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Research Article

AMAZONITE (A MICROCLINE FELDSPAR) -AN ANTI-CANCEROUS GEO-BIOTECHNOLOGICAL APPROACH FOR H460: HUMAN LUNG CANCER WITH VALIDATION VIA ROS ANTI-OXIDANT ANALYSIS

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ABSTRACT

The research delves into the medicinal properties of amazonite, with a particular focus on its potential application in nanomedicine for combating lung cancer. Employing the MTT Assay, the authors observed amazonite's impact on H460 lung cancer cells, revealing its cytotoxic effects and thereby suggesting its promise as an anti-cancer agent. Moreover, through ROS antioxidant analysis, the study unveiled amazonite's ability to regulate reactive oxygen species levels, indicating its potential in mitigating oxidative stress-induced damage, a hallmark of cancer progression. FTIR spectroscopy emerged as a crucial tool in the research, offering insights into amazonite's molecular composition and its possible interactions with biological molecules. Of paramount importance is the toxicity analysis conducted in the study, which revealed amazonite's low toxicity profile. This aspect is particularly significant as it underscores the safety of amazonite for potential therapeutic applications, including in conjunction with treatments like radiotherapy. The assurance of low toxicity is pivotal in ensuring the viability of amazonite as a candidate for clinical use, instilling confidence in its safety profile and minimizing adverse effects on patients. Overall, the findings of the research underscore amazonite's potential as a safe and effective nanomedicine for the treatment of lung cancer. The observed cytotoxic effects on cancer cells, coupled with its ability to modulate reactive oxygen species levels and low toxicity profile, position amazonite as a promising candidate for further exploration in the realm of cancer therapy. However, the study also highlights the need for continued research to fully elucidate the underlying mechanisms of amazonite's action and to explore its potential clinical applications comprehensively.

INTRODUCTION

About Amazonite- It is a type of crystal, a common microcline feldspar usually transparent along with textures of white, cream to pale yellow, pink to red or bright green to blue-green mineral. The bright green variety of microcline is called Amazonite or Amazonstone and is prized as a Gemstone.

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Amazonite crystals mostly have two sets of fine lines set at right angles to each other, an effect termed as cross-hatch twinning that creates a “plaid” effect. This distinguishes them from variety of feldspars and from green jade. Single crystals from granite pegmatites can weigh several tonnes and can be tens of metres long [5]. Although named after the river Amazon, no deposits of Amazonite have been found there. The Pikes Peak district of Colorado, USA, is the primary source of Amazonite from the southern front range of the Rocky Mountains at well over 4000 metres. In an archaeological dig undertaken during Southern Jordan, more than 2000 fragments of amazonite jewellery were discovered via panning etc. dating back to neo-lithic times (stone- age man) around 10,000 years ago! Nowadays, Amazonite is predominantly sourced from

regions across East Asia, such as China and Mongolia, as well as from the Ural Mountains in Russia and various locations in southern and eastern Africa. Additionally, deep green varieties of Amazonite have been discovered in the Kola Peninsula of Russia, the renowned mines of Minas Gerais in Brazil, Mogok in Burma (Myanmar), and the Sidamo-Borana Province in Ethiopia. [7].

It is one of the oldest used gemstones.

Amazonite – Gemological Properties

Chemical formula	$KAlSi_3O_8$: Potassium Aluminium Silicate
Crystal structure	Triclinic, Prismatic
Colour	Green, blue, gray, multicolour (white colour of streak)
Hardness	6- 6.5 on Mohs Scale
Density	2.56-2.58
Cleavage	Perfect
Refractive index	1.522-1.530
Transparency	Translucent to opaque
Luster	Vitreous, dull
Double refraction or birefringence	-0.008
Fluorescence	Weak, olive- green

Amazonite Green: For most of its wide life, it was thought that amazonite was green because of the presence of copper in its upbringing however when scientist investigated it was decided that lead impurities were the actual reason behind its lovely green colour, unfortunately more scientists had sometime on their hands and the latest theory is iron impurities.

It is a type of feldspar which is a mineral which engages up a full half of the world's crust! Other types of feldspar include moonstone, sunstone and labradorite which are also well-known renounced gemstones.

Amazonite History: It is a trade name for microcline. The name was coined by German mineralogist Johann Breithaupt in 1847 but we are not sure why he finalized up with this name since this gemstone is not found in Amazon River or Forest. Perhaps the name sounded a bit more flavoured than microcline or other, Green Feldspar. This gemstone, using different names, has been in use since at least the time of the Pharaohs of Ancient Egypt as cut and polished jewellery and beads have been found in tombs of the period, including King Tutankhamun around 1300 BC. In 2006, two ancient amazonite or microcline mines were discovered in the mountains of the Eastern Desert in Egypt which were the earliest mines found so far, dating back to 1800 BC.

