AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

ANORTHOSITE (LUNAR ROCK REPLICANT) - AN ANTI-CANCEROUS MEDICINAL APPROACH FOR H460: HUMAN LUNG CANCER WITH VALIDATION VIA ROS ANTI-OXIDANT ANALYSIS

Yamini Malhotra[1]*, SR Singara Subramanian[2]
*[1]Research Scholar, [2]Professor & Former Head, Dept of Earth Sciences, Annamalai University, Tamil Nadu, India.

Article info
Article History:
Received: 21-09-2023
Accepted: 18-10-2023
Published: 05-11-2023

KEYWORDS:
Anorthosite, Lunar Simulant, Cancer Drug Agent, Lung cancer- H460.

ABSTRACT

Anorthosite, a commonly occurring mineral found in lunar regolith, holds the promise of a groundbreaking dual application in the fields of cancer therapy and space medicine.

In the context of lung cancer therapy, anorthosite nanoparticles were synthesized and subjected to MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay along with FTIR to assess their cytotoxic effects on H460 lung cancer cells. The results revealed that anorthosite nanoparticles exhibit a dose-dependent cytotoxic effect on H460 cells, suggesting their potential as a novel treatment modality for lung cancer with Inhibitory Concentration IC50 value at 50% to be 21.65 which simply gestures of how toxic it could be if being treated as a cancer drug via chemo/immune/radio or target cell therapies, even when merged with ayurvedic discipline. These findings warrant further investigations into the precise mechanisms of anorthosite-mediated cytotoxicity and its therapeutic potential in vivo.

Moreover, anorthosite's role in space medicine was explored by assessing its ability to mitigate oxidative stress, a major concern for astronauts exposed to the harsh space environment. Reactive oxygen species (ROS) generated during space travel can have detrimental effects on an astronaut's health. Anorthosite nanoparticles were tested for their capacity to scavenge ROS in an in vitro model. The results demonstrated that anorthosite nanoparticles possess potent antioxidant properties, indicating their potential in safeguarding astronauts from oxidative stress-induced health issues during prolonged space missions.

This dual-application research on anorthosite nanoparticles highlights their therapeutic potential for lung cancer treatment and their utility in protecting astronauts from oxidative stress in space.

INTRODUCTION

Anorthosite is a rock comprised of the chemical components as aluminium, calcium, and silicon. Its potential has already been proven in numerous industries with revenue streams spanning from marine to automobile to windmill blades and turbines, further to insulation materials, aerospace, chemical/medical industry and many more. Proven technical advantage of anorthosite is that it escalates more environmentally friendly production processes than current sources such as bauxite and kaolin. It is a monomineralic feldspathic rock and is known to host crucial ore deposits such as ilemenite, an excellent source for high quality rock aggregate. Being primarily composed of plagioclase feldspar, most of Proterozoic anorthosites appears to be greyish blue. Huge amount of it on the Moon reveals that moon was entirely molten at some point in history because of feldspar crystals that began to crystallise out of the melt were less dense than the melt itself. It is a rare rock because it is made up of calcium plagioclase and relatively rare because of the forms of the mechanisms we don't fully understand till date. It is abundant in Europe. Here in this study, the authors collected the same from Salem, Tamil Nadu, India and tested its viability in triplicates at 570nm in MTT Assay for H460- Lung Cancer and found some amazing results with IC50 as 21.65, which indicates drug with high toxicity.

