



Research Journal of Pharmaceutical, Biological and Chemical Sciences

Insilico Analysis and Identification of Gene in Annona muricata

Monika Kushwaha*, T Jayalakshmi, PB Ramesh Babu, and P Krishnamoorthy

School of Bio-engineering, Dept of Bioinformatics, Bharath University, Chennai.

ABSTRACT

Annona muricata (Graviola) is a small evergreen, flowering tree native to tropical regions. It consists of prickly fruit which can kill the tumor cells more effectively in comparison to chemotherapy without producing harming normal cells [9]. The Lung Cancer (Bronchoalveolar carcinoma) is the deadly disease which causes more deaths in a year in comparison to other type of cancer. Lung cancer is the condition in which there will be uncontrolled cell growth in the tissues of lungs especially bronchiolar region, and if it will remain undetected it will spread to nearby tissues. Most of the Lung cancer symptoms are being observed in the people with "Tobacco Smoke or Alcohol Consumption" (80-90%). But it can also occur in Passive smokers (10-15%). The major one reason for tumor formation is the malfunctioning of gene involved in normal cell growth (eg. p53, Rb2) due to the chemicals present in Tobacco like Nitrosamines and Polycyclic Aromatic hydrocarbons. The sequence of Annona muricata have been taken and detailed study was made by using the Bioinformatics Tool i.e. Apollo and Biology WorkBench and the databases are collected from NCBI, KEGG. **Keywords:** Graviola, Lung cancer, Nitrosamines, Apollo, Biology WorkBench.

*Corresponding author monika.27bioinfo@gmail.com

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Graviola is a small, upright evergreen tree growing 5 to 6 meters in height with large dark green and glossy leaves. It is indigenous to most of the warmest tropical areas in South and North America including the Amazon [1][9].

It produces a large "Heart shaped" edible fruit that is 6-9", yellow green in color, with white flesh. All parts of the Graviola tree are used in natural medicine in the tropics including the bark, leaves, roots, fruit and fruit-seeds [3][4]. Different properties and uses are attributed to the different parts of the tree. Generally the fruit and fruit juice is taken for worms and parasites, to cool fevers, to increase mother's milk after childbirth (lactagogue), and as an astringent for diarrhea and dysentery [4][6]. The crushed seeds are used as Antibacterial [8], Vermifuge and Anthelmintic against internal and external parasites and worms. The bark leaves and roots are considered Sedative, Antispasmodic, Hypotensive and Nervine and a tea is made for various disorders for those purposes [1].

Lung cancer is the most common cause of death due to cancer in both men and women throughout the world [2]. It is the condition in which there will be uncontrolled cell-growth in tissues of the lung. It widely spreads by the metastasis into nearby tissues if left untreated [2][3]. Lung cancer is basically observed in the old age people; almost 70% of people diagnosed with lung cancer are over 65 years of age, while less than 3% of lung cancers occur in people under 45 years of age. Lung cancer has been increased dramatically for last few years because of "Tobacco smoke" and Some other reasons like exposure to harmful chemicals [6][3].

Types of cancer:

- Non small cell Lung cancer.[10]
- Small cell lung cancer.

METHODS AND MATERIALS

Sequence Retrieval:

NCBI: Data for the area of interest has been collected from NCBI. It develops software tools for analyzing Genomic data and disseminates biomedical information affecting human health and diseases.

Tools used for Genome Annotation & Genome Analysis:

- Apollo: A GUI [graphical user interface]-based tool for editing genome annotations.
- **Biology WorkBench**: A web-based tool for biologists for variety of analysis and modeling tools.
- **CLUSTAL W:** A progressive multiple alignment program.
- TEXSHADE: A web implementation of TeXshade, based on Local alignment of sequences.

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 BOXSHADE: A GIF and postscript plots of pre-aligned multiple sequences works by Global alignment of all sequences and shows the conserved, identical and similar residues.

