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Evaluating the Antagonistic Activity of Annona Muricata against Bronchogenic Carcinoma and Associated Microbial Infections in the Context of Chemotherapy-An Alternative Therapy Approach

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ABSTRACT: Cancer is a complex disease characterized by the uncontrolled growth and spread of abnormal cells in the body. Natural anticancer drugs have shown potential in treating various types of cancer, with lower toxicity and increased efficacy making them an attractive alternative to traditional chemotherapy. Genetic mutations play a crucial role in cancer development, and changes in genes such as proto-oncogenes, tumor suppressor genes, and DNA repair genes can drive the transformation of normal cells into cancerous ones. Lung cancer, a serious health issue causing severe harm and death, is a significant public health concern, causing a considerable number of deaths globally. Soursop, also known as graviola, contains bioactive compounds such as acetogenins, alkaloids, phenols, and essential oils, which have been studied for their antioxidant, anti-inflammatory, antimicrobial, and antitumor effects. However, chemotherapy-induced immunosuppression is a significant concern in cancer treatment, as it can weaken the body's ability to mount an effective anti-tumor immune response and increase the risk of infections and other complications.

KEYWORDS: Lung cancer, Alternative therapy, Microbial disease, Immuno comprised.

I. INTRODUCTION

Cancer is a complex group of diseases characterized by the uncontrolled growth and spread of abnormal cells in the body. These cells can originate in almost any part of the body;Natural anticancer drugs are a promising approach to cancer treatment, with a long history of use in traditional medicine. These drugs have shown potential in treating various types of cancer, and their lower toxicity and increased efficacy make them an attractive alternative to traditional chemotherapy.(Vander Heiden M.G., DeBerardinis et al. 2017) Genetic mutations play a crucial role in the development of cancer. These mutations can arise from errors during cell division, exposure to environmental carcinogens like tobacco smoke or UV radiation, or inherited genetic predispositions. Changes in genes such as proto-oncogenes, tumor suppressor genes, and DNA repair genes can drive the transformation of normal cells into cancerous ones. (Hanselmann R.G, et al 2016). Lung cancer is a type of cancer that starts when abnormal cells grow in an uncontrolled way in the lungs. It is a serious health issue that can cause severe harm and death. Symptoms of lung cancer include a cough that does not go away, chest pain, and shortness of breath. It is important to seek medical care early to avoid serious health effects. Treatments depend on the person's medical history and the stage of the disease. Lung cancer is a significant public health concern, causing a considerable number of deaths globally... (American- Cancer-Society. Cancer facts & figures 2014.)Sour sop, also known as graviola

The anticancer activity of acetogenins is primarily attributed to their ability to inhibit the mitochondrial complex I enzyme, which plays a crucial role in energy production within cells. (Moghadamtousi S., Fadaeinasab M et al 2015) The compounds present in soursop Annona muricata include a variety of bioactive compounds

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that contribute to its beneficial properties. Some of the key compounds found in soursop are acetogenins, alkaloids, phenols, and essential oils. These compounds have been studied for their diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and antitumor effects. (Newman D. J., Cragg G. M. et al 2015)Acetogenins are a class of natural compounds found in soursop that have been extensively studied for their potential health benefits. These compounds have shown promising antitumor properties and are being investigated for their cytotoxic effects on cancer cells.Soursop contains alkaloids, which are nitrogen-containing organic compounds known for their pharmacological activities. Alkaloids isolated from soursop have demonstrated various bioactivities, including antimicrobial and anti- inflammatory effects. Phenolic compounds are another group of bioactive substances present in soursop. These compounds have antioxidant properties and play a role in protecting cells from oxidative damage caused by free radicals. (Schmidt B., Ribnicky D. M. et al 2008)