Access this article online
Quick Response Code
https://doi.org/10.47070/ayushdhara.v10i5.1319

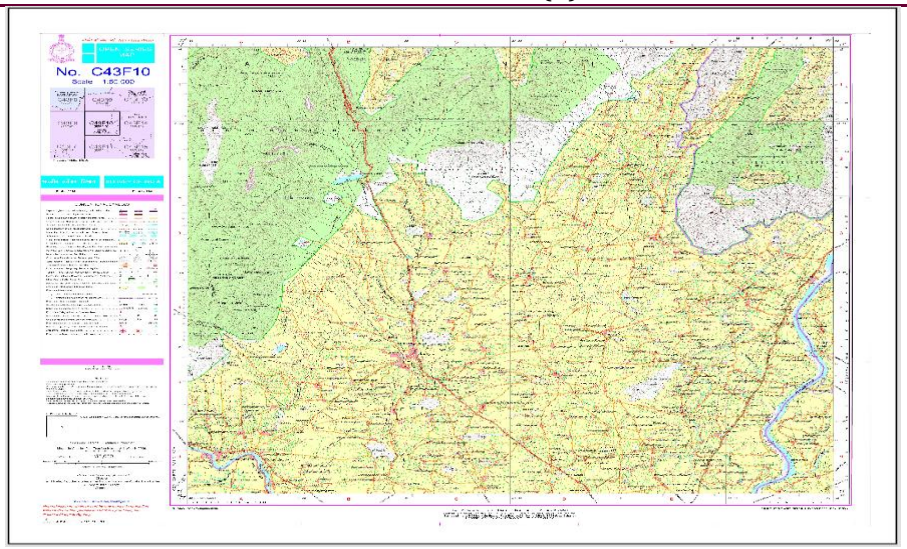
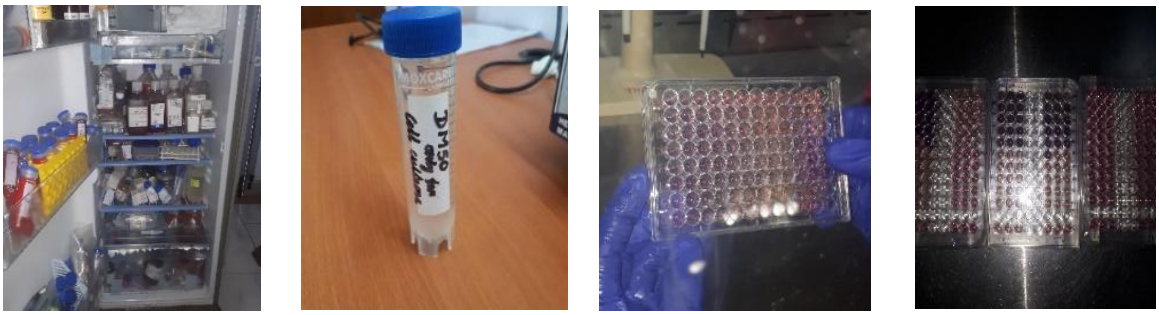


Figure 1- Topological sheet of study/field/ geographical area provided by Survey Of India.

a. b. c. d. e.

f. g. h. i.



j. k. l. m.

Figure 2 (a.-m.)- Stages of Anorthosite, 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) were 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 degrees Celsius along with 5% of CO_2.

MTT Assay

Anorthosite 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 15ml 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°C, cells were incubated in a humidified 5% CO_2 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 (100µL) was added to dissolve formazan crystals, and the plate was shaken for 4 to 5 minutes. Absorbance for each well was measured at 570nm 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.

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

Sample- Anorthosite	Concentration in ug/ml	OD	Control OD	%
	0	0.716	0.716	100
	5	0.683	0.716	95.3911
	10	0.623	0.716	87.0112
	15	0.538	0.716	75.1397
	20	0.348	0.716	48.6034
	50	0.126	0.716	17.5978
	75	0.075	0.716	10.4749
	100	0.036	0.716	5.02793
	150	0.028	0.716	3.91061
	200	0.017	0.716	2.3743
	300	0.015	0.716	2.09497
	400	0.013	0.716	1.81564
	500	0.011	0.716	1.53631

