TO STUDY THE THERAPEUTIC EFFICACY OF ANNONA MURICATA (LAKSHAMANAPHALA) FRUIT EXTRACT FOR ORAL CANCER AND IDENTIFY MOLECULAR TARGETS

Project Reference No.: *47S_BE_1589*

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Keywords:

Cancer, Oral cancer, Green synthesis, Acetogenins, Silver nanoparticles, Cytotoxicity, Gene expression analysis

Introduction:

Cancer is the second leading cause of death worldwide. Cancer accounts for about one in six deaths. Oral cancer poses significant health risks globally, emphasising the need for novel treatment options. Annona muricata L., a tree from the Annonaceae family, is a traditional medicinal plant found and cultivated in subtropical and tropical climates known for its anticancer properties. This study investigates the therapeutic potential of Annona muricata (Lakshmanaphala) fruit extract and Silver nanoparticles synthesised from the fruit extract for oral cancer treatment, focusing on molecular targets. Many phytomedicine and extracts of plant despite of their surprising potential in-vitro finding, exhibit least or no significant in-vivo activity due to their poor solubility, poor lipid solubility and improper size result in poor absorption and bioavailability. Silver nanoparticles offer several advantages that can enhance the anticancer properties of Annona muricata fruit extract. They are effective in cancer treatment due to their small size, which enables them to penetrate cancer cells more effectively, their inherent cytotoxicity against cancer cell lines, and their ability to exhibit synergistic effects when combined with natural compounds, leading to enhanced anticancer efficacy. Green synthesis of Silver nanoparticles (AgNPs) have gained attention in oncotherapy due to their notable In-Vitro and In Vivo antiproliferative effects on cancer cells and malignant tumors, its cost-effectiveness and sustainability. By evaluating Annona muricata acetogenins through computational approach this study aims to elucidate its anticancer properties. Characterization of the fruit extract was done using GC-MS and characterization of silver nanoparticles was done using FTIR and TEM. This study aims to evaluate and compare the cytotoxicity of the crude extract of Annona muricata fruit and its combination with nanoparticles on Oral cancer cell line (KB cell line). AgNPs generate reactive oxygen species (ROS) that modulate oxidative stress in the cancer cells, which is evaluated through ROS assay. Additionally, gene expression analysis

of oral cancer cell lines treated with fruit extract and AgNPs will provide insights into its mechanisms of action at the genetic level.

Objectives:

1. ***Evaluation of Annona muricata acetogenins as a potential anticancer agent through computational approach.***

This involves using in silico techniques such as molecular docking, molecular dynamics simulations, and other bioinformatics tools. These computational tools help predict how acetogenins interact with cancer-related biological targets, evaluate their potential efficacy, and understand their mechanism of action at the molecular level.

2. ***Synthesis of silver nanoparticle of Annona muricata Fruit extract.***

This study focuses on producing silver nanoparticles (AgNPs) using environmentally friendly methods involving fruit extracts. This method offers several advantages over traditional chemical synthesis: it is eco-friendly, cost-effective, and sustainable as it avoids the use of toxic chemicals and harsh conditions. Additionally, the phytochemicals in the fruit extract can also stabilize the nanoparticles, enhancing their functionality and biocompatibility.

3. ***Gene expressions analysis of oral cancer cell line treated with Annona muricata Fruit extract.***

This study aims to evaluate the anti cancer efficacy of Annona muricata by analyzing the changes in the expression levels of cancer specific genes such as EGFR, p53, Bax, Bcl2. By using techniques such as qRT-PCR, we aim to identify which genes are upregulated or downregulated in response to the treatment. The analysis helps to validate the efficacy of Annona muricata fruit extract combined with AgNP as a potential natural anticancer agent by demonstrating its impact at the genetic level.

Methodology:

Preparation of fruit extract:

- The fruit was dried for a week at 60°c in the hot air oven and powdered. The powdered fruit was extracted using soxhlet extraction at 65°c and Rotary dried at 45°c to concentrate the sample. This extract is characterized using GC-MS.

In silico analysis:

- Molecular docking was carried out for four cancer receptors (EGFR, p53, Bax, Bcl2) with Annona muricata Phyto-ligands derived from database as well as GC-MS results.
- Molecular dynamics simulations of 10 ns followed by 50 ns were run using Desmond to check the overall stability and flexibility of the ligand-protein complexes.

Synthesis of Silver nanoparticles:

- The fruit extract was added dropwise to 1mM AgNo3 solution with continous stirring and incubated overnight. The color change to dark brown indicates the formation of silver nanoparticles.
- The synthesized silver nanoparticles were characterized using FTIR and TEM.

Cytotoxic study against KB Cell line:

- The percentage of cell viability was assessed for different concentrations of AgNPs in combination with the methanolic fruit extract by using MTT assay. The IC50 was determined after 24hrs of treatment.

Gene expression analysis:

- The human oral cancer (KB) cell line was used in this study and sourced from NCCS Pune.
- RNA extraction was done using RNA extraction Trizol kit-Genei Labs Lot no. FC642129.
- cDNA synthesis was done using VNIR - Cat no. VNIR109.
- qRT-PCR amplification was carried out using Qiaquant 96.

Conclusion:

GC-MS for the fruit extract revealed multiple phytochemicals out of which those having anticancer potential were identified and used for in silico studies. Molecular docking results revealed that few compounds out of all the screened compounds identified from A. muricata (soursop) had high binding affinity to the receptors. The synthesized nanoparticles were characterized using FTIR and the peak values identified coincided with that of Acetogenins. MTT results indicated that there was a 5-fold increase in the cytotoxicity of fruit extract against Oral cancer cell line when combined with Silver nanoparticles. (IC50 for fruit extract: 184.9 ug/ml; IC50 for fruit extract combined with AgNP: 40.295 ug/ml). Dose dependent treatment was carried out against the cell lines to assess the gene expression at various concentrations of combination of fruit extract and nanoparticles. At IC50 no substantial change in the expression levels was observed. Treatment of cells with 5×IC50 is ongoing.

Scope for future work:

1. Targeted Drug Delivery

Enhanced Efficacy: Nanoparticles can improve the bioavailability and solubility of the active compounds in Annona muricata, allowing for more effective targeting of cancer cells.

Reduced Side Effects: By targeting cancer cells more precisely, these nanoparticles can potentially reduce the harmful side effects associated with conventional chemotherapy.

2. Synergistic Effects

Combination Therapies: Annona muricata-synthesized nanoparticles can be used in conjunction with other chemotherapeutic agents to enhance overall treatment efficacy through synergistic effects.

3. Mechanistic Insights

Apoptosis Induction: Studies suggest that Annona muricata extracts can induce apoptosis in cancer cells. Nanoparticles can facilitate the delivery of these extracts to tumor sites, maximizing this effect.

Anti-Metastatic Properties: Nanoparticles can help inhibit metastasis by targeting cancer cells more effectively, preventing their spread to other parts of the body.

4. Bioactive Compounds:

Nanoparticles can encapsulate bioactive compounds such as acetogenins, which are found in Annona muricata, ensuring their stability and providing controlled release at the tumor site.

5. Biocompatibility and Safety:

Research can focus on ensuring that these nanoparticles are biocompatible and safe for human use, minimizing potential toxicity.

6. Regulatory and Production Challenges:

Scalability: Developing cost-effective and scalable methods for synthesizing these nanoparticles is crucial for widespread clinical use.

Regulatory Approval: Navigating the regulatory landscape to gain approval for new nanoparticle-based therapies will be an important step.