Chemotherapy-induced immunosuppressant is a significant concern in cancer treatment as chemotherapy, while targeting cancer cells, can also affect the immune system. The effects of chemotherapy on the immune system can lead to immune suppression, weakening the body's ability to mount an effective anti-tumor immune response and increasing the risk of infections and other complications. (Rady I.,Siddiqui I. A. et al 2017)The study discussed the distribution of pathogenic bacteria in lower respiratory tract infections in lung cancer patients after chemotherapy. The findings revealed that lung cancer patients after chemotherapy exhibited a high resistance to commonly used antimicrobial drugs, emphasizing the importance of monitoring pathogenic microorganism resistance in clinical practice. (Welte T, Torres a, Nathwani D. et al). Pseudomonas aeruginosa is also a notable pathogen found in lung cancerpatients with infections. Klebsiella pneumoniae is a significant pathogen. It is a gram-negative bacterium that has been identified in respiratory tract infections in lung cancer patients after chemotherapy. They can lead to serious infections and is known for its resistance to commonly used antimicrobial drugs. Aspergillus sps is a common fungus that can cause infections in lung cancer patients called Aspergillosis. It can lead to symptoms such as shortness of breath, cough, wheezing, fever, chest pain, and coughing up blood. In imaging studies, they may appear as nodules or spots on the lungs. (Wang Q,Zhang et al 2016) Even the normal flora of the human body cause chronic infection because of their uncontrolled growth in the micro biota they can colonize in the lower respiratory tract and cause symptomatic or asymptomatic diseases due the immune suppressionon account of the chemotherapy treatment. (Didkowska J, Wojciechowska et al)

II. MATERIALS AND METHODS

1. Sample collection:

Sour sop leaves and fruits collected from commercial store free fromcontaminants and then examined for its overall quality.

2. Pre treatment:

The skin, pulp, seed were separated from fruit and washed thoroughly with distilled water including leaves then they are dried under shade until the slight change in color. Then they are subjected to surface sterilization with 70% ethanol to remove microbes and other contaminants. All the contents are then dried to remove moisture in a hot air oven at 75ºc until they become dry.] (Badoni, A., Chauhan, J. S. et al)

3. Extraction:

The extraction was carried out by using probe sonicator [Ultrasound assisted extraction technique] (24 kHz, 100% sonication amplitude, 2s pulse cycle) by using conc. methanol as solvent. (Xian H.W., Sidik N.A.C et al.,)

4. Concentration:

The solid contents were filtered out and crude extract is obtained by removing the solvent by rotary evaporator at [65ºc (water bath), 100 rpm] (Zhang Q., Lin L., Ye W.)

5. Phytochemical analysis:

The sample was subjected to phyto chemical analysis to check the presence of pharmacological compounds as follows (Cheesman MJ, et al.,2019)

a. <u>Flavanoids:</u>

- Small amount of sample taken in a test tube.
- Add a few drops of dilute ammonia solution to the sample.
- Follow by adding a few drops of concentrated sulfuric acid.
- Observe for color changes.

b. <u>Anthocyanin:</u>

- Prepare a dilute solution of sodium hydroxide (NaOH).

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- Take a small amount of the extracted compound and add a few drops of theNaOH solution to it. Mix well to ensure uniform distribution.
- Observe any color changes in the mixture after adding NaOH.

c. Tannin:

- Take 2 mL of the plant extract and add a few drops of 10% Ferric chloride solution to it.
- After adding the Ferric chloride solution, observe any color change in themixture.

d. Quinone:

- Take a small amount of the extracted compound in a test tube.
- Add a few drops of dilute hydrochloric acid (HCl) to the test tube containingthe compound.
- Mix the contents gently by swirling the test tube.
- Observe any color changes or formation of precipitates in the solution.

6. Identification and quantification:

The sample was identified by the chromatography technique by High performance liquid chromatography [HPLC C18 column].

7. Determination of anti microbial activity:

- The antibacterial and antifungal activity of the compound is determined by the minimal inhibitory concentration and by minimal bactericidal concentration.
- A series of seven test tubes were taken and then suitable broth (5ml) was poured and then the organisms of interest were inoculated.
- Then the obtained crude extract is added in increasing concentrations [µl].
- Further the tubes were incubated at 37ºc for 18-24 hours.
- Then the turbidity of all tubes was noted by using a photometer.
- The lower turbid value tubes were plated by using spread plate technique.
- Then the colonies were observed. (Clin. Microbiol. Infect. 1998;4:291–296)

8. Evaluation of free radical scavenging activity (DPPH assay):

The free radical scavenging ability is analyzedby DPPH(2, 2-Diphenyl-1- picrylhydrazyl) assay (Brainina K.Z., Ivanova A.V et al., 2007)

Step 1 – DPPH solution preparation:

- Dissolve 7.89 mg of DPPH (0.2 mM) in 99.5 % of ethanol and fill 100 mL offlask to obtain a constant volume.
- Keep it in dark for 2 h until the absorbance is stabilized.
- In a tube test, put 1 mL of the DPPH solution, 200 µL of ethanol, and 800 µLof 0.1 tris amino methane hydrochloride (Tris-HCL) buffer (pH 7.4).
- In the blank tube, put 1.2 mL of ethanol and 800 µL of Tris-HCL buffer.
- After mixing, measure the absorbance at 517 nm and store the DPPH solutionin the dark at room temperature.

