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# ISOLATION, SEPARATION AND DOCKING ANALYSIS OF β CAROTENE AND LYCOPENE FROM VARIOUS FRUITS AND VEGETABLES

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## ABSTRACT

Cancer is the leading cause of death today and mostly in developed countries. Regular consumption of fruits and vegetables is associated with reduced risks of Cancer, Cardiovascular diseases, Stroke, Alzheimer's disease, Cataract etc. Prevention is more effective strategy then is the treatment of chronic disease .Functional foods that contain significant amount of bioactive components that may provide desirable health benefits beyond basic nutrition and play an important role in prevention of chronic diseases. The literature survey revealed that intake of antioxidants like beta Carotene, Vitamin C ,Vitamin E etc reduce oxidative stress induced carcinogenesis .Keeping this in view the study of qualitative analysis of antioxidants β-carotene and Lycopene in various fruits and vegetable sources are investigated. The biological work involved in isolating the antioxidants from different sources like tomato, red pepper, spinach, yellow pepper, carrot and papaya, separating the isolated samples by thin layer chromatography. The docking studies of β-carotene and Lycopene with EGFR receptor was carried out which indicated their effect of mitigating the symptoms of lung cancer. Study showed that the binding efficiency of β Carotene is much better than Lycopene.

**KEYWORDS:** Lycopene, β carotene, antioxidants, EGFR Receptor, Docking studies.

**Introduction:**

Antioxidants are helpful in inhibiting the oxidation process which can produce free radicals. A free radical contains an unpaired electron in an atomic orbital. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. Free radicals attack important macromolecules leading to cell damage. Oxidative stress arises as a result of an imbalance between free radical production and antioxidant defences and is associated with damage to cellular molecules like lipids, nucleic acids and proteins .Reactive oxygen species play an important role in carcinogenesis. Plants and animals maintain complex systems of multiple types of antioxidants such as Glutathione, Vitamin C, Vitamin E as well as enzymes such as catalase, superoxide dismutase and Peroxides. The administration of a mixture of the Beta carotene, Vitamin C, Vitamin E antioxidant s revealed the highest reduction in risk of developing cardiac cancer. Antioxidants can decrease oxidative stress induced carcinogenesis by a direct scavenging of ROS and/or by inhibiting cell proliferation secondary to the protein phosphorylation. B-carotene and Lycopene may be protective against cancer through their antioxidant function. Recent research indicates that antioxidant may even positively influence life span. (V.Lobo et al., 2010)

Lycopene is the red pigment of the tomato, a C40-carotenoid made up of eight Isoprene units making it a tetraterpene and β-Carotene, the yellow pigment of the carrot is an isomer of Lycopene .( Anil Kumar et al.,2014) The epidermal growth factor receptor (EGFR) belongs to the ErbB family of receptor tyrosine kinases (RTK). These trans-membrane proteins are activated following binding with peptide growth factors of the EGF-family of proteins. Evidence suggests that the EGFR is involved in the pathogenesis and progression of different carcinoma types. (Normanno et al.,2006)

The EGFR was the first receptor to be proposed as a target for cancer therapy, and after 2 decades of intensive research, there are several anti-EGFR agents available in the clinic.( Seshacharyulu et al.,2012) These receptors are anchored in the cytoplasmic membrane and share a similar structure that is composed of an extracellular ligand-binding domain, a short hydrophobic trans membrane region, and an intra cytoplasmic tyrosine kinase domain.(Yarden et al.,2001).
Lung cancer is one of the leading causes of cancer-related deaths among both men and women, and there continues to be limited treatment options available for advanced-stage disease. Upon binding of a specific ligand (eg. epidermal growth factor), the normally functioning EGFR undergoes conformational change and phosphorylation of the intracellular domain occurs, leading to downstream signal transduction by various pathways. Depending on the pathway, the end result is cell proliferation or cell maintenance by inhibition of apoptosis (Gillian Bethune., et al 2010)

The present study involves qualitative analysis of antioxidants (β Carotene and Lycopene) in various fruits and vegetables. Isolation of Lycopene and β Carotene from different sources like tomato, Red pepper, Spinach, Yellow pepper, Carrot and Papaya samples, separation of isolated samples using Thin layer chromatography.

Further the docking study of β Carotene and Lycopene with EGFR Receptor indicated their effect of mitigating the symptoms of cancer.

**Materials and Methods:**

Extraction and isolation of carotene and lycopene was carried out as described by L. Jeyanthi Rebecca et al.,2014 with slight modifications.