Medicinal Importance of Amazonit: The gemstone is not only used for centuries as an amulet, carved ornament, and decoration, but too as an antidote for one of the modern world's most potent disorders-electromagnetic pollution. With all the smartphones, laptops, computers, WiFi, bluetooth devices, GPS circling around us everywhere we go are constantly exposed to electromagnetic radiation. This led to an increase in biological effects on every one of us such as lack of concentration and sleep disorders, an unbalanced nervous system, mental issues, stress, metabolic disturbances, weakened immune system and many more.

The health advantages of Amazonite are attributed to its association with the throat and heart chakras, which directly influences physical wellness, particularly concerning lung and liver health. It possesses the potential to enhance metabolic functions and promote restful sleep, offering support for a healthy lifestyle. It has a direct effect on anything to do with calcium in the body, so will help prevent osteoporosis, tooth decay and any form of calcium deficiency.

Discovery of Radiotherapy: Madam Curie has left a great deal to the world. Through her pioneering efforts, she spearheaded advancements in nuclear energy and the application of radiotherapy (RT) in cancer treatment. Her contributions not only revolutionized medical science but also positively impacted the perception of scientific endeavours. The roots of radiotherapy can be traced back approximately 125 years to the serendipitous discovery of X-rays in 1895 by the German physicist W.C. Roentgen. By experimenting with these potentially hazardous X-rays, scientists stumbled upon the groundbreaking realization that they could be

harnessed for the treatment of cancer, laying the foundation for modern radiation therapy techniques. When the German physicist Wilhelm Conrad Roentgen announced his discovery of the X-rays in December of 1895, he was lauded on the front page of just about every newspaper in the world.

Radiotherapy

Radiation therapy, also known as radiotherapy, is a cancer treatment method that administers high levels of radiation to eliminate cancer cells and reduce tumor size. At lower doses, radiation is employed in medical imaging, such as X-rays for dental or bone examinations, to visualize internal structures. However, at higher doses, radiation therapy disrupts the DNA of cancer cells, either killing them outright or impeding their growth. When cancer cell DNA sustains irreparable damage, they cease dividing or perish. Subsequently, the body breaks down and eliminates these damaged cells. The effects of radiation therapy are not immediate; it takes several days or even weeks of treatment before the DNA damage reaches a critical level, prompting cancer cells to perish. Even after radiation therapy concludes, cancer cells may continue to die off over subsequent weeks or months. There are two primary types of radiation therapy: external beam, which is administered from outside the body, and internal radiation therapy, where radioactive material is placed directly into or near the tumour site.

External Beam Radiation Therapy- It comes from a machine that aims radiation at the cancerous area. The machine is large and noisy. It does not touch you, but can move around you, sending radiation to the parts of your body from many directions. Its' a local treatment treating specific part of your body. For example, if you have cancer in your breast, you will have radiation only to your breast, not to entire body.

Internal radiation therapy involves the introduction of a radiation source into the body for treatment. This source of radiation can either be in solid or liquid form. When utilizing a solid radiation source, the procedure is termed brachytherapy. During brachytherapy, small objects like seeds, ribbons, or capsules containing the radiation source are inserted into or near the tumour. Similar to external beam radiation therapy, brachytherapy targets a specific area of the body, providing localized treatment. The radiation emitted by the source within the body continues to exert its effects over time. Conversely, internal radiation therapy utilizing a liquid source is referred to as systemic therapy. This approach involves the administration of radiation via the bloodstream, allowing it to reach various tissues throughout the body and target cancer cells for destruction. One receives systematic radiation therapy by swallowing through a vein via an IV line, or through an injection. With systematic radiation, your body fluids, such as urine, sweat, and saliva will give off radiation for a while [8].

Cancer Treatments Causing Skin

Cancer treatments usually causes skin and nails as part of side effects. While skin problems caused by radiation therapy and chemotherapy are often mild, they maybe more severe if you are receiving a stem cell transplant, targeted therapy, or immunotherapy.

