



## Amazonite (A Microcline Feldspar) - An Anti-Cancerous Geo-Biotechnological Approach for MDA-MB-231: Human Breast Cancer with Validation Via ROS Anti-Oxidant Analysis

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### Abstract

The present study investigates the preliminary in vitro cytotoxic and oxidative stress-inducing potential of processed amazonite extracts against the MDA MB 231 human breast cancer cell line. Cytotoxicity was evaluated using the MTT assay, while intracellular reactive oxygen species (ROS) generation was assessed qualitatively using DCFH DA fluorescence staining. Fourier Transform Infra-red Spectroscopy (FTIR) was employed only for surface functional group observation and not to infer intrinsic organic composition.

The results demonstrate a concentration dependent reduction in cell viability, with an  $IC_{50}$  value of 64.85  $\mu\text{g/mL}$ , indicating moderate cytotoxic activity under in vitro conditions. Qualitative ROS imaging revealed increased intracellular fluorescence in treated cells, suggesting oxidative stress involvement in cell death. However, the absence of normal cell line controls, quantitative ROS analysis, physicochemical nanoparticle characterization, and in vivo validation restricts therapeutic interpretation.

This study should be considered exploratory and hypothesis generating, providing preliminary data that warrant further mechanistic, toxicological, and translational investigations before any biomedical or radiotherapeutic relevance can be proposed.

**Keywords:** Amazonite; MDA MB 231; Breast Cancer; MTT Assay; Reactive Oxygen Species; In vitro Cytotoxicity

### Abbreviations

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide; FTIR: Fourier Transform Infrared Spectroscopy; ROS: Reactive Oxygen Species/Oxygen Radical;  $IC_{50}$ : 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.

### Introduction

#### About amazonite

A widely occurring feldspar, microcline shows a range of colours including colourless, white, cream, pale yellow, salmon pink to red, and bright green to blue-green. The striking green form, called *amazonite* or *amazonstone*, is often used as a gemstone.

Amazonite crystals commonly display two sets of fine lines intersecting at right angles, a feature known as crosshatch twinning that produces a "plaid" appearance. It is this characteristic that dif-

ferentiates amazonite from both other feldspars and green jade. Individual crystals found in granite pegmatites may reach lengths of tens of metres and can weigh several tonnes [5]. The mineral is named for the Amazon River, but no known amazonite deposits exist in that area. Located at elevations exceeding 4000 metres, Colorado’s Pikes Peak district is the chief source of amazonite in the southern Front Range of the Rockies. More than 2,000 fragments of amazonite jewellery, dating to the Neolithic era some 10,000 years ago, were discovered during an archaeological excavation in southern Jordan. In modern times, amazonite is primarily sourced from China and Mongolia in East Asia, and from the southern and eastern regions of Russia’s Ural Mountains. Occurrences of deep green amazonite include the Kola Peninsula in Russia, the famous Minas Gerais mines of Brazil, Mogok in Myanmar, and Ethiopia’s Sidamo–Borana Province [7].

Breast cancer remains one of the leading causes of cancer-related mortality worldwide, necessitating the exploration of novel materials with potential anticancer properties. Natural minerals and inorganic compounds have increasingly attracted interest for their biological interactions at the cellular level. Amazonite, a green variety of microcline feldspar ( $KAlSi_3O_8$ ), is widely distributed in granitic pegmatites and is primarily valued as a gemstone.

Despite anecdotal and traditional claims regarding its medicinal relevance, systematic biological evaluation of amazonite remains extremely limited. Therefore, the present work aims to assess only the *invitro* cytotoxic and oxidative stress-associated responses of breast cancer cells exposed to processed amazonite extracts.

It’s the oldest prevailed 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

Table a

Amazonite green

For many years, people assumed that copper gave amazonite its green colour. When scientists examined it more closely, they concluded that lead impurities were actually responsible. Later still, new studies put forward another idea—that iron impurities may be the real source of the colour.