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

OD	Control OD	%	Concentration in ug/ml
0.716	0.716	100	0
0.672	0.716	93.8547	5
0.586	0.716	81.8436	10
0.541	0.716	75.5587	15
0.352	0.716	49.162	20
0.143	0.716	19.9721	50
0.083	0.716	11.5922	75
0.039	0.716	5.44693	100
0.024	0.716	3.35196	150
0.019	0.716	2.65363	200
0.016	0.716	2.23464	300
0.014	0.716	1.95531	400
0.012	0.716	1.67598	500

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

0.716	0.716	100	0
0.649	0.716	90.6425	5
0.567	0.716	79.1899	10
0.526	0.716	73.4637	15
0.346	0.716	48.324	20
0.163	0.716	22.7654	50
0.095	0.716	13.2682	75
0.041	0.716	5.72626	100
0.035	0.716	4.88827	150
0.024	0.716	3.35196	200
0.017	0.716	2.3743	300
0.014	0.716	1.95531	400
0.012	0.716	1.67598	500

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

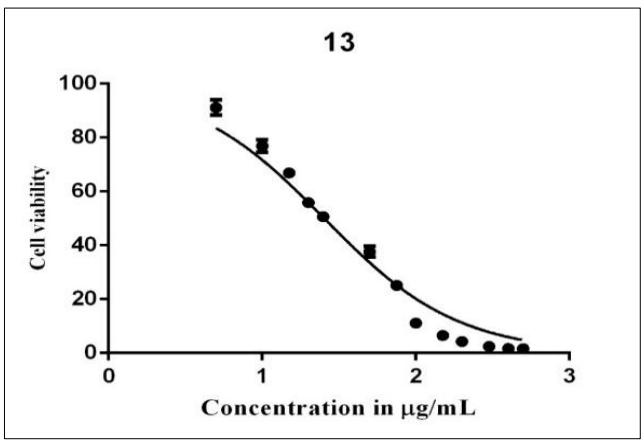
Concentration in ug/ml		%		Average %
Con.c	1st trial	2nd trial	3rd trial	
0	100	100	100	100
5	95.3911	93.8547	90.6425	93.2961
10	87.0112	81.8436	79.1899	82.6816
15	75.1397	75.5587	73.4637	74.7207
20	48.6034	49.162	48.324	48.6965
50	17.5978	19.9721	22.7654	20.1117
75	10.4749	11.5922	13.2682	11.7784
100	5.02793	5.44693	5.72626	5.40037
150	3.91061	3.35196	4.88827	4.05028
200	2.3743	2.65363	3.35196	2.7933
300	2.09497	2.23464	2.3743	2.23464
400	1.81564	1.95531	1.95531	1.90875
500	1.53631	1.67598	1.67598	1.62942

Table 5: Different log values so obtained with IC50 as 21.65

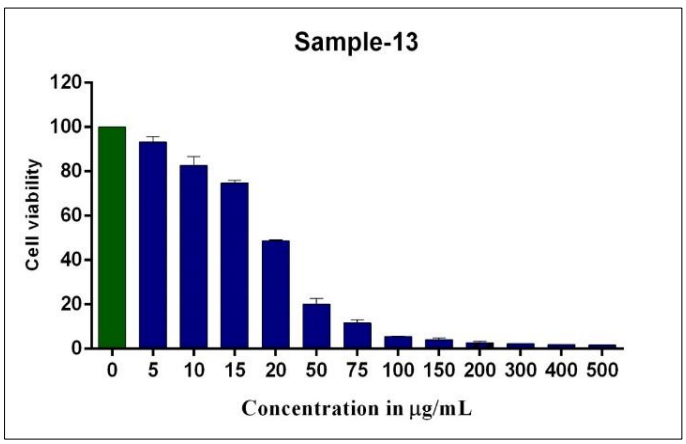
Log (inhibitor) vs. normalized response	
Best-fit values	
LogIC50	1.335
IC50	21.65
Std. Error	
LogIC50	0.04447
95% Confidence Intervals	
LogIC50	1.245 to 1.426
IC50	17.58 to 26.65
Goodness of Fit	
Degrees of Freedom	35
R square	0.9173
Absolute Sum of Squares	3463
Sy.x	9.947
Number of points	
Analyzed	36