Step2 - DPPH assay procedure:

- In a 96-well micro titer plate, prepare various concentrations of test sample by dilution method using Dimethyl sulfoxide (DMSO).
- In each well, add 800 µL of 0.1 M Tris-HCL buffer (pH 7.4) and 1 mL of the DPPH solution to 200 µL of each concentration of sample test. In the blank well, add 1.2 mL of ethanol and 800 µL of tris-HCL buffer.
- Mix immediately the plate for 10s and keep it at room temperature in the dark.
- After 30 min, measure the absorbance of the solution at 517 nm.

9. Cell proliferation assay (2.9. MTT assay):

- MTT is soluble in water, ethanol, buffered salt solutions, and culture media. Itis recommended to use a 5 mg/mL solution in PBS.
- After adding MTT, filters sterilize the solution.
- Store the MTT solution at -20°C for long-term stability.
- The MTT solvent consists of 4 mM HCl and 0.1% NP40 in isopropanol.
- Discard media from cell cultures by aspirating carefully for adherent cells or spinning down suspension cells.
- Add 50 µL of serum-free media and 50 µL of MTT solution into each well of aclear cell culture plate.
- Incubate the plate at 37°C for 3 hours to allow the cells to metabolize the MTT reagent.
- After incubation, add 150 µL of MTT solvent into each well to solubilize the formazan crystals.
- Wrap the plate in foil and shake on an orbital shaker for 15 minutes to ensure complete dissolution.
- Read the absorbance at OD=590 nm using a micro plate reader within one hourafter adding

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the solvent. (Stockert J.C., Horobin R.W., Colombo L.L etal.

III. RESULTS AND DISCUSSION:

(Fig: 1) The leaves and fruit are being dried in a hot air oven after surfaces sterilization.

1. Extraction:

(Fig: 2)Probe Sonicator

- The samples were subjected to sonication process for obtaining extract.
- The probe generates sound waves to agitate the contents and the cavities areformed in the sample.
- Then the compounds can be obtained in the solvent.

2. Concentration:

(Fig: 3) Rotary evaporator

(Fig: 3.1) Crude extract

- The concentration of the sample is achieved by rotary evaporation.
- Sample is filled in the flask and vacuum is created to lower the boiling pointthe reduced pressure ensure faster evaporation of the solvent.
- The flask is rotated at the desired speed to ensure the spread of uniform heat.
- Then the solvent is evaporated and then condensed by condenser coil by meansof flowing cold water.
- Then the crude extract is obtained.

3. Phyto-chemical

(Fig3.3.1): Flavanoids ,Anthroquinone, Tanin
(fig3.3.2): Quinones, Phlobatannin, Saponin

Phytochemical analysis	Result
Flavanoids	+ ve
Anthocyanin	+ ve
Tannins	+ ve

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Quinones	+ ve
Phlobatannin	-- ve
Saponin	+ ve

(Table: 1)

- Shinoda test is carried out to detect presence of flavanoids reaction involves complex processes that result in the formation of colored compounds due to conjugated π-electron systems within flavonoids.
- Anthocyanin phytochemical test involves utilizing specific chemical reactionsto detect the presence of anthocyanins in a sample. Anthocyanins are water- soluble pigments that exhibit color changes based on their pH environment. The test typically involves using a reagent that reacts with anthocyanins to produce a visible color change, indicating the presence of these compounds.
- Tannins are polyphenolic compounds that can form complexes with proteins and other macromolecules. The test is based on the ability of tannins to react with certain chemicals, resulting in visible changes that indicate the presence oftannins in the sample.
- The addition of dilute Naoh to the sample forms a color change when the Quinones are present due to the reaction of Naoh to the carbonyl group.
- Phlobatannins are a group of water-soluble polyphenolic compounds that are known for their ability to form insoluble complexes with proteins. In the test,the presence of phlobatannins is typically indicated by the formation of a reddish precipitate while addition of 1% HCL.