This mineral belongs to the feldspar family, which collectively accounts for about half of the Earth’s crust. Other feldspar varieties

include moonstone, sunstone, and labradorite, all of which are well-known gemstones.

Amazonite history

It is a modernised cum trade initial for microcline. The term was introduced in 1847 by German mineralogist Johann Breithaupt, though the reason for the name remains unclear, as the gemstone is not found in the Amazon River or rainforest. The name may have been selected simply for sounding more exotic than microcline

or its alternative, green feldspar. Known under different names, this gemstone has a history of use dating back to the pharaohs of Ancient Egypt; polished jewellery and beads have been found in tombs from that period, such as King Tutankhamun's (c. 1300 BC). In 2006, two ancient amazonite (microcline) mines were uncovered in the mountains of Egypt's Eastern Desert; these are the earliest known deposits, dating back to about 1800 BC.

### Medicinal importance of amazonite

Beyond its traditional use as an amulet, carved ornament, and decorative piece, amazonite has long been valued for its reputed ability to counteract electromagnetic pollution. Everywhere we go, the presence of smartphones, laptops, WiFi, Bluetooth devices, and GPS keeps us continually exposed to electromagnetic radiation. As a result, everyone is affected biologically, experiencing issues such as reduced concentration, sleep disorders, nervous system imbalance, mental challenges, stress, metabolic disruptions, a compromised immune system, and other health problems.

### Health benefits of amazonite

Amazonite, associated with the throat and heart chakras, is thought to have a direct physical effect on these regions, particularly the lungs and liver. It may boost the metabolic rate and aids in getting sound sleep. By influencing calcium in the body, it may aid in preventing osteoporosis, tooth decay, and various calcium deficiencies.

### Radiotherapy discovery

Madam Curie left a great advancement to the world. Through her work, nuclear energy and radiotherapy for treating cancer were developed, and she also enhanced the standing of science in society. The origins of radiotherapy can be traced to 1895, when German physicist W. C. Roentgen discovered X-rays, about 125 years ago. Anyhow swinging with the dangerous x-rays lead to the formulation of radiation treatment for cancer. In December 1895, German physicist Wilhelm Conrad Roentgen revealed his discovery of X-rays, earning front-page coverage in newspapers across the globe.

### Radiotherapy

Radiation therapy (also called radiotherapy) is a cancer treatment that uses high doses of radiation to kill cancer cells and shrink tumours. Radiation has different effects depending on the dose. At low levels, it is used in X-rays to view the inside of the body, such as examining teeth or detecting broken bones. At higher doses, radiation therapy works by damaging the DNA of cancer cells, causing them to stop dividing or to die. The body then breaks down and removes these damaged cells. Because this process takes time, radiation therapy does not destroy cancer cells immediately. Several days or weeks of treatment are usually required for enough DNA damage to occur, and cancer cells may continue to die for weeks or even months after treatment ends.

There are 2 kind of Radiotherapy- EXTERNAL BEAM and INTERNAL.

### External beam radiation therapy

External beam radiation therapy uses a machine that directs radiation toward the cancer. The machine is large and may be noisy, but it never touches the patient. Instead, it moves around the body, delivering radiation from multiple angles to the specific area being treated. Because it targets only one region, it is considered a localized therapy. For instance, if the cancer is in the breast, only the breast receives radiation—not the whole body.

### Internal radiation therapy

Internal radiation therapy involves placing a radiation source inside the body. This source may be either solid or liquid.

- When a solid radiation source is used, the treatment is called brachytherapy. Small seeds, ribbons, or capsules containing radioactive material are positioned in or near the tumour. Like external beam radiation, brachytherapy treats only the targeted area. The implanted material emits radiation for a limited period.
- When a liquid radiation source is used, the treatment is known as systemic therapy. Because it travels through the bloodstream, it reaches tissues throughout the body, seeking out and destroying cancer cells. Systemic radiation can be given orally, through an IV, or by injection. After treatment, body fluids such as urine, sweat, and saliva may temporarily emit radiation.