Table 6: OD values in triplicates at 570nm

S.No	**Treated sample concentration (µg/mL)**	**OD value at 570 nm (Triplicates)**		
1	0	0.716	0.716	0.716
2	5	0.683	0.672	0.649
3	10	0.623	0.586	0.567
4	15	0.538	0.541	0.526
5	20	0.348	0.352	0.346
6	50	0.126	0.143	0.163
7	75	0.075	0.083	0.095
8	100	0.036	0.039	0.041
9	150	0.028	0.024	0.035
10	200	0.017	0.019	0.024
11	300	0.015	0.016	0.017
12	400	0.013	0.014	0.014
13	500	0.011	0.012	0.012
14	0	0.716	0.716	0.716



Plot 1- Cell Viability at different concentrations



Plot 2- Bar chart representation of con.c

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	5	95.3911	93.8547	90.6425	93.2961
3	10	87.0112	81.8436	79.1899	82.6816
4	15	75.1397	75.5587	73.4637	74.7207
5	20	48.6034	49.162	48.324	48.6965
6	50	17.5978	19.9721	22.7654	20.1117
7	75	10.4749	11.5922	13.2682	11.7784
8	100	5.02793	5.44693	5.72626	5.40037
9	150	3.91061	3.35196	4.88827	4.05028
10	200	2.3743	2.65363	3.35196	2.7933
11	300	2.09497	2.23464	2.3743	2.23464
12	400	1.81564	1.95531	1.95531	1.90875
13	500	1.53631	1.67598	1.67598	1.62942