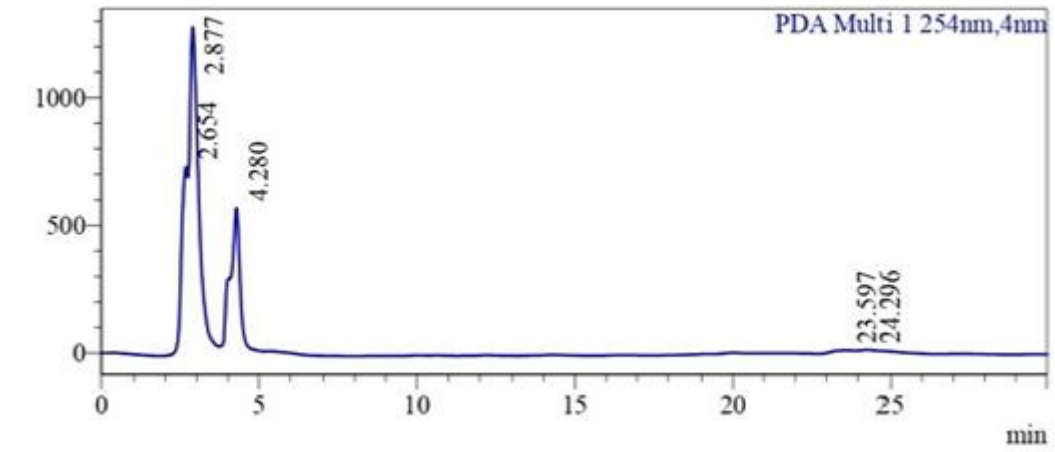
4. Identification and quantification:

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: Crude extract		
Sample ID	:		
Data Filename	: Crude extract.lcd		
Method Filename	: Kowsalya 1.lcm		
Batch Filename	: Kowsalya 2.lcb		
Vial #	: 1-9	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 4/9/2024 7:38:59 AM	Acquired by	: Vasanth
Date Processed	: 4/9/2024 8:09:01 AM	Processed by	: Vasanth

<Chromatogram>



<Peak Table> PDA Ch1 254nm

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Peak #	Ret. Time	Area	Height	Conc.	Unit	Mark
1	2.654	10497153	740875	19.43		
2	2.877	26525365	1287589	49.098		V
3	4.28	15181026	579367	28.1		SV
4	23.597	599622	13431	1.11		
5	24.296	1222457	16043	2.263		V
Total		54025623	2637304			

(Table: 2)

- The presence of compound is identified and quantified by High performance liquid chromatography HPLC C18 column.
- The separation in High Performance Liquid Chromatography (HPLC) is basedon the distribution of analytes between a mobile phase and a stationary phase.
- Depending on the chemical structure of the analyte, the molecules are retardedwhile passing the stationary phase.
- The specific intermolecular interactions between the molecules of a sample andthe packing material define their time "on-column".

5. Determination of anti microbial activity by Minimal inhibitory concentration [MIC]:

Dilution µL

Organism	(-ve) Cnt.	(+ve) Cnt.	20	40	60	80	100
Klebsiella pnuemoniae (Pc- Ciprofloxacin)	0.81	0.14	0.58	0.57	0.51	0.56	**0.40**
Pseudomonas sps (Pc - amikacin)	0.65	0.16	0.61	0.73	0.55	**0.35**	0.38
Staphylococcus aureus (Pc - erythromycin)	0.67	0.20	0.77	0.73	0.69	0.62	**0.60**
Aspergillus flavus (Pc - Amphotericin)	0.75	0.17	0.40	0.48	0.41	0.38	**0.30**

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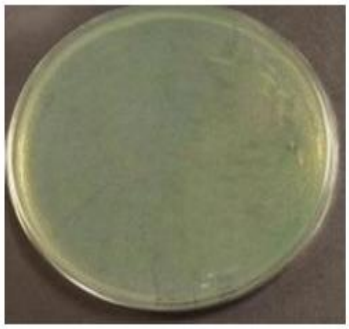