### Skin effects from cancer treatment

Cancer treatments often affect the skin and nails as side effects. Radiation therapy and chemotherapy usually cause mild skin reactions, but these problems can become more severe in patients undergoing stem cell transplants, targeted therapies, or immunotherapy.

Radiotherapy can sometimes affect the skin in the area being treated. The skin may become dry, start to peel, or feel itchy (a condition known as pruritus). It can also turn red or appear darker than usual. In many cases, the treated skin looks sunburned or becomes swollen and puffy. Some people develop painful, wet sores that may become infected—this reaction is referred to as a moist reaction.

Certain chemotherapy drugs can also lead to skin changes. Your skin might become dry, itchy, darker, or begin to peel. A mild rash may appear, and you may sunburn more easily, a sensitivity known as photosensitivity. Some individuals notice changes in their skin

pigmentation. Your nails may darken, develop cracks, or cause discomfort around the cuticles.

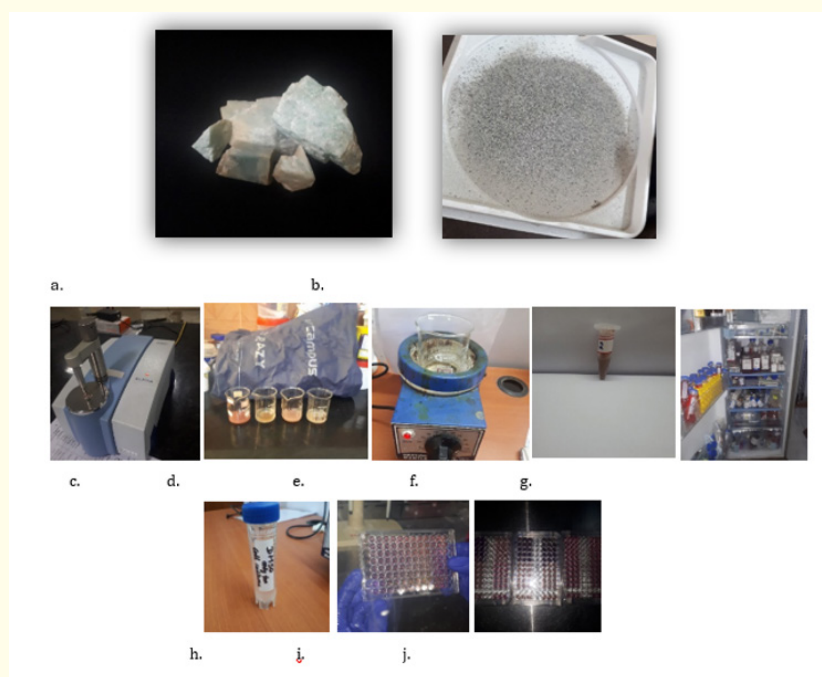
If you have had radiation therapy in the past, the area that was previously treated may unexpectedly become red, blistered, painful, or start peeling again. This reaction is known as radiation recall. Chemotherapy can also trigger allergic responses such as sudden rashes, hives, or a burning sensation.

Stem cell transplants may lead to graft-versus-host disease (GVHD), which can manifest as skin rashes, blisters, or thickened skin. Certain forms of immunotherapy may cause severe or widespread rashes, sometimes with blistering. Targeted therapies can also contribute to skin dryness, rashes, and nail problems.

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 64.85 which indicates drug with good toxicity.



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



**Figure 2:** (a-j)- 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 coloured 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. Surviving cells(n) is directly proportional to the leveled 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

MDA-MB-231 human breast cancer cell line was obtained from NCCS, Pune, and cultured in DMEM medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin. The cells were maintained at 37 °C in a humidified incubator with 5% CO<sub>2</sub>.