Sometimes, radiotherapy can cause the skin on the part of your body receiving radiation to become dry and peel, itch called pruritus, and turn red or darker. After undergoing certain medical treatments, your skin might exhibit various reactions. It could appear sunburned, swollen, or inflamed, and you may experience the formation of painful, wet, and infected sores, known as a moist reaction. Additionally, specific chemotherapy regimens might induce dryness, itching, redness, darkening, or peeling of the skin, along with increased sensitivity to sunlight, termed photosensitivity, which can lead to minor rashes or sunburns. Skin pigmentation changes may also occur. Nail abnormalities like darkening, cracking, and painful cuticles could develop. If you've undergone radiation therapy previously, the treated skin area may exhibit redness, blistering, peeling, or discomfort, known as radiation recall. Allergic reactions to chemotherapy may manifest suddenly with severe rashes, hives, or a burning sensation. Stem cell transplants may trigger graft vs host disease (GVHD), resulting in skin issues such as rashes, blisters, or thickened skin. Certain immunotherapy treatments might lead to severe rashes with dry or blistered skin. Some targeted therapies could cause dry skin, rashes, and nail complications.

Here in this study, the authors tested the Amazonite sample from Brazil and tested its viability in triplicates at 570 nm in MTT Assay for H460- Lung Cancer and found some amazing results with IC50 as 47.29, which indicates drug with high toxicity.

FIGURES

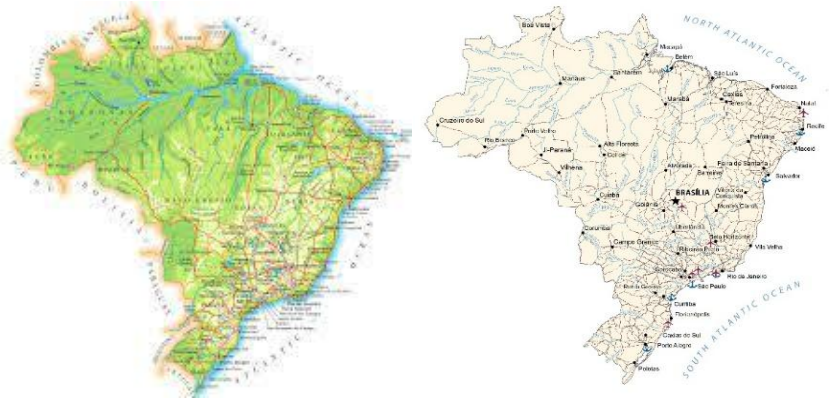


Figure 1(A, B)- Topological map of geographical area from where Amazonite came in this study

Figure 2: Stages of Amazonite, from raw rock to sediments to extraction to DMSO concentration to induction to cells

MTT assay for cell cytotoxicity

Principle

(3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) MTT assay is the capability of mitochondrial dehydrogenase enzyme from viable cells to cleave the rings from tetrazolium pale yellow MTT and hence to form blue colored formazan crystals that are largely impermeable to cell membranes, thus outputting in its accumulation within healthy cells. By the addition of detergents like DMSO, solubilization of cells results in the liberation of crystals that are soluble. Number of surviving cells is directly proportional to the level of formazan so created. We can quantify the color using a multi-well plate reader.

Materials Required

Dulbecco's modified eagle medium (DMEM) [1,2], antibiotic solutions and Fetal Bovine serum (FBS) were brought from Gibco, USA. Dimethyl sulfoxide (DMSO) and 3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide (MTT) (5mg/ml) were landed from Sigma, USA. 1X Phosphate Buffer Saline (PBS) was from Himedia, INDIA. Wash beakers and Tissue culture plates with 96 wells were from Tarson, INDIA.

Procedure

Cell culture

Human lung cancer cell line (H460) was purchased from NCCS, Pune, and were cultured in liquid medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100ug/ml of penicillin and 100µg/ml of streptomycin and are maintained within an atmosphere of 37°Celsius along with 5% of CO_2.

MTT Assay

Amazonite crude sample was tested for in vitro cytotoxicity, using H460 cells by MTT assay. Briefly, the cultured H460 cells were harvested by trypsinization, pooled in a 15 ml tube. At a density of $1*10^5$ cells/ ml cells, cells were plated with cells/well (200µL) into 96- well tissue cultured plate in DMEM containing 10% of FBS and 1% of antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of

the 5 sample in a serum free DMEM medium. Each sample was in replication for three times and at 37 degrees C, cells were incubated in a humidified 5% CO2 for 24 hours. After incubation period, MTT (20µL of 5mg/ml) was inserted into each well and cells were incubated for another 2 to 4 hours until purple precipitates were clearly seen under inverted microscope. Finally, the medium along with MTT (220µL) were aspirated off the wells and washed after that with 1X PBS (200µl). Further-after, DMSO (100 µL) was added to dissolve formazan crystals, and the plate was shaken for 4 to 5 minutes. Absorbance for each well was measured at 570 nm using micro plate reader (Thermo Fisher Scientific, USA) and IC50 and Percentage cell viability was calculated using Graph Pad Prism 6.0 Software, USA [3,4].