EXPERIMENTAL PROTOCOL

During Project execution, the following procedural steps have been followed:

- Known gene for Lung cancer of Rattus norvegicus was retrieved.
- The sequence of Annona muricata was retrieved.
- Sequence Analysis:
 - Multiple Sequence Analysis
 - Global and Local Alignment
- Phylogenetic Analysis
- Study of Metabolic Pathways

RESULTS AND DISCUSSION

ANNOTATION RESULTS

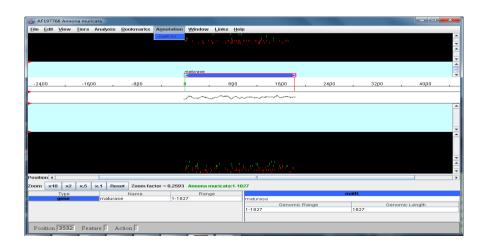


Figure1: Annotation Result of Annona muricata using Apollo Tool

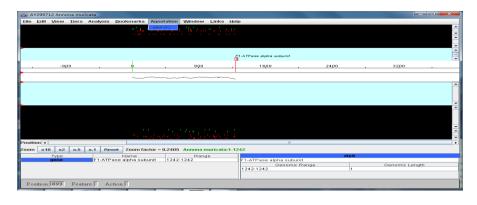


Figure 2: Annotation Result of Annona muricata using Apollo Tool

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TEXSHADE Results (Local Alignment):

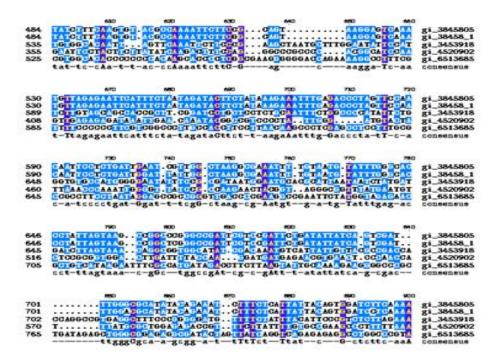


Figure3: TEXSHADE result for Annona muricata

COLOUR	STATUS
Blue	Conserved
Pink	Similar
Purple	All Match
White	Non Conserved



BOXSHADE Results (Global Alignment):



Figure 4: BOXSHADE result for Annona muricata

COLOUR	STATUS
Red	Conserved
Green	Identical
Cyan	Similar

TABLE No. 1: TEXSHADE: LOCAL ALIGNMENT

RATIO
Mostly
Partially

TABLE No. 2: BOXSHADE: GLOBAL ALIGNMENT

RATIO	
Mostly	
Partially	



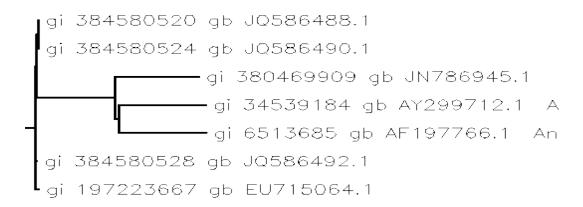


Figure5: Phylogenetic Analysis of Annona muricata

CLUSTAL W (MSA):

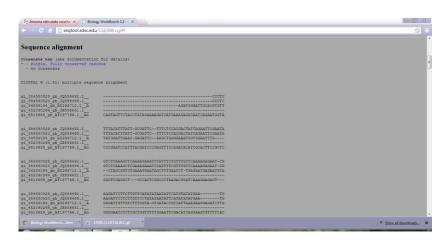


Figure6: Multiple sequence Alignment

DISCUSSION

- From the Results I understood that there could be a cure for lung cancer with no side-effects.
- Malfunctioning of p53 gene which is involved in regulation of normal cell growth that has been caused because of some interruption in Pathway of p53 gene.
- The lung cancer gene "Tumor protein p53" taken as a molecular target for the study.

CONCLUSION

• In the study attempt was made to Detect/Identify inhibitor for "Tumor Protein p53" using database available for Graviola, a natural product.

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Two genes namely "Maturase" and "F1-ATPase alpha subunit" were identified by using Apollo Tool which can inhibit the ATP cycle of tumor cells and can actually help in cure of the cancer without making any harm to normal cell.

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