### MTT assay

Amazonite crude sample was tested for *in vitro* cytotoxicity, using MDA-MB-231 cells by MTT assay. Briefly, the cultured MDA-MB-231 cells were harvested by trypsinization, pooled in a 15 ml

tube. At a density of  $1 \times 10^5$  cells/ml cells, cells were plated with cells/well (200  $\mu$ L) 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% CO<sub>2</sub> for 24 hours. After the incubation period, 20  $\mu$ L of MTT (5 mg/mL) was added to each well, and the cells were incubated for an additional 2–4 hours until purple precipitates became clearly visible under the inverted microscope. Finally, the medium along with MTT (220  $\mu$ L) were aspirated off the wells and washed after that with 1X PBS (200  $\mu$ L). Further-after, DMSO (100  $\mu$ L) was added to dissolve formazan crystals, and the plate was shaken for 4 to 5 minutes. The absorbance of each well was recorded at 570 nm using a microplate reader (Thermo Fisher Scientific, USA), and cell viability percentages along with the IC<sub>50</sub> value were determined using GraphPad Prism 6 [3,4].

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

### Result/(s)

MDA-MB-231 cells/Breast 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.

MTT assay revealed a concentrationdependent decrease in cell viability following 24hour exposure to amazonite extract. The calculated IC<sub>50</sub> value was 64.85  $\mu$ g/mL.

The observed dose–response relationship demonstrated a moderate correlation ( $R^2 = 0.7798$ ), indicating biological variability.

### Trial 1<sup>st</sup>

Sample- Amazonite	Concentration in ug/ml	OD	Control OD	& Age
	0	0.956	0.956	100
	50	0.788	0.956	82.42678
	75	0.479	0.956	50.1046
	100	0.339	0.956	35.46025
	150	0.228	0.956	23.84937
	200	0.194	0.956	20.29289
	300	0.024	0.956	2.51046
	400	0.022	0.956	2.301255
	500	0.018	0.956	1.882845

**Table 1:** Different Concentration trials (1<sup>st</sup>) with OD and Controlled OD.

### 2<sup>nd</sup> Trial

Concentration in ug/ml	OD	Control OD	% Age
0	0.948	0.948	100
50	0.796	0.948	83.96624
75	0.481	0.948	50.7384
100	0.344	0.948	36.28692
150	0.237	0.948	25
200	0.186	0.948	19.62025
300	0.025	0.948	2.637131
400	0.021	0.948	2.21519
500	0.019	0.948	2.004219

**Table 2:** Different Concentration trials (2<sup>nd</sup>) with OD and Controlled OD.



### 3<sup>rd</sup> Trial

Concentration in ug/ml	OD	Control OD	% Age
0	0.959	0.959	100
50	0.793	0.959	82.6903
75	0.479	0.959	49.94786
100	0.341	0.959	35.55787
150	0.242	0.959	25.23462
200	0.178	0.959	18.561
300	0.028	0.959	2.919708
400	0.025	0.959	2.606882
500	0.018	0.959	1.876955

**Table 3:** Different Concentration trials (3<sup>rd</sup>) with OD and Controlled OD.

### Merged trials with average

Concentration in ug/ml		%Age		Average %Age
	1 <sup>st</sup> trial	2 <sup>nd</sup> trial	3 <sup>rd</sup> trial	
0	100	100	100	100
50	82.42678	83.96624	82.6903	83.02778
75	50.1046	50.7384	49.94786	50.26362
100	35.46025	36.28692	35.55787	35.76835
150	23.84937	25	25.23462	24.69466
200	20.29289	19.62025	18.561	19.49138
300	2.51046	2.637131	2.919708	2.6891
400	2.301255	2.21519	2.606882	2.374442
500	1.882845	2.004219	1.876955	1.92134