Cell viability (%) = Test OD/Control OD X 100

RESULT/(s)- H460 cells/ Lung cancer cells were treated for 24 hours treatment covering 10000 cells/ wall. After getting readings from Elisa Plate Reader and then processing through Graph Prism Software. Cell death infringes the target drug to be effective with high toxicity at different concentrations.

Trial 1st

Table 1: Different Concentration trials (1st) with OD and Controlled OD

Concentration in ug/ml	OD	Control OD	%
0	0.689	0.689	100
50	0.536	0.689	77.7939
60	0.359	0.689	52.1045
75	0.211	0.689	30.6241
100	0.149	0.689	21.6255
150	0.109	0.689	15.82
200	0.038	0.689	5.51524
300	0.027	0.689	3.91872
400	0.019	0.689	2.75762
500	0.018	0.689	2.61248

2nd Trial

Table 2: Different Concentration trials (2nd) with OD and Controlled OD

Concentration in ug/ml	OD	Control OD	%
0	0.689	0.689	100
50	0.549	0.689	79.6807
60	0.361	0.689	52.3948
75	0.223	0.689	32.3657
100	0.137	0.689	19.8839
150	0.107	0.689	15.5298
200	0.054	0.689	7.83745
300	0.032	0.689	4.64441
400	0.019	0.689	2.75762
500	0.016	0.689	2.32221

3rd Trial

Table 3: Different Concentration trials (3rd) with OD and Controlled OD

Concentration in ug/ml	OD	Control OD	%
0	0.689	0.689	100
50	0.549	0.689	79.6807
60	0.353	0.689	51.2337
75	0.241	0.689	34.9782
100	0.132	0.689	19.1582
150	0.128	0.689	18.5776
200	0.048	0.689	6.96662
300	0.034	0.689	4.93469
400	0.025	0.689	3.62845
500	0.017	0.689	2.46734

Merged Trials with Average

Table 4: All trials combined with different concentration with Average %

Concentration in ug/ml		%		Average %
	1st trial	2nd trial	3rd trial	
0	100	100	100	100
50	77.7939	79.6807	79.6807	79.0518
60	52.1045	52.3948	51.2337	51.911
75	30.6241	32.3657	34.9782	32.656
100	21.6255	19.8839	19.1582	20.2225
150	15.82	15.5298	18.5776	16.6425
200	5.51524	7.83745	6.96662	6.7731
300	3.91872	4.64441	4.93469	4.49927
400	2.75762	2.75762	3.62845	3.0479
500	2.61248	2.32221	2.46734	2.46734

For log analysis

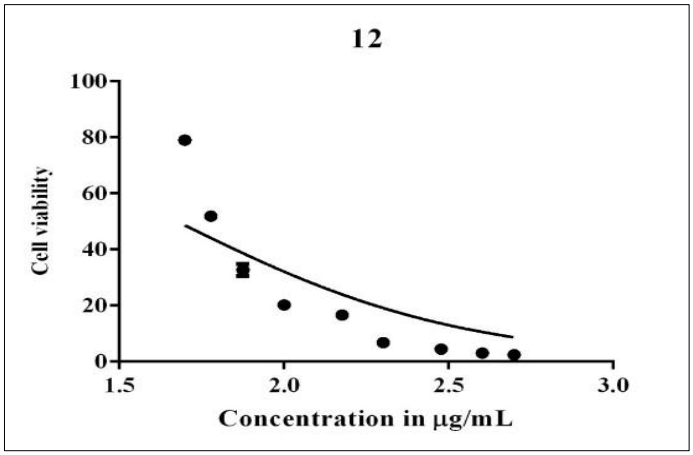
Table 5: Different log values so obtained with IC50 as 47.29