Fig: 5) **S.aureus** (Fig: 5.1)**pseudomonas sps**

(Fig: 5.2) **Klebsiella pnuemoniae** (Fig: 5.3) **C. albicans**

(Fig: 5.4) **Aspergillus flavus**

6. Evaluation of free radical scavenging activity (DPPH assay)

Set: 1

Con.µl	Blank	Sample	% Rsa
50	0.901	0.493	45.28302
100	0.901	0.393	56.3818

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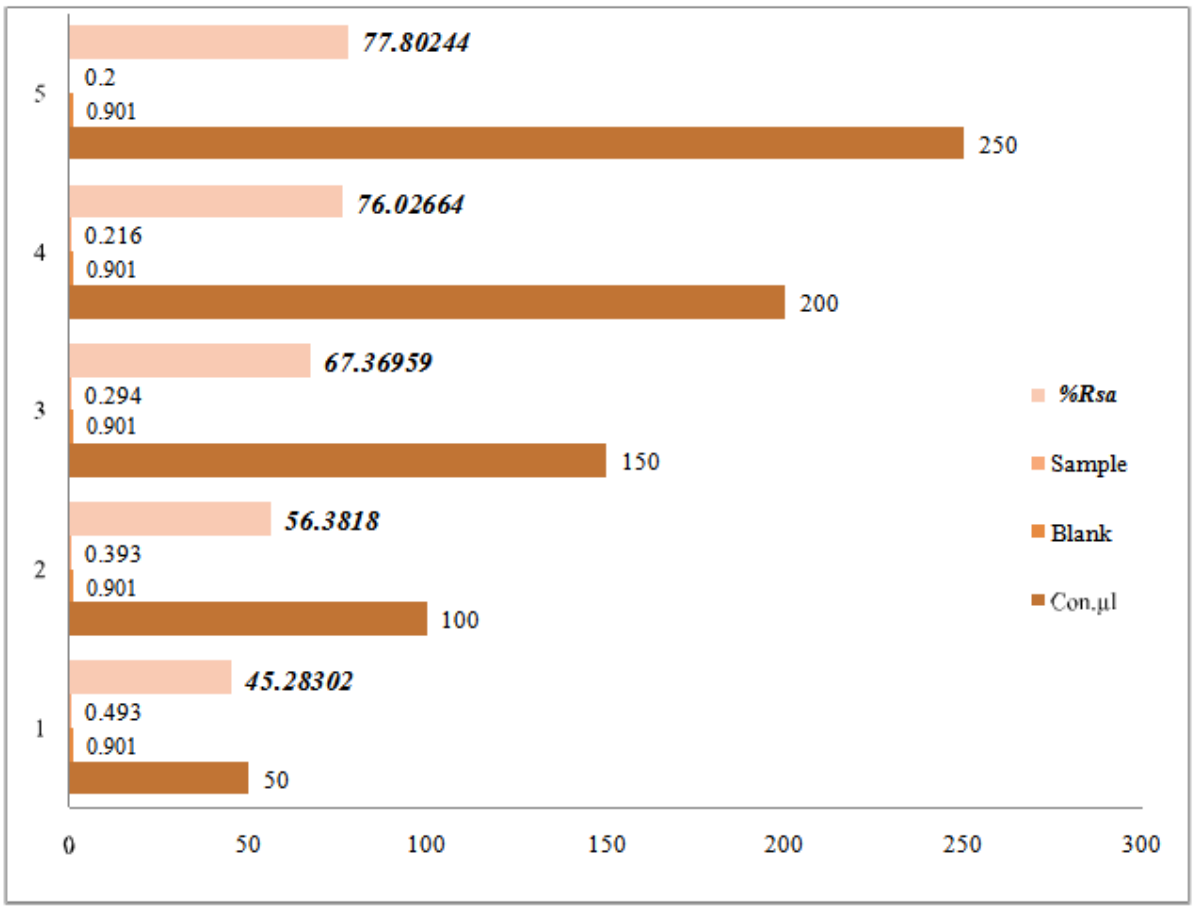
150	0.901	0.294	67.36959
200	0.901	0.216	76.02664
250	0.901	0.2	**77.80244**

(Table: 4)



% Rsa - Radical scavenging activity

Set: 2

Con.µl	**Blank**	**Sample**	**% Rsa**
50	0.901	0.497	44.83907
100	0.901	0.4	55.60488