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

### For log analysis

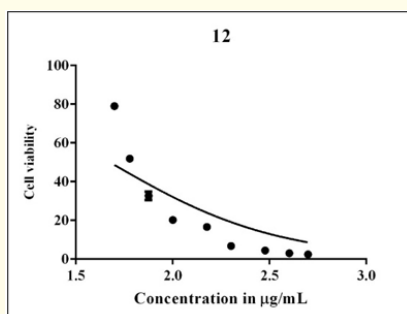
log(inhibitor) vs. normalized response	
Best-fit values	
LogIC50	1.812
IC50	64.85
Std. Error	
LogIC50	0.05792
95% Confidence Intervals	
LogIC50	1.692 to 1.932
IC50	49.21 to 85.45
Goodness of Fit	
Degrees of Freedom	23
R square	0.7798
Absolute Sum of Squares	3729
Sy.x	12.73
Number of points	
Analyzed	24

**Table 5:** Different log values so obtained with IC50 as 64.85.

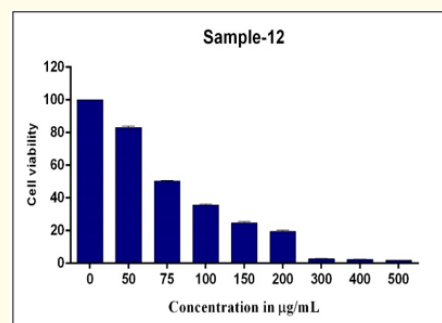
## At 570 nm

S. No	Treated sample concentration (µg/mL)	OD value at 570 nm (Triplicates)		
1	0	0.956	0.948	0.959
2	50	0.788	0.796	0.793
3	75	0.479	0.481	0.479
4	100	0.339	0.344	0.341
5	150	0.228	0.237	0.242
6	200	0.194	0.186	0.178
7	300	0.024	0.025	0.028
8	400	0.022	0.021	0.025
9	500	0.018	0.019	0.018

**Table 6:** OD values in triplicates at 570nm.



**Plot 1:** Cell Viability at different concentrations.



**Plot 2:** Bar chart representation of conc.

S. No	Treated sample concentration (µg/ml)	Cell viability (%) (In Triplicate)			Mean Value (%)
1	0	100	100	100	100
2	50	82.42678	83.96624	82.6903	83.02778
3	75	50.1046	50.7384	49.94786	50.26362
4	100	35.46025	36.28692	35.55787	35.76835
5	150	23.84937	25	25.23462	24.69466
6	200	20.29289	19.62025	18.561	19.49138
7	300	2.51046	2.637131	2.919708	2.6891
8	400	2.301255	2.21519	2.606882	2.374442
9	500	1.882845	2.004219	1.876955	1.92134

**Table 7:** Mean Value %age at different concentrations.



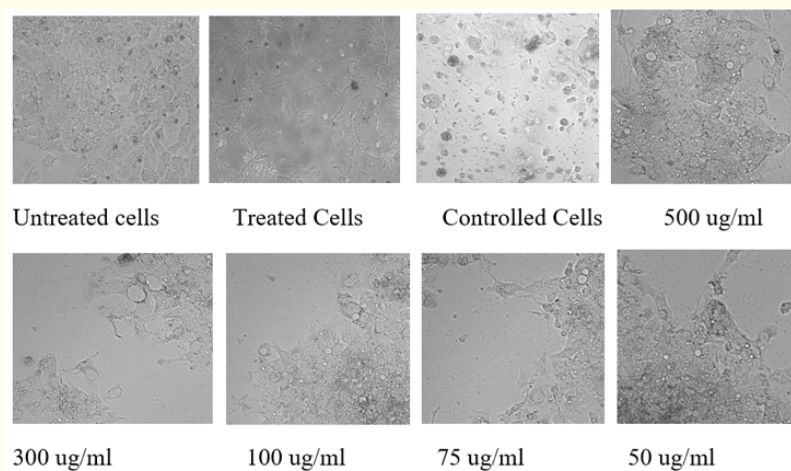
## Morphology

Microscopic examination revealed morphological changes such as cell shrinkage and reduced adherence at higher concentrations. These observations are qualitative and supportive only.