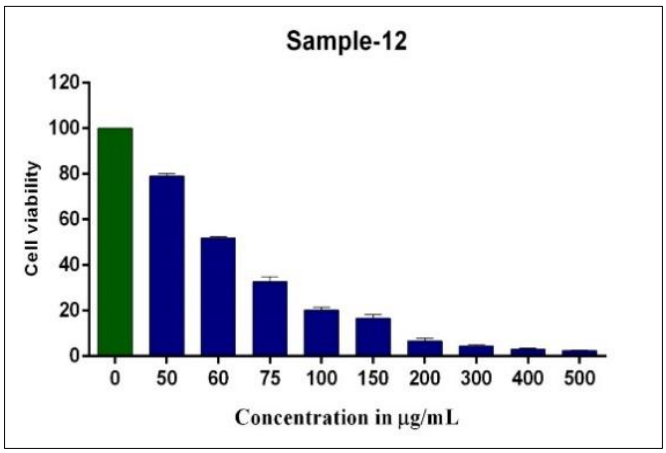
Log (inhibitor) vs. normalized response	
Best-fit values	
LogIC50	1.675
IC50	47.29
Std. Error	
LogIC50	0.06009
95% Confidence Intervals	
LogIC50	1.551 to 1.798
IC50	35.58 to 62.86
Goodness of Fit	
Degrees of Freedom	26
R square	0.7188
Absolute Sum of Squares	4675
Sy.x	13.41
Number of points	
Analyzed	27

At 570 nm

Table 6: OD values in triplicates at 570nm

S. No	Treated sample concentration (µg/mL)	OD value at 570 nm (Triplicates)		
1	0	0.689	0.689	0.689
2	50	0.536	0.549	0.549
3	60	0.359	0.361	0.353
4	75	0.211	0.223	0.241
5	100	0.149	0.137	0.132
6	150	0.109	0.107	0.128
7	200	0.038	0.054	0.048
8	300	0.027	0.032	0.034
9	400	0.019	0.019	0.025
10	500	0.018	0.016	0.017





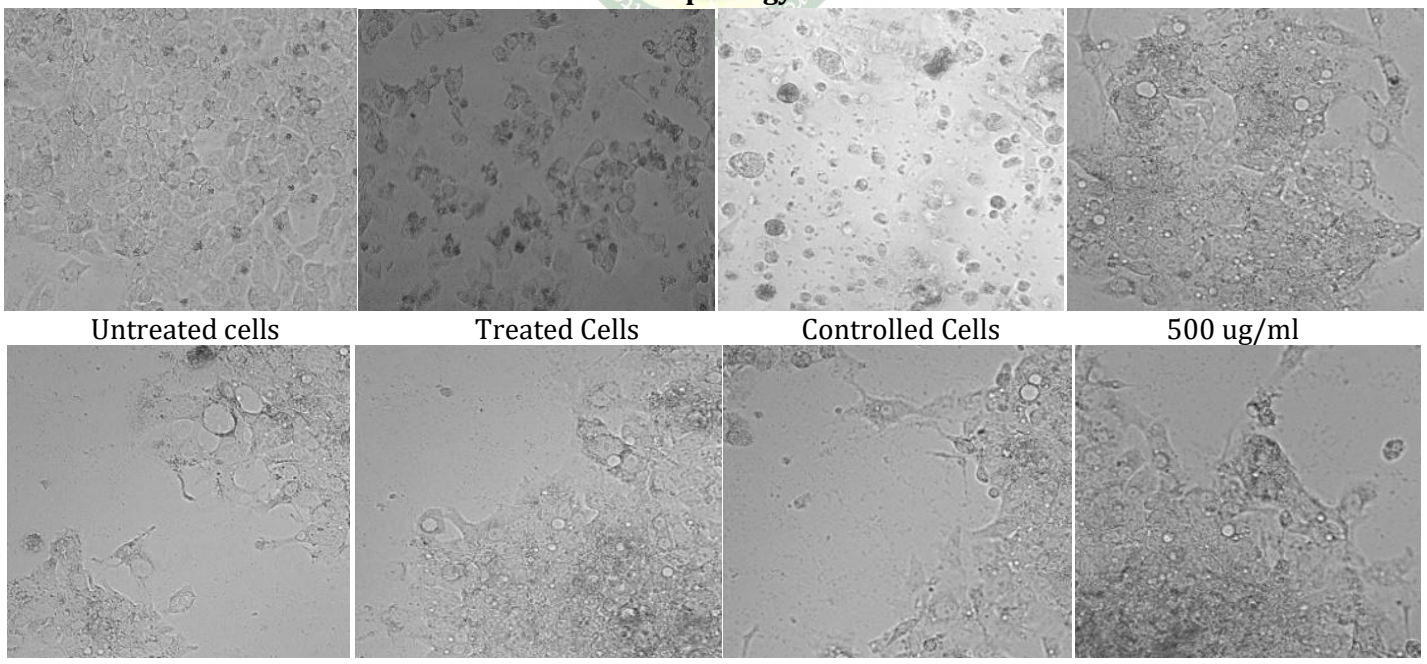
Plot 1- Cell Viability at different concentrations Plot 2- Bar chart representation of concentrations