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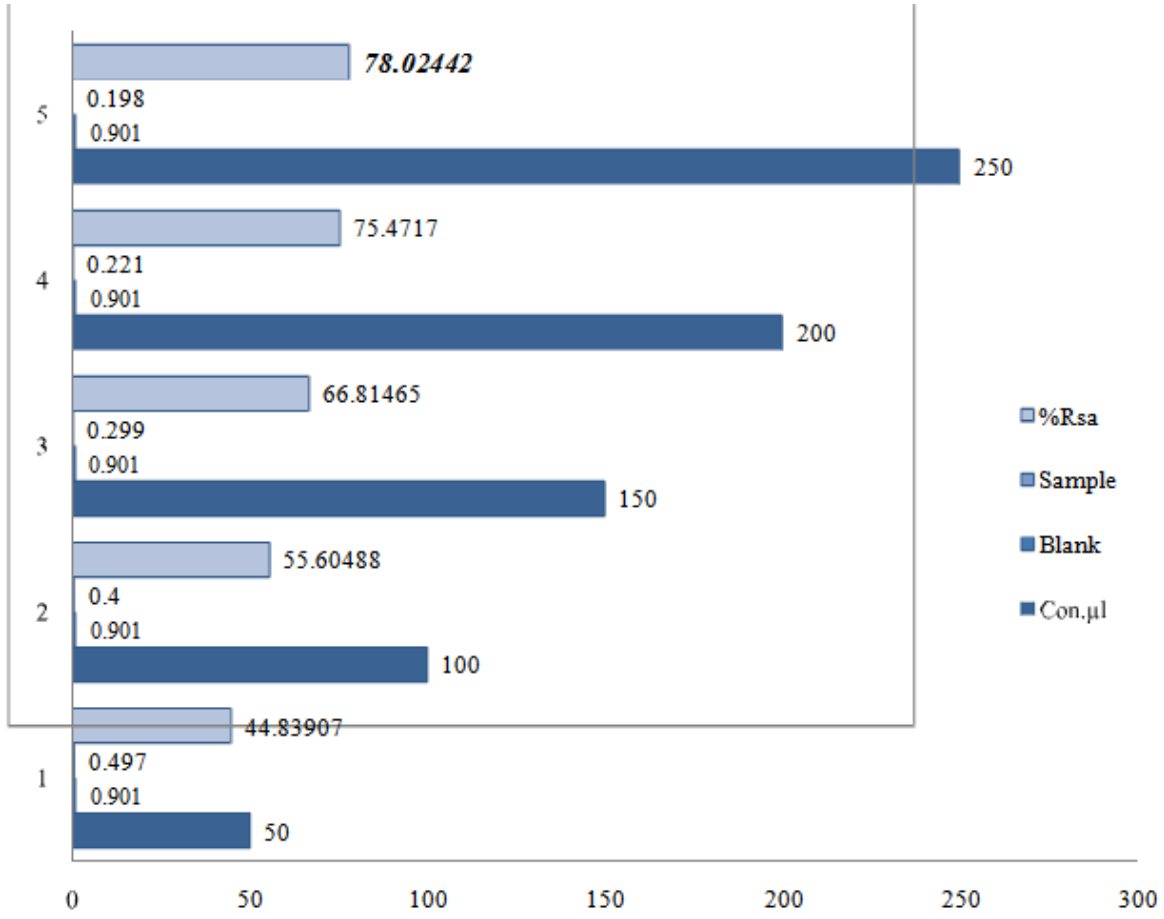
150	0.901	0.299	66.81465
200	0.901	0.221	75.4717
250	0.901	0.198	**78.02442**

(Table: 5)



% Rsa - Radical scavenging activity

Average Rsa %

Set 1	**Set 2**	**Average [% Rsa]**	**Std.dev [% Rsa]**
45.28302	44.83907	45.06104	0.313921