Morphology

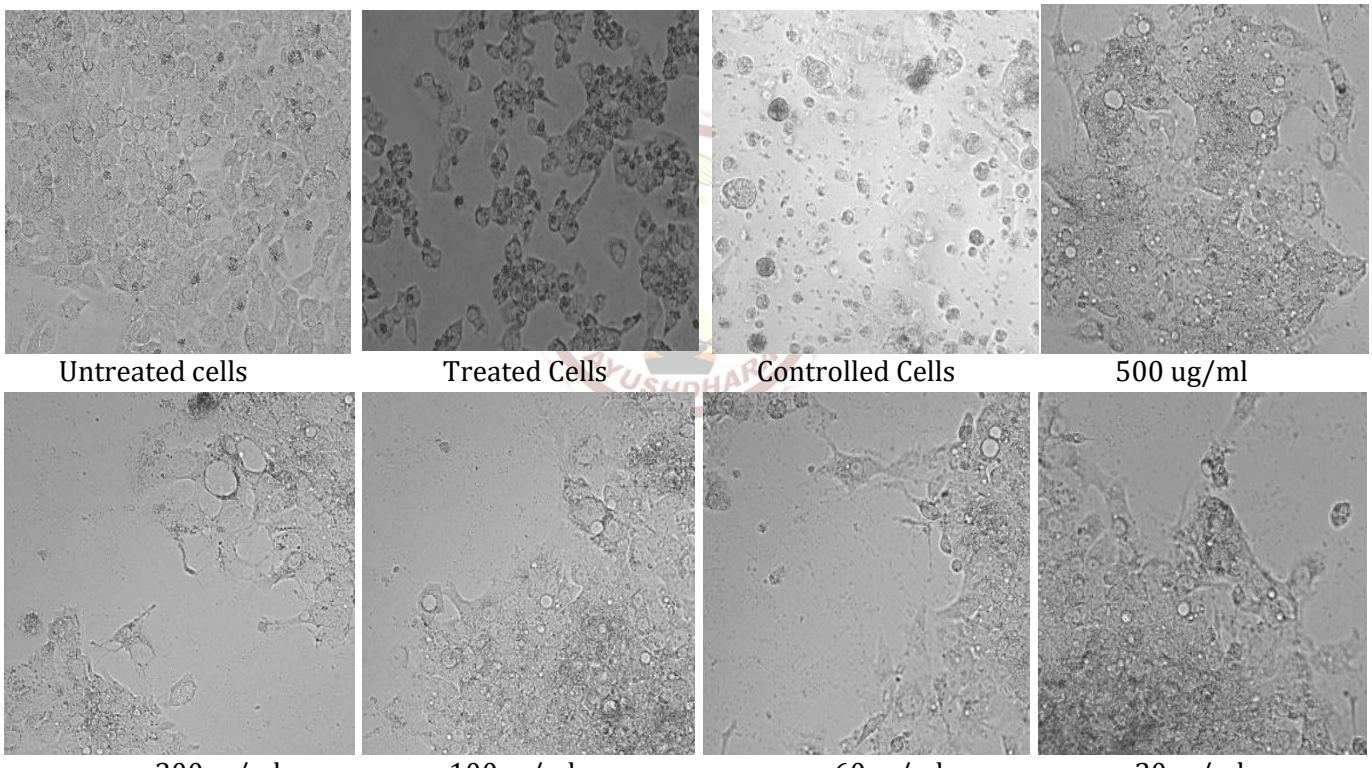


Figure 3: Images captured from Elisa Plate Reader at different concentrations- Cell Death Experience via targeted drug therapy

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 microcopy using 20× magnification fields (Life Technology, USA).

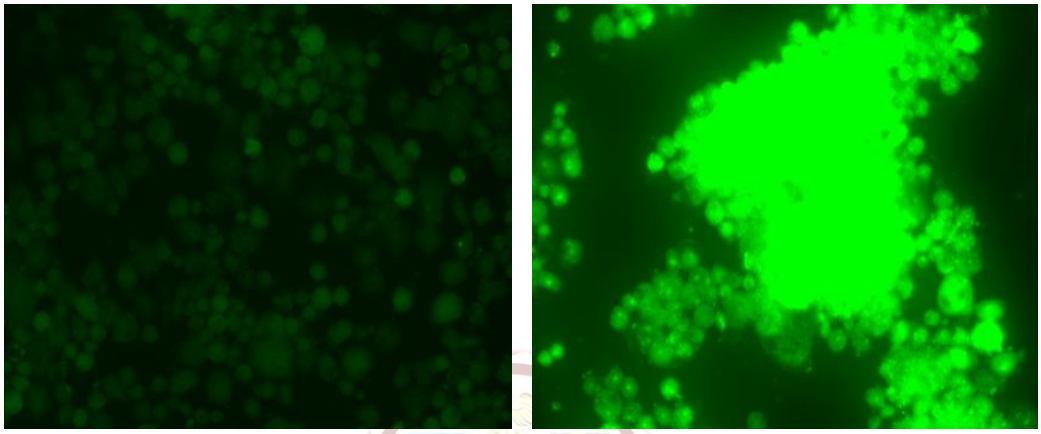
Materials Required: The IC50 Treated cells in experiment plate, 1× PBS solution, DCFH-DA (10mg/mL of DMSO), fluorescent microscope and Pipette.

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 microcopy (Floid imaging station, Life Technologies, USA). The used scale bar is 125µm with 20× magnification lens.