Mean Values

Table 7: Mean Value % at different concentrations

S.No	Treated sample concentration (µg/ml)	Cell viability (%) (In Triplicate)			Mean Value (%)
1	0	100	100	100	100
2	50	77.7939	79.6807	79.6807	79.0518
3	60	52.1045	52.3948	51.2337	51.911
4	75	30.6241	32.3657	34.9782	32.656
5	100	21.6255	19.8839	19.1582	20.2225
6	150	15.82	15.5298	18.5776	16.6425
7	200	5.51524	7.83745	6.96662	6.7731
8	300	3.91872	4.64441	4.93469	4.49927
9	400	2.75762	2.75762	3.62845	3.0479
10	500	2.61248	2.32221	2.46734	2.46734

Morphology



Untreated cells | Treated Cells | Controlled Cells | 500 ug/ml

300 ug/ml | 100 ug/ml | 75 ug/ml | 50 ug/ml

Figure 3- Images captured from Elisa Plate Reader at different concentrations- Cell Death Experience via targeted drug therapy

Anti Oxidant Test

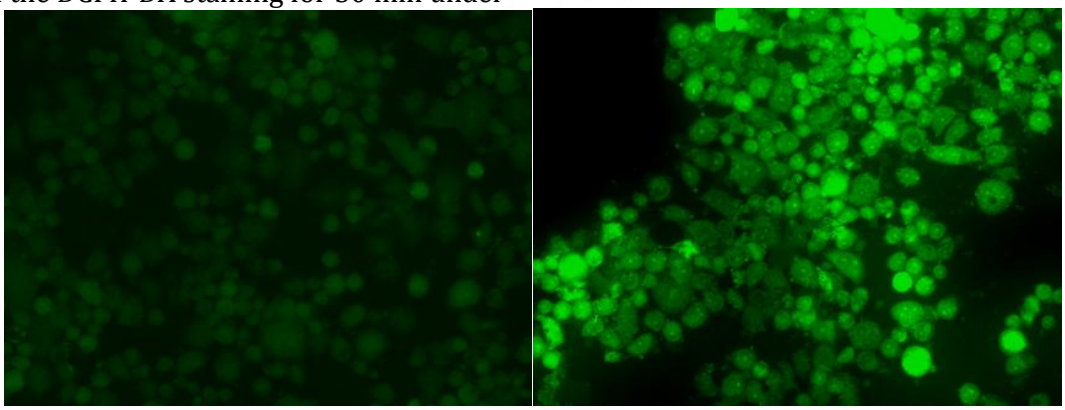
Intra-Cellular Ros Determination/H460 Lung Cancer Principle

The assay employs the cell-permeable fluorogenic probe DCFH-DA, which diffuses into cells and is deacetylcated by cellular esterases into the non-fluorescent DCFH. In the presence of ROS, DCFH is rapidly oxidized to highly fluorescent DCF. Then the images are captured by the fluorescence microscopy using 20 × magnification fields (Life Technology, USA).

Materials Required: The IC_{50} treated cells in experiment plate, 1× PBS solution, DCFH-DA (10mg/ mL of DMSO), fluorescent microscope and pipette.

