadhari, Rupali and Pusa ruby showed lesser antioxidant activity than Solo variety of papaya. These results are not in accordance with reports of other workers (26) where tomato showed higher antioxidant activity. However when this was compared to standard ascorbic acid, it was seen that all extracts had lesser antioxidant activity than standard ascorbic acid.

The antioxidant assay of lycopene was carried out by using potassium permanganate assay.

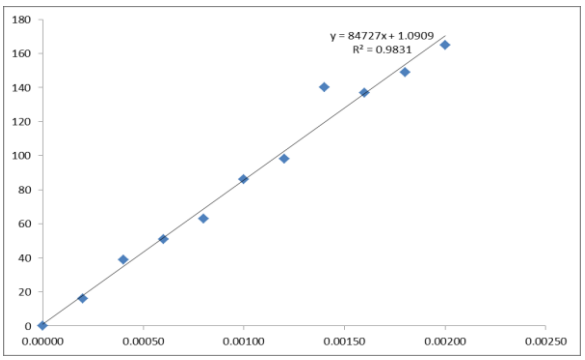

Fig. 2: $KMnO_4$ calibration curve, indicating antioxidant activity of Lycopene

Lycopene has also shown its promising antibacterial effect on *E. coli*, *S. aureus* and antifungal effect on *C. albicans*. The antifungal assay carried out for *C. albicans* using fluconazole and ketoconazole as control did not show inhibition effect on growth *C. albicans*. Antifungal activity was assessed in lycopene extracts of tomato and papaya. However the extract containing approximately 100µg of lycopene failed to show antifungal effect. On contrary standard antibiotic showed effective antifungal activity against *C. albicans*. This lack of reactivity may be due to presence of other metabolites and compounds in the preparation of samples which may hinder the effect of lycopene on *C. albicans* (Fig. 3).

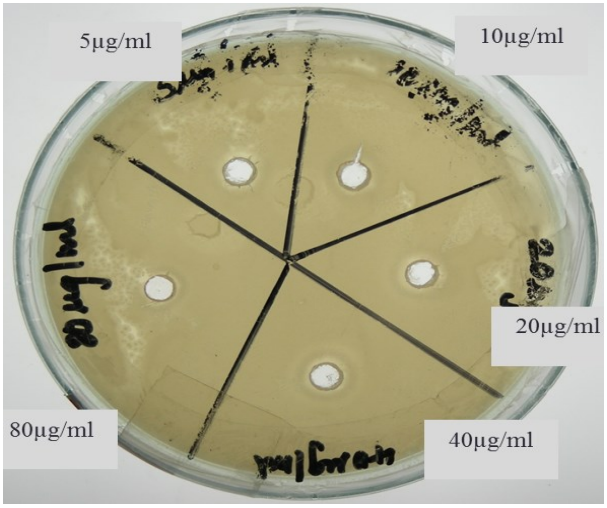

Fig. 4: No antifungal activity against *C. albicans* in varying concentration of Lycopene extract.

Considering beneficial effects of lycopene in regulating oxidative stress due to its antioxidant activity and ability to prevent bacterial and fungal contamination, it is clearly evident that lycopene rich sources should be incorporated into individual's staple diet. Hence studies to assess lycopene content in various food sources should be undertaken to estimate nutritional significance in them.

IV. Conclusion

Lycopene has an efficient antioxidant property which helps in minimizing the risk associated with the ailments of oxidative stress. No studies have been done to estimate the lycopene content from any source in Karnataka. Hence, this study was carried out to assess the estimation of lycopene, and antioxidant and antifungal activity of lycopene. The results point out clearly that tomato is a rich source of lycopene, and also papaya has considerable amount of lycopene. Though lycopene content is less in papaya, it has shown highest antioxidant activity than tomato. But however this antioxidant property was lesser when compared to vitamin C. This calls for using few more methods of antioxidant assay among food sources. Tomato and papaya extract did not show any antifungal activity against *C. albicans* even at a concentration of 100µg/ml. However, the standard antibiotics showed antimicrobial effect on *C. albicans* at 100µg concentration. Antimicrobial effect can also be studied on other fungal species apart from *C. albicans*. In future, such studies are required to assess the lycopene content in other sources such as processed tomato products and other lycopene rich food sources.

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REFERENCES

[1] Sung WS, Lee IS, Lee DG. Damage to the cytoplasmic membrane and cell death caused by lycopene in Candida albicans. Journal of microbiology and biotechnology. 2007 Nov;17(11):1797-804. PubMed PMID: 18092463. Epub 2007/12/21. eng.

[2] Vogele AC. EFFECT OF ENVIRONMENTAL FACTORS UPON THE COLOR OF THE TOMATO AND THE WATERMELON. Plant Physiology. 1937;12(4):929-55. PubMed PMID: PMC439348.

[3] Schunck C. The xanthophyll group of yellow colouring matters. Proceedings of the Royal Society of London. 1904;72(477-486):165-76.

[4] Kong K-W, Khoo H-E, Prasad KN, Ismail A, Tan C-P, Rajab NF. Revealing the power of the natural red pigment lycopene. Molecules. 2010;15(2):959-87.