**Extraction of lycopene:** Lycopene was extracted from tomatoes, red pepper, while β- carotene was extracted from carrots, papaya, yellow pepper and spinach. 25gms of tomatoes, red pepper were smoothed in a blender with acetone. The acetone extract was filtered through a vacuum filtration pump using what Mann no 42 filter paper on a Buchner funnel using acetone until all the colour is extracted to this filtrate, petroleum ether was added in on a Buchner funnel using where two layers separate out. Lycopene gets into upper layer the lower layer is discarded. Ethereal layer containing lycopene is collected; solid Na2SO4 is added and evaporated by an open dish evaporation method. After evaporation the dry residue is re dissolved in petroleum ether/hexane and used for TLC analysis and purified by column chromatography. (Naren Kumar Datta .,2005)

**Extraction of beta carotene:** β- Carotene was isolated from carrots, papaya, yellow pepper and spinach. 25gms of sample was smoothed in a blender with acetone and petroleum ether in 7:3 ratio. After overnight incubation, sample was filtered through what Mann no1 filter paper on a Buchner funnel using vacuum pump until all the colour is extracted and colorless residue remains. The filtrate is then taken in on a Buchner funnel and 20ml of petroleum ether,20ml of 10 % NaCl solution is added. Two layers separate out, lower layer discarded and upper layer is washed with 10ml of distilled water twice. To this a spatula tip of $Na_2SO_4$ is added and evaporated by an open dish evaporation method. The sample was reconstituted using petroleum ether and used for TLC analysis. (D.M. Sahabi et al.,2012) (SK Sawhney et al.,1996)


**Figure 1.Samples and Sample extracts**

**Thin layer chromatography:**

Crude extracts of the sample, were used for TLC analysis. Silica gel plates were used for TLC analysis after activation of the plates at 110° C in a hot air oven. Using a capillary tube, one spot each from the colored crude extract of the six samples (tomato, red pepper spinach, yellow pepper, carrot and papaya) was spotted on the silica gel side of the TLC plate along the pencil line (origin line 1.5cms from the bottom plate) the plate was then developed in a TLC chamber saturated

with petroleum ether and acetone taken in a 7: 3 ratio. As all were colored extracted spots were visible as yellow, green, red and orange bands during the development .All visible spots were circled in pencil and Rf values were calculated to compare with the standard Rf values of Beta carotene and lycopene (L. Jeyanthi Rebecca et al.,2014)

**Docking studies:**

**1.Identification of protein responsible for Lung cancer using Gene Cards:**

**The Gene Cards** database was used to identify the Protein Responsible for Lung Cancer. This is a secondary database containing annotated collection of only Human genes and proteins. The sequence of the protein was also collected from the same database. It has direct link to several other data bases like Uniprot, PDB, CDD, Kegg etc.( Rappaport N et al., 2014) ( Stelzer G et al.,2011)

**2. Retrieval of 3 D structure of the protein using RCSB Protein data Bank:**

**RCSB Protein data Bank** is one of the very few data bases that has the 3D structure script for all the proteins structured till date. The X ray Crystallography and NMB Based structures generated are stored in this database. Each data set is given a unique code called PDB id so as to make its retrieval easy. PDB has not only the structure related data but also the other information like Research Articles related to the structure development, Source of organism, Molecular properties etc. (D.W.Heinz et al.,1993)

**3. Retrieval of pharmacological action using PUBCHEM:**

It is a byproduct of NCBI specific to store the data related to chemicals and Substances. All the chemical structures, related annotation like Molecular Properties, Representation Formats, Bioavailability, Bioactivity, Pharmacological action etc can be obtained from the same data base. (Bolton E et al., 2008)

**4. Calculation of molecular properties using Dundee ProDrg Server:**

It is an online tool that has been developed in order to calculate the molecular properties, ambiguities of the chemicals their various structure formats etc. The server will identify the bond angles, bond lengths, torsions etc in the structures of the molecules. ( A.W.Schüttelkopf et al.,2004)

**5. Docking analysis was done using HEX software:**

**HEX software** it is standalone software used for the molecular docking studies. It performs both Ligand –Receptor as well as Protein -Protein dockings. The docking efficacy can be calculated based on the E total released after the docking calculations. The software being user friendly also has access to change the visualization of the Compounds in the docking pair as per the user's interest. To summarize, hex is automated docking software enabling the user to study the protein ligand and protein -protein interactions. (A.W. Ghoorah et al., 2013)

**Results and Discussion:**

**Thin layer chromatography** of crude extracts of all the samples was performed on silica gel plates using petroleum ether and Acetone (7:3) as mobile phase .Samples formed yellow, orange colored spots on the plate. Rf values were calculated and compared with standard Rf values of β-carotene and lycopene.