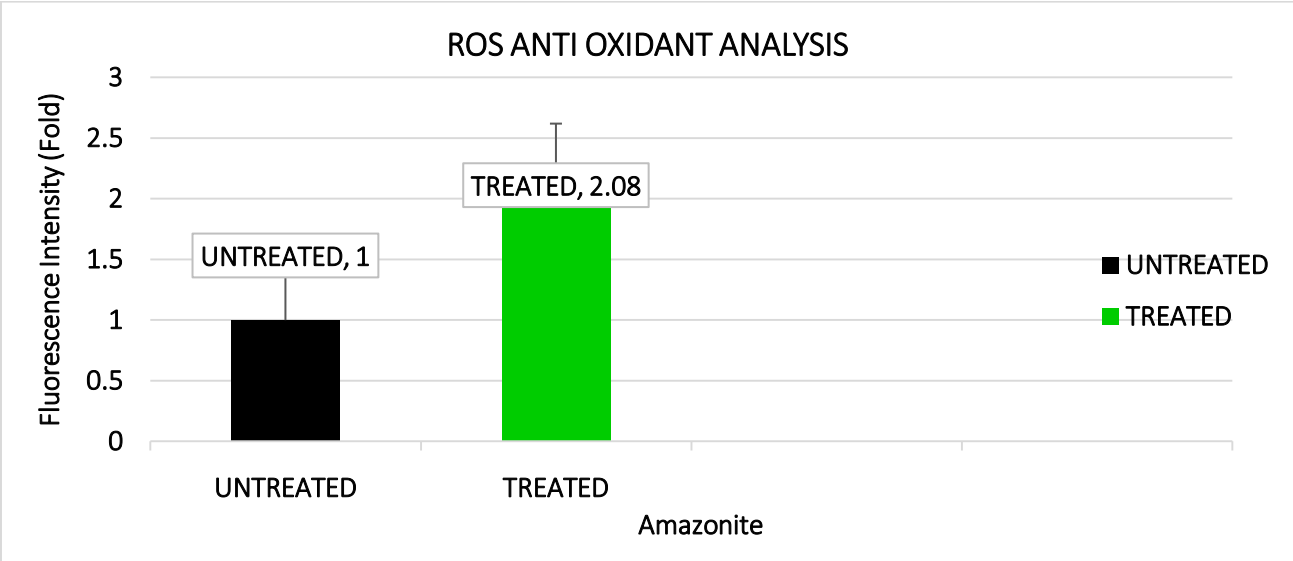
Procedure: For the determination of intracellular ROS molecules, this study adopted the DCFH-DA staining analysis. Briefly, The H460 cells were seeded on (1×10^5 cells/well) six-well plate and allowed them for overnight for maturation of cells. The next day, the old medium was aspirated with new medium containing with different concentration of sample(s) and incubated for 24 hrs. Afterwards, the plate was incubated with the DCFH-DA staining for 30 min under dark condition. Further the plate was subjected into fluorescence staining analysis by florescence microscopy (Floid imaging station, Life Technologies, USA). The used scale bar is 125µm with 20× magnification lens.

Interpretation: The ROS molecules play a vital role in cellular mechanism and it play important role in the cellular apoptosis. Apoptosis is mediated by extrinsic and intrinsic signaling pathways. Reactive oxygen species (ROS) are short-lived and highly reactive molecules. Low doses of ROS activate cell survival signaling pathways: UPR, Nrf2. High doses of ROS activate cell death signaling pathways: apoptosis and necroptosis. ROS activate mitochondrial, death receptor and ER pathways of apoptosis. In our results, all the samples have the capable of inducing the ROS accumulation in the cell cytoplasm and caused the cell death in lung cancer cell line (H460). The obtained data judged that the intended target samples are more capable to generate the cell death.



Untreated Treatment **After ROS**

Figure 4- Untreated and after treatment images so captured from Elisa Plate Reader reflecting cell death with highlighted bright green



Plot 3- Bar chart of ROS representing Untreated and Treated Cells

RESULTS

Reactive Species Oxygen- An antioxidant analysis after MTT Assay is a validation that target sample is a good cancer agent and is responsible for cell death. Further infringes that it can be used as a suitable chemo or immune medicine.

Fourier Transform Infrared Spectroscopy (FTIR)- Fast Fourier Transform Method on which the modern FTIR Spectroscopy is based was introduced by Turskey and Cooley in 1965. First FTIR spectrum was recorded by Peter Fellgett in 1949. This spectrometer uses infrared light to scan test samples with an observation on chemical properties. It is a quick analysis to identify compounds with functional groups and classes. FTIR provides higher wavelength accuracy with widest possible wavelength range.

