Anti cancerous effect of bioactive compounds derived from the aqueous extract of Seaweed *Laurencia karachiana* Bibi

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Abstract
Cancer is one of the major non-communicable disease affecting 18 million patients worldwide. It is reported to be the second most lethal disease in the world. Natural products are attractive sources for the development of new medicinal and therapeutic agents for their cell selective and fewer adverse effects. Natural origins are the main origins for approved drugs in the treatment of cancer, occupying almost 60%. In this context, it is significant to develop natural products in cancer treatment. A large number of studies have uncovered the anti-cancer activities of seaweeds and numerous seaweed-derived compounds have been shown to be effective through multiple mechanisms such as the inhibition of cancer cell growth, invasiveness and metastasis as well as by the induction of apoptosis in cancer cells. Therefore, the present investigation undertakes to explore the anticancer activity of Laurencia karachiana Bibi.

The A549 (Human lung adenocarcinoma cell line) and L929 (Mouse embryo fibroblast cell line) were purchased from NCCS, Pune, India. MTT assay was conducted to find out the antioxidant and anti-cancerous activity. The aqueous extract of L. karachiana shows a concentration dependent anti-oxidant activity and is cytotoxic in nature on human lung cancer (A549) cells with IC_{50} value of 56.85 µg/ml and non-cytotoxic in nature on mouse embryo fibroblast (L929) cells with IC_{50} value of 219.05 µg/ml. The MTT assay results suggest that the given test compound exhibited cytotoxic and anti-cancer activity against human lung cancer cells without affecting any toxic potency on normal mouse fibroblast cells and further studies have to be conducted to determine the molecular mechanism behind cytotoxicity properties of the test compound.

Keywords: Macroalgae, *Laurencia karachiana*, Anticancer activity, Phytochemical analysis.

Introduction

Cancer is one of the non-communicable disease. Cancer is a disease in which some of the body cells grow uncontrollably and spread to other parts of the body[7]. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Cancer diagnosed and treated in the early stages is usually not life threatening. Cancer can kill when it invades essential organs like your