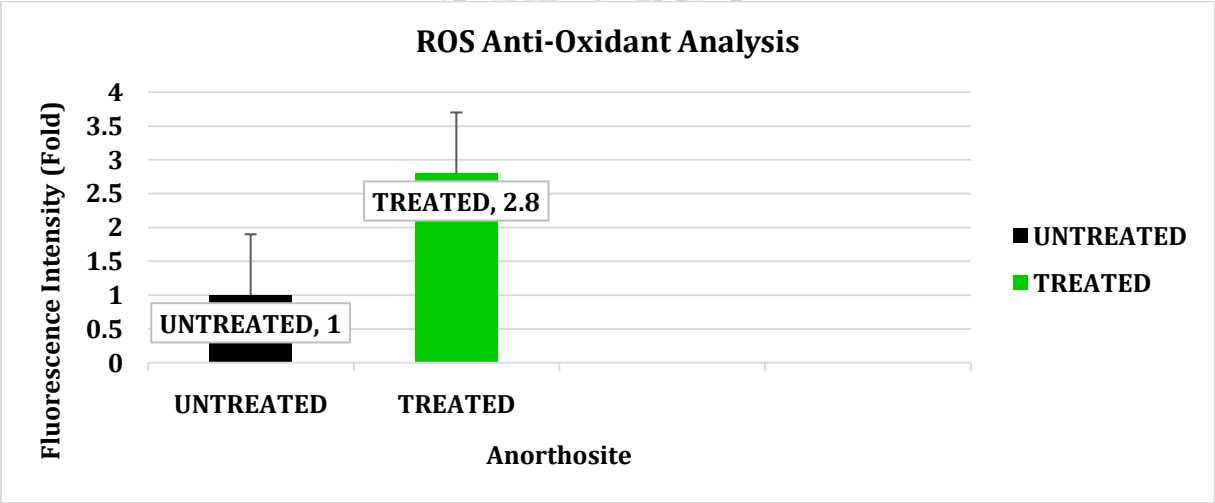
Interpretation- The ROS molecules play a vital role in cellular mechanism and it play important role in the cellular apoptosis. Apoptosis is mediated by extrinsic and intrinsic signalling pathways. Reactive oxygen species (ROS) are short-lived and highly reactive molecules. Low doses of ROS activate cell survival signalling 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.

Results



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

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.

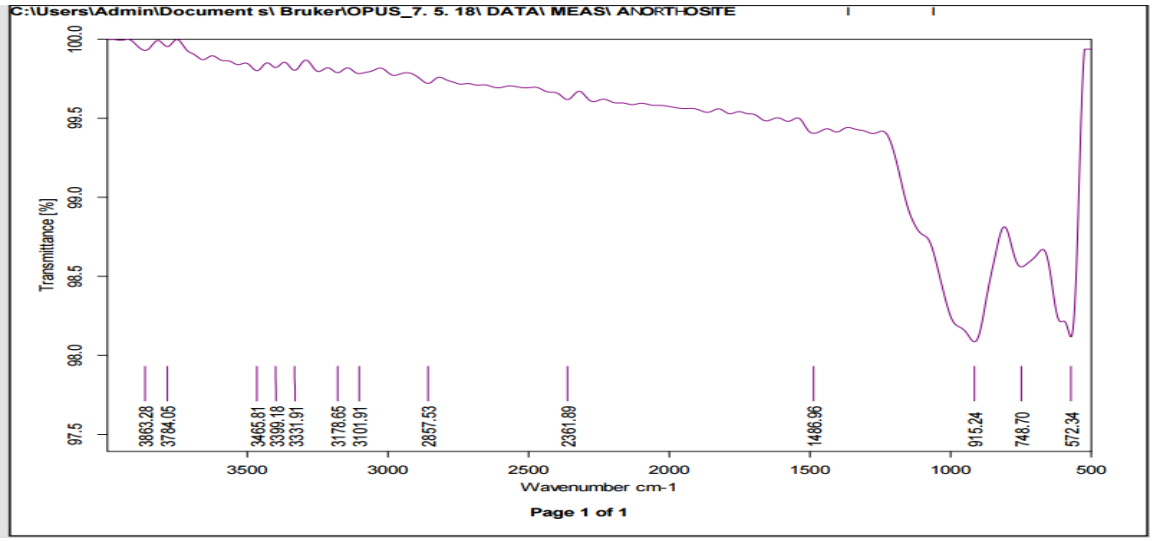


Figure 6: IR representation of Anorthosite with different peak values and transmittance