## Anti oxidant test

### Intra-cellular ROS determination/MDA-MB-231 breast cancer

INTRACELLULAR ROS ANALYSIS ROS generation was assessed using DCFHDA staining and fluorescence microscopy. Treated cells



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

exhibited increased green fluorescence compared to untreated controls.

- ROS assessment was qualitative.
- Increased ROS levels indicate oxidative stress-mediated cytotoxicity.

## Principle

In this assay, the fluorogenic probe DCFH-DA diffuses across the cell membrane and is converted by cellular esterases into the non-fluorescent compound DCFH. When reactive oxygen species (ROS) are present, DCFH is quickly oxidized into the highly fluorescent compound DCF. Then the images are captured by the fluorescence microscopy using 20 × magnification fields (Life Technology, USA).

## Materials required

The IC<sub>50</sub> 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, MDA-MB-231 cells were seeded on (1×10<sup>5</sup> cells/well) six-well plate and allowed them for overnight for maturation of cells. The very next day, old medium was aspirated with new medium containing 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 fluorescence microscopy (Fluorescence imaging station, Life Technologies, USA). The used scale bar is 125µm with 20× magnification lens.

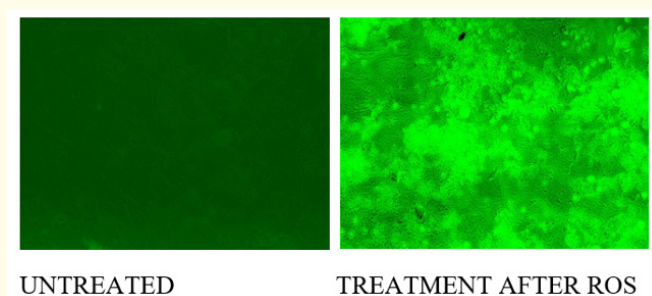
## Interpretation

The ROS molecules play a vital role in cellular mechanism and it plays an important role in the cellular apoptosis. Apoptosis is intermediated with extrinsic and intrinsic signaling pathways. Reactive oxygen species (ROS) that lived short are highly reactive molecules.

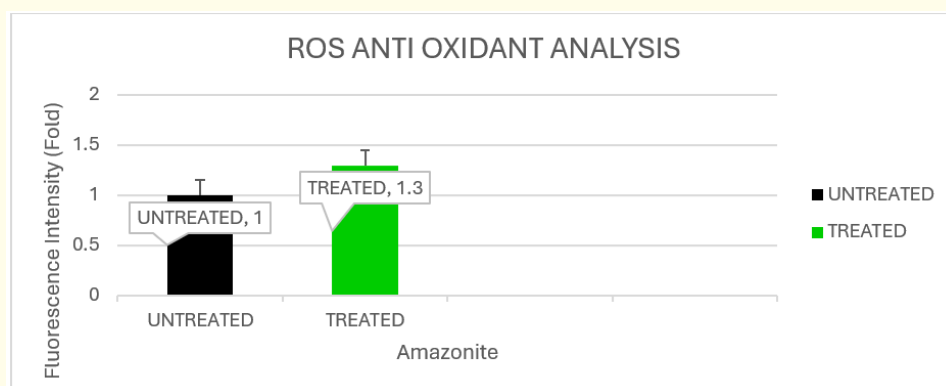
Lower doses of ROS activates cell survival signaling pathways: UPR, Nrf2. High dose of ROS activates cell death signaling pathways: apoptosis and necroptosis. ROS trigger apoptosis through the mitochondrial, death-receptor, and endoplasmic-reticulum (ER) pathways. In our results, all the samples have the capable of inducing the ROS accumulation in the cell cytoplasm and caused

the cell death in breast cancer cell line (MDA-MB-231). The obtained data judged that the intended target samples are more capable to generate the cell death.

## Results



**Figure 4:** Untreated and Treated scans captured from Elisa Plate Reader reflecting cell death with highlighted bright green.