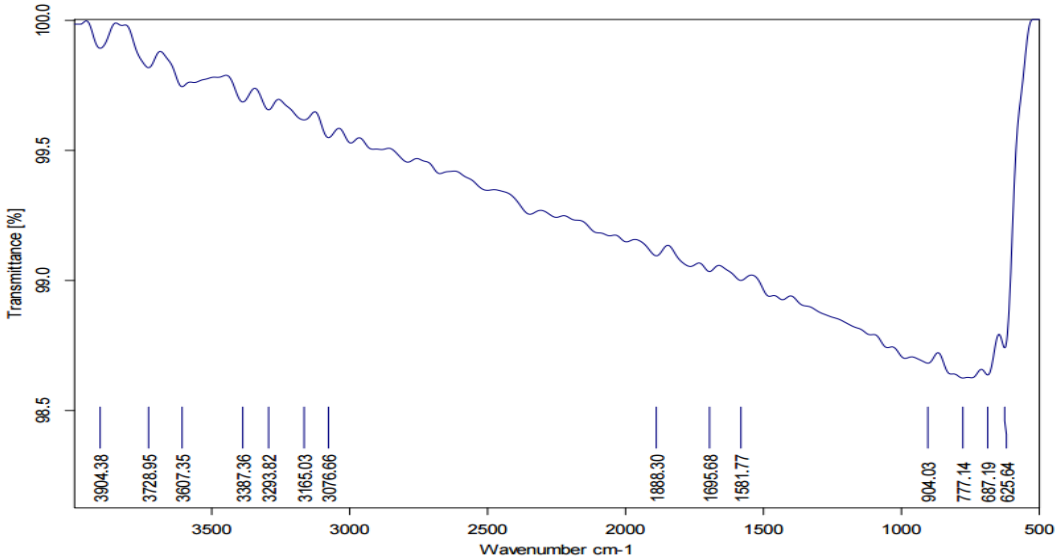


Figure 6: IR representation of Anorthosite with different peak values and transmittance

FTIR Spectrum Table

Table 7: Conclusion table from Infrared Spectrum

Wave number highest peaks)	Transmittance %	Functional class	Stretching vibration
3904.38	100	Alcohols / Phenols	O-H (free), usually sharp
3728.95	99.8	Alcohols/Phenols	O-H(H-bonded), usually broad
3607.35	99.6	Amine/ Amide	N-H stretch

DISCUSSION

The authors demonstrated and designed the entire experiment in order to find the anti-cancerous properties of phytochemicals present in the sample-amazonite. To begin with, the authors picked the top map of Brazil and studied the seasonal variations of different Land Use Land Patter via satellite data so provided by NASA, USGS. Samples were stored safely in laboratory.

The authors performed FTIR- Fourier Transfer Infrared Spectroscopy to find the functional group/s where the different peak falls.

Samples were isolated, soxhelated and extracted using Distilled cow urine at first when was going through cold isolation for 4 to 6 days. Cell line experiments involving MTT and subsequent analysis of Reactive Oxygen Species (ROS) imaging and antioxidant properties revealed the presence of toxicity in the sample, resembling anti-cancer medication. It's plausible that the compounds within the sample vials possess broader significance beyond cancer treatment, potentially impacting various other health conditions or disabilities.

Furthermore, the phytochemicals present in them that are bioactive and have toxicity reflects the medicinal importance of the Amazonite. Proceeded with Lung cancer for in-vitro experimentation, cell lines of H460 lung cancer was given treatment with our drug in different concentrations and we found that Amazonite is one of the best fit to be an anti-cancer agent which simply means that we can use it in the treatment for human lung cancer as Nano Medicine with different solvents, let it be distilled cow urine, DMSO D6 etc. which proves that yes, Amazonite is having anti-cancerous properties which can be used as in a form of pellet or vaccine or drug etc.- whether Ayurvedic, homoeopathy, allopathy, or Unani or may use as a nano powdered in radiotherapy for lung cancers.

CONCLUSION

The authors concluded that Amazonite as Nano medicine, when interfered with distilled cow urine and DMSO as solvents, results as a suitable agent being capable of eradicating lung cancer. In coming future, astronauts can treat themselves onboard with 3D Amazonite medication whenever they will be exposed to higher level of radiation. The authors too demonstrated the anti-cancer toxicity of Brazilian Amazonite via MTT Assays for in-vitro cell lines of human lung cancer and found amazing results which itself is a proof that it can be used as a drug with few solvents to treat cancer patients, not only on earth but too into space for astronauts that are exposed to high radiation yielding cancer as a result. In order to re-examine, the authors performed ROS anti-oxidant analysis and found drastic cell death and concluded Amazonite as an anti-cancerous agent.

Data Availability: The authors confirm that the data supporting the findings of this study are available within this article.

ABBREVIATIONS

MTT-3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide

FTIR- Fourier transform infrared spectroscopy

ROS- Reactive Oxygen Species/Oxygen radical

IC50- Inhibitory Concentration at 50%

DMSO- Dimethyl Sulfoxide

FBS- Fetal Bovine Serum

CO_2- Carbon Dioxide

DMEM- Dulbecco's modified Eagle medium

PBS- Phosphate Buffer Saline

H460- Hypotriploid human lung (cell line slides), hypoxanthine guanine phosphoribosyltransferase

OD- Optical density

NASA- National Aeronautics Space Administration

USGS- United States Geological Survey

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