**Table 1. Rf values of samples compared to standard Rf Values**

| S. No | Sample Extract | Number of spots | Colour of spot | Observed RF value | Pigment | Standard RF value |
|---|---|---|---|---|---|---|
| 1 | Tomato | 1st spot | Yellow Orange | 0.94 | β-carotene | 0.94 |
| | | 2nd spot | Red Orange | 0.82 | lycopene | 0.81 |
| 2 | Papaya | 1st spot | Yellow | 0.98 | Carotene Yellow | 0.98 |
| 3 | Carrot | 1st spot | Yellow | 0.98 | Carotene Yellow | 0.98 |
| 4 | Yellow Bell Pepper | 1st spot | Yellow | 0.98 | Carotene Yellow | 0.98 |
| | | 2nd spot | Yellow | 0.52 | Xanthophyll | 0.5 |
| 5 | Red Bell Pepper | 1st spot | Yellow | 0.98 | Carotene yellow | 0.98 |
| | | 2nd spot | Red Orange | 0.82 | Lycopene | 0.81 |
| | | 3rd spot | Yellow Orange | 0.97 | α-carotene | 0.97 |
| 6 | Spinach | 1st spot | Yellow | 0.95 | β-carotene | 0.94 |
| | | 2nd spot | Orange | 0.83 | Lycopene | 0.81 |
| | | 3rd spot | Yellow | 0.50 | xanthophyll | 0.5 |

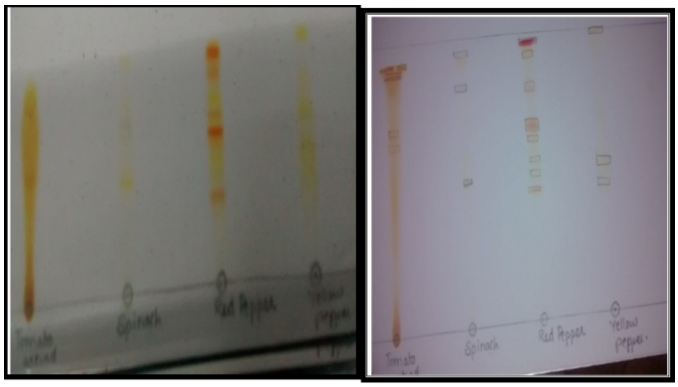

**Figure 2.Thin layer chromatogram of the crude extracts of tomato, spinach, red pepper, yellow pepper, papaya and carrot done in triplicates**

**Docking Studies:**

EGFR is selected as the receptor for the lung cancer study as it is already proved in several research articles. The protein encoded by this gene is a Transmembrane glycoprotein that is a member of the protein kinase super family. This protein is a receptor for members of the epidermal growth factor family. EGFR is a cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine auto phosphorylation leading to cell proliferation. Mutations in this gene are associated with lung cancer. Multiple alternatively spliced transcript variants that encode different protein isoforms have been found for this gene

**Structure of EGFR has been obtained from PDB**

**EGFR Protein study:**

**Protein Sequence of EGFR from NCBI:**

>gi|110002567|gb|AAI18666.1| EGFR protein [Homo sapiens]
MAAEDVIICAQQCSGRCRGKSPSDCCHNQCAAGCTGPRESDCLVCRK
FRDEATCKDTCPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYV
VTDHGSCVRACGADSYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFK
DSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKT
VKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITS
LGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGE
NSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLE
GEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCV
KTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPT
NGPKIPSIATGMVGALLLLLVVALGIGPLHGR