FTIR Spectrum Table

Table 7: Conclusion table from Infrared Spectrum

Wave Number (Highest Peaks)	Transmittance %	Functional Class	Stretching Vibration
3863.28	100	Alcohols / Phenols	O-H (free), usually sharp
3784.05	100	Alcohols/Phenols	O-H(H-bonded), usually broad
3465.81	99.8	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- Anorthosite. To begin with, the authors picked the toposheet of Salem, South India from Survey of India and studied the seasonal variations of different Land Use Land Patter via satellite data so provided by Indian Space Research Organisation, India. There-after, sampling trip was conducted along with GPS tracker to find the desired location of Anorthosite site in Salem. Samples were collected 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 Anorthosite.

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 Anorthosite 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, Anorthosite 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.

CONCLUSION

The authors concluded that anorthosite as nano medicine, when interfered with distilled cow urine and DMSO as solvents, results as a suitable agent being capable of eradicating lung cancer. As Moon is full of anorthosite, hence, in coming future, astronauts can treat themselves onboard with 3D anorthosite medication whenever they will exposed to higher level of radiation. The authors too demonstrated the anti-cancer toxicity of Salem's anorthosite via MTT Assays for in-vitro cell lines of human lung Cancer and found amazing results which itself is a proof that anorthosite 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 Anorthosite as an anti-cancerous formulation.

Acknowledgments- Thankful to Tri-Biotech Research Lab, and Bharathidasan University, Trichy, Tamil Nadu for getting the tests done under their payable facility for university scholars.

Credit Authorship Contribution Statement- Authors designed the performed experiments, and wrote the manuscript. All authors are reviewed in the original article.

Additional Information- Corresponding Author got Women Icon Asia Technology Award in the year 2022 and Woman Ph.D. Scholar Award for 2023.

Presentations of Corresponding Author- Presented first in Indian Cancer Congress 2013, New Delhi with published abstract in Indian Journal of Cancer- Poster. Then, to ISO, ISMPO, RGCON etc. till April 2022- Oral and Poster both, was in top 4. Having all certificates.

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

REFERENCES

1. Mosmann, Tim. "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays." Journal of immunological methods 65, no. 1-2 (1983): 55-63.
2. Marshall, N. J., C. J. Goodwin, and S. J. Holt. "A critical assessment of the use of microculture tetrazolium assays to measure cell growth and function." Growth regulation 5, no. 2 (1995): 69-84.
3. Kim H, Xue X. Detection of Total Reactive Oxygen Species in Adherent Cells by 2',7'-Dichlorodihydrofluorescein Diacetate Staining. J Vis Exp. 2020 Jun 23; (160): 10.3791/60682. doi: 10.3791/60682. PMID: 32658187; PMCID: PMC7712457.
4. Aranda A, Sequedo L, Tolosa L, Quintas G, Burello E, Castell JV, Gombau L. Dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay: a quantitative method for oxidative stress assessment of nanoparticle- treated cells. Toxicol In Vitro. 2013 Mar; 27(2): 954-63. doi: 10.1016/j.tiv.2013.01.016. Epub 2013 Jan 26. PMID: 23357416.
5. Yamini, SR Singara Subramanian., Lunar Geology- An Overview., Journal of Emerging Technologies and Innovative Research, pages. 861-868., http://doi.one/10.1729/Journal.29323

Cite this article as:
Yamini Malhotra, SR Singara Subramanian. Anorthosite (Lunar Rock Replicant) - An Anti-Cancerous Medicinal Approach for H460: Human Lung Cancer with Validation via Ros Anti-Oxidant Analysis. AYUSHDHARA, 2023;10(5):11-19.
https://doi.org/10.47070/ayushdhara.v10i5.1319
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. Yamini Malhotra
Research Scholar
Dept of Earth Sciences,
Annamalai University,
Tamil Nadu, India.
Email:
yaminipriya2008@gmail.com