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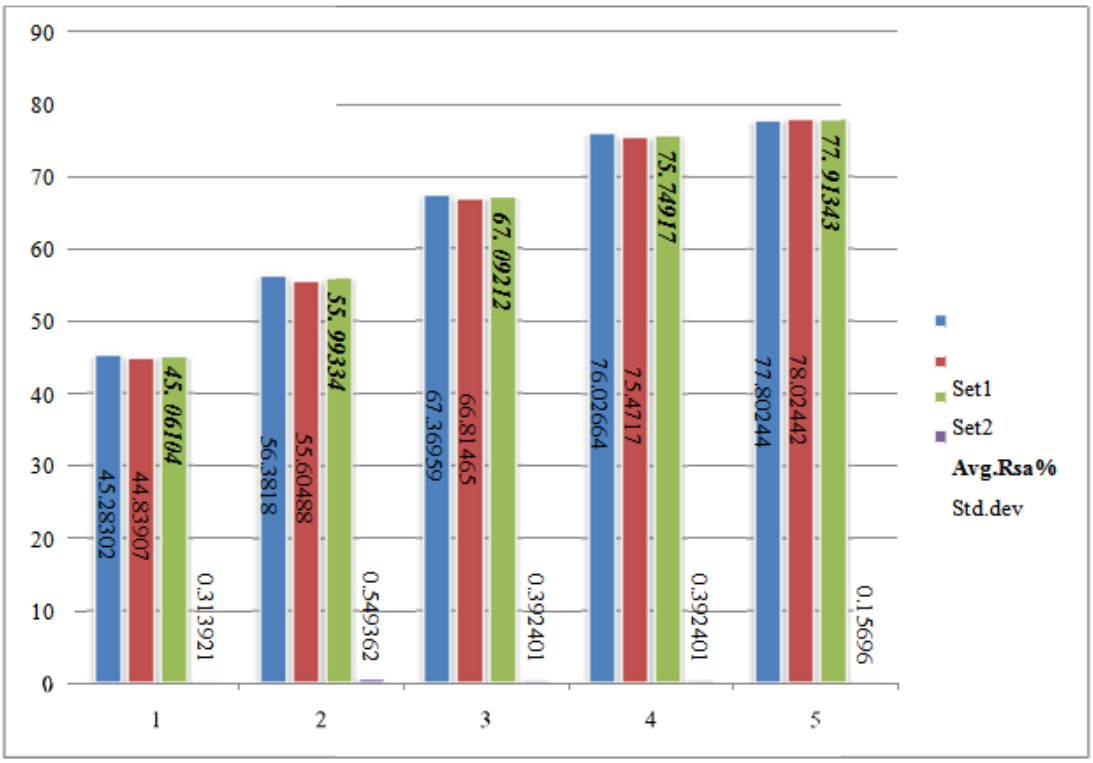
56.3818	55.60488	55.99334	0.549362
67.36959	66.81465	67.09212	0.392401
76.02664	75.4717	75.74917	0.392401
77.80244	78.02442	**77.91343**	0.15696

(Table: 6)



% Rsa - Radical scavenging activity

Std.dev - standard deviation

The DPPH assay, which stands for 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging activity, is a widely used method to evaluate the antioxidant potential of compounds, extracts, or biological sources. This assay involves measuring the ability of antioxidants to scavenge the stable free radical DPPH by donating a hydrogen atomto it. The reduction of DPPH results in a color change from purple to yellow, indicating the antioxidant activity present in the sample being tested.

The DPPH assay is based on the principle that antioxidants can interrupt the oxidative process caused by free radicals at different stages - initiation, propagation,and termination. The reaction between DPPH and an antioxidant leads to the formation of a reduced form of DPPH with a loss of violet color, which is indicativeof antioxidant activity.

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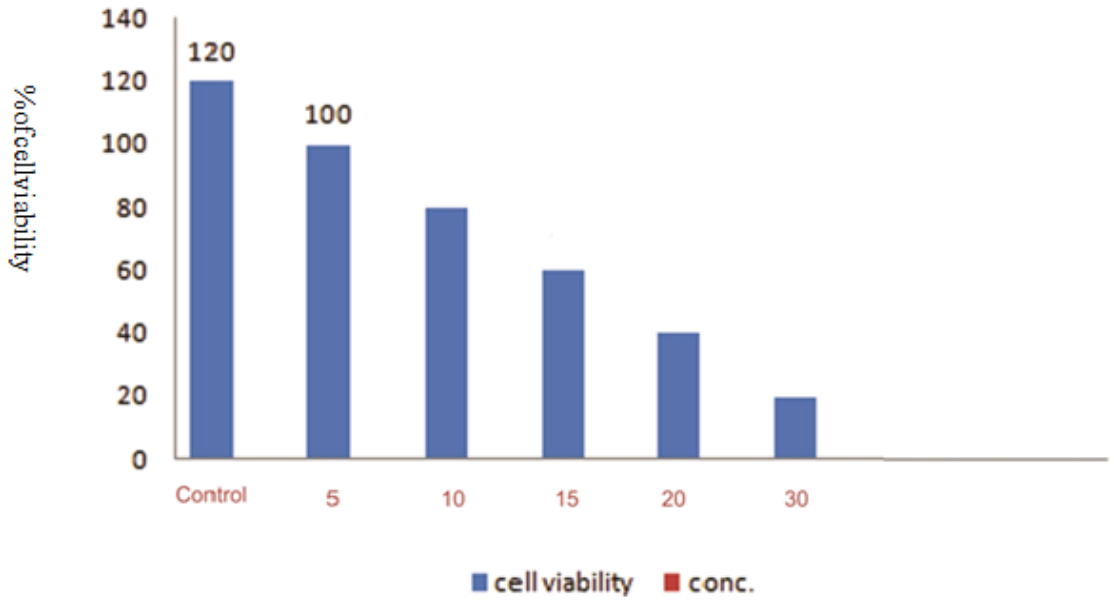
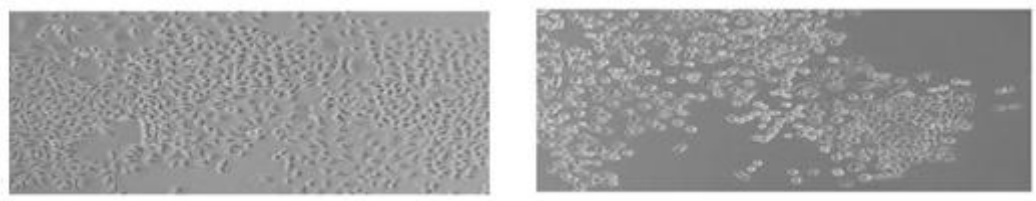
7. Cell proliferation assay- 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl- 2H-tetrazolium bromide) [MTT] assay:

MTT assay A-549 cells



Con. of Annona muricata SeNps (µg/ml)

SeNPs-Selenium nanoparticles



Control A.muricata SeNps (20 µg/ ml)

The cytotoxicity level of A. muricata SeNPs through MTT assay and revealed that 40µg/ml of AM- SeNPs inhibited almost 80% of the lung cancer cells (A-549) in 24 h.

MTT assay involves the reduction of a water-soluble, yellow-colored tetrazolium salt called MTT to a non-water-soluble purple formazan crystal. This reduction process occurs when metabolically active cells are introduced to the MTT reagent. The conversion of MTT to formazan is primarily facilitated by the succinate dehydrogenase system present in active mitochondria.

The formation of purple formazan crystals, they are dissolved in organic solvents to create a colored solution. This solution's optical density (OD) is then measured at a specific wavelength between 500 and 600 nm using a spectrophotometer or a plate reader. The amount of formazan produced is directly correlated with the metabolic activity of the cells, providing insights into cell viability.

IV. SUMMARY AND CONCLUSION:

Lung cancer remains a significant global health challenge, being the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) accounts for the majority of cases and presents a poor prognosis due to the lack of early diagnostic tools. Despite recent advancements in diagnosis and treatment strategies, the overall five-year survival rate for NSCLC patients remains low at less than 15%. Epidemiology of infections in cancer patients has changed across the globe overtime and is characterized by a shift from gram-negative bacteria (1960s and 1970s) to gram-

positive ones (1980s). Gram-negative bacteria have predominated the scene as a major cause of infections in cancer patients in the last 20 years across the globe in many countries The increasing development of resistance to existing antimicrobials necessitates need to develop novel agents at a rate faster than the development of resistance. It is of utmost importance to restrict the use of antibiotics in all clinical practices, using narrow-spectrum antibiotics based on culture reports wherever possible. This may come a long way in improving the situation of patients with life-threatening infections, especially in those who are immune compromised.

Infections among cancer patients are a major challenge to deal with. They are due to the delivery of chemotherapy which leads to poor treatment outcome, adds to cost of management, and contributes to increased morbidity. To successfully prevent, identify, and treat infections, knowledge of the changing epidemiology of infections isessential. The Annonaceae family has been extensively studied recently because of its potential as a therapeutic plant, and it has a long history of documented medicinal uses. This particular species has recently drawn attention owing to its bioactivity and long-standing use. The treatment of chronic degenerative illnesses has received clinical attention since they have reached widespread proportions and are now recognized as major health issues.Biologically Soursop is potent against micro-organisms is due the presence of acetogenins. These are bioactive compounds found in the annonacea family, these acetogenins, are known to have anti-malarial, anti-helmintic, and anti- microbial effects, suggesting many potentially useful application.The plant's acetogenins and other secondary metabolites, such as alkaloids, flavanoids, tannins, phenols have been shown to inhibit cancer and microbial growth, and this potential could be fully explored, these compounds could provide great benefits by making effective in cancer treatment as an alternative therapyand control the microbial infection in lung cancer patient due to immune suppression on accountof chemotherapy.

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