**Structure of EGFR has been obtained from PDB:**

The PDB ID selected was **3QWQ**

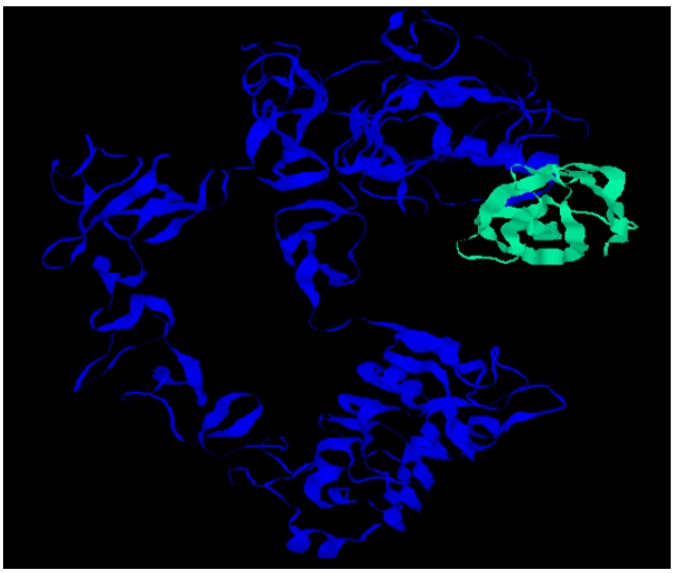

**Figure 3. Structure of EGFR as seen in RASMOL:**

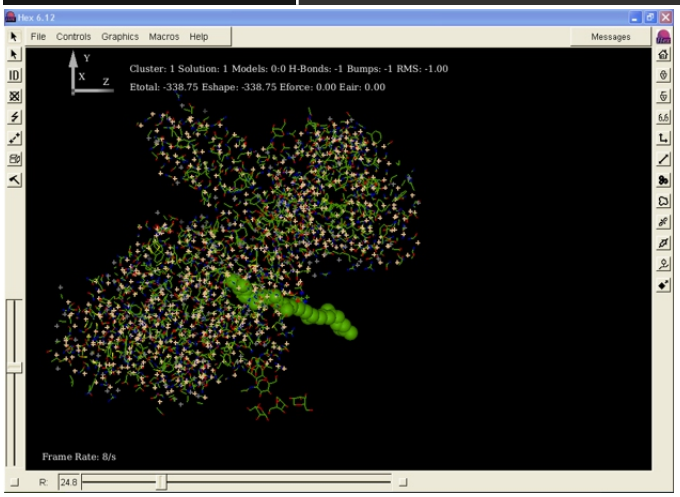


**Figure 4. Docking OF Lycopene (Compound) with EGFR receptor as seen in HEX 6.12:**

**Inference:** From the above docking result it can be inferred that the Lycopene compound binds to the EGFR Receptor with the E total of -338. This is an indication for very good docking pair. This concludes that the EGFR receptor has very good binding/ docking interaction with Lycopene. Thus Lycopene can be used as a very good compound in mitigating the symptoms of Cancer by docking to EGFR receptor

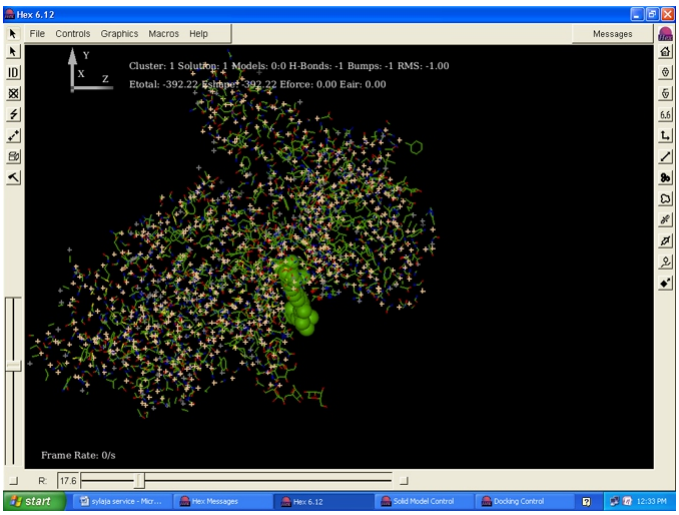


**Figure 5. Docking of Beta Carotene to the EGFR Receptor**

**Inference:** From the above docking result it can be inferred that the Beta Carotene compound binds to the EGFR Receptor with the E total of -392.22. This indicates that the binding efficiency of carotene is much better than Lycopene. Thus Beta carotene can also be used as a very good compound to act against EGFR receptor and mitigate the complications in Lung cancer.

**Conclusion:**

Thin layer chromatography of the crude extracts of tomato showed the presence of β carotene (0.94) and Lycopene (0.82). Papaya and carrot extract showed the presence of yellow carotene (0.98). Yellow bell pepper showed the presence of yellow carotene (0.98) and xanthophyll (0.52). Red bell pepper showed the presence of β carotene (0.94), Lycopene (0.82) and α- carotene (0.97) and Spinach showed the presence of β carotene(0.95), Lycopene(0.83) and xanthophyll (0.50).

The crude extracts were preserved for further purification and spectral analysis. The docking studies of β carotene and Lycopene can be inferred as both of them can be used as a ligand in mitigating symptoms of lung cancer

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