**Plot 3:** Bar chart of ROS representing Untreated and Treated Cells.

## Fourier Transform Infrared Spectroscopy (FTIR)

The relationship between a spectrum and its interferogram through a Fourier transform was first recognized by Lord Rayleigh in 1892. Fellgett was the first to convert an interferogram into its corresponding spectrum. The modern FTIR spectrometer is based on the Fast Fourier Transform (FFT) method, introduced by Cooly and Tukey 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.

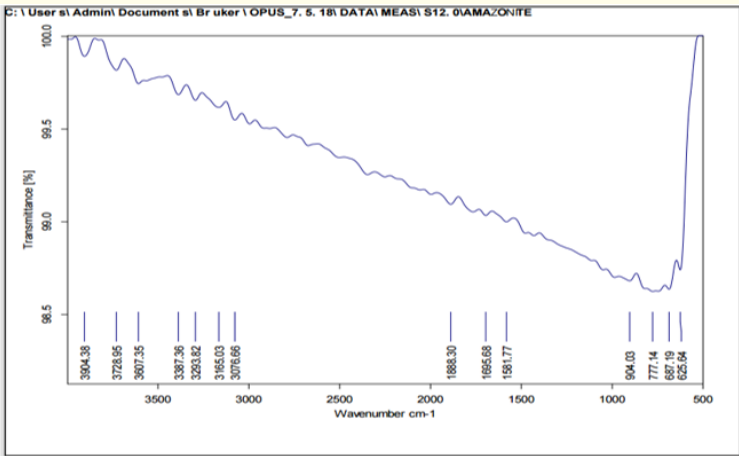


Figure 5: IR representation of Anorthosite with different peak values and transmittance.

FTIR spectrum table

Wavenumber (Highest Peaks)	Transmittance % age	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

Table 8: Conclusion table from Infrared Spectrum.

FTIR ANALYSIS FTIR spectra revealed peaks corresponding to hydroxyl and aminelike vibrations.

Given the inorganic aluminosilicate nature of amazonite, these peaks are likely attributable to surfaceadsorbed water, environmental contamination, or solvent residues rather than intrinsic organic functional groups.

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 arrived easily and stored 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. MTT Cell line studies and there after ROS Imaging Anti-Oxidant Analysis were performed and it is observed that toxicity was present in the sample and was a look alike of anti-cancer medication. May be the compounds present in the vials, in sample are having different importance that are not only confined to cancer but too to other disabilities/diseases. Furthermore, the phytochemicals present in them that are bioactive and have toxicity reflects the medicinal importance of the Amazonite. Proceeded with breast cancer for *in-vitro* experimentation, cell lines of MDA-MB-231 breast can-

cer 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.

The present study demonstrates that processed amazonite extracts can induce cytotoxicity and oxidative stress in MDAMB231 breast cancer cells under *invitro* conditions.

## Conclusion

The authors concluded that Amazonite as suppressed mineral suspension, when interfered with distilled cow urine and DMSO as solvents, results as a suitable agent being capable of eradicating breast cancer. The authors too demonstrated the anti-cancer toxicity of Brazilian Amazonite via MTT Assays for *in-vitro* cell lines of human breast Cancer and found anti-cancerous. 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. This work provides preliminary *invitro* evidence that amazonite extracts exhibit moderate cytotoxic and ROSassociated effects against MDAMB231 breast cancer cells.

## Data Availability

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

## Declaration of Competing Interest

The author shows no conflict of interest.

## Acknowledgments

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## Credit Authorship Contribution Statement

The authors designed the performed experiments, and wrote the manuscript. All authors are reviewed in the original article.

## Additional Information

Principal Author got Women Icon Asia Technology Award in the year 2022 and Woman Ph.D. Scholar of the year award 2023.

## Source of Support/Funding

No funding received. Corresponding author carried out the research by her family support.

## Miscellaneous images

Supporting Morphological analysis -Images captured during process



Figure 6

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