[5] van Breemen RB, Pajkovic N. Multitargeted therapy of cancer by lycopene. Cancer letters. 2008;269(2):339-51.

[6] Rao AV, Agarwal S. Role of antioxidant lycopene in cancer and heart disease. Journal of the American College of Nutrition. 2000;19(5):563-9.

[7] Palozza P, Catalano A, Simone RE, Mele MC, Cittadini A. Effect of lycopene and tomato products on cholesterol metabolism. Annals of Nutrition and Metabolism. 2012;61(2):126-34.

[8] Bose K, Agrawal B. Effect of lycopene from cooked tomatoes on serum antioxidant enzymes, lipid peroxidation rate and lipid profile in coronary heart disease. Singapore medical journal. 2007;48(5):415.

[9] Karppi J, Kurl S, Ronkainen K, Kauhanen J, Laukkanen JA. Serum carotenoids reduce progression of early atherosclerosis in the carotid artery wall among Eastern Finnish men. PLoS One. 2013;8(5):e64107.

[10] Sesso HD, Buring JE, Norkus EP, Gaziano JM. Plasma lycopene, other carotenoids, and retinol and the risk of cardiovascular disease in men–. The American journal of clinical nutrition. 2005;81(5):990-7.

[11] Sesso HD, Liu S, Gaziano JM, Buring JE. Dietary lycopene, tomato-based food products and cardiovascular disease in women. The Journal of nutrition. 2003;133(7):2336-41.

[12] Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, et al. cis-trans lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiology and Prevention Biomarkers. 1996;5(10):823-33.

[13] Campbell JK, Canene-Adams K, Lindshield BL, Boileau TW-M, Clinton SK, Erdman JW. Tomato phytochemicals and prostate cancer risk. The Journal of nutrition. 2004;134(12):3486S-92S.

[14] Kelkel M, Schumacher M, Dicato M, Diederich M. Antioxidant and anti-proliferative properties of lycopene. Free radical research. 2011;45(8):925-40.

[15] Holzapfel NP, Holzapfel BM, Champ S, Feldthusen J, Clements J, Hutmacher DW. The potential role of lycopene for the prevention and therapy of prostate cancer: from molecular mechanisms to clinical evidence. International journal of molecular sciences. 2013;14(7):14620-46.

[16] Wang L, Liu S, Pradhan AD, Manson JE, Buring JE, Gaziano JM, et al. Plasma lycopene, other carotenoids, and the risk of type 2 diabetes in women. American journal of epidemiology. 2006;164(6):576-85.

[17] Cuevas-Ramos D, Almeda-Valdés P, Chávez-Manzanera E, Meza-Arana CE, Brito-Córdova G, Mehta R, et al. Effect of tomato consumption on high-density lipoprotein cholesterol level: a randomized, single-blinded, controlled clinical trial. Diabetes, metabolic syndrome and obesity: targets and therapy. 2013;6:263.

[18] Rao L, Rao A. Oxidative stress and antioxidants in the risk of osteoporosis—role of phytochemical antioxidants lycopene and polyphenol-containing nutritional supplements. Phytochemicals-Isolation, Characterisation and Role in Human Health: InTech; 2015.

[19] Evans JA, Johnson EJ. The role of phytonutrients in skin health. Nutrients. 2010;2(8):903-28.

[20] Butnariu MV, Giuchici CV. The use of some nanoemulsions based on aqueous propolis and lycopene extract in the skin's protective mechanisms against UVA radiation. Journal of nanobiotechnology. 2011;9(1):3.

[21] Calvo M, Garcia M, Selgas M. Dry fermented sausages enriched with lycopene from tomato peel. Meat science. 2008;80(2):167-72.

[22] Salem RH. Quality characteristics of beef sausages with tomato peel as a colour and functional additive during frozen storage. World Applied Sciences Journal. 2013;22(8):1085-93.

[23] Kim I-S, Jin S-K, Mandal PK, Kang S-N. Quality of low-fat pork sausages with tomato powder as colour and functional additive during refrigerated storage. Journal of food science and technology. 2011;48(5):591-7.

[24] Devitt LC, Fanning K, Dietzgen RG, Holton TA. Isolation and functional characterization of a lycopene β-cyclase gene that controls fruit colour of papaya (Carica papaya L.). Journal of Experimental Botany. 2009;61(1):33-9.

[25] 2Katoch R. Analytical techniques in biochemistry and molecular biology: Springer Science & Business Media; 2011.

[26] Cacig SI, Szabo-Raluca MI, Lupea AXD. Spectrophotometric method for the study of the antioxidant activity applied on Ziziphus jujuba and Hydrangea paniculata aqueous extract. Zbornik Matice srpske za prirodne nauke. 2006 (111):87-93.

[27] Erdman Jr JW, Ford NA, Lindshield BL. Are the health attributes of lycopene related to its antioxidant function? Archives of biochemistry and biophysics. 2009;483(2):229-35.

[28] Alda LM, Gogoasa I, Bordean D-M, Gergen I, Alda S, Moldovan C, et al. Lycopene content of tomatoes and tomato products. Journal of Agroalimentary Processes and technologies. 2009;15(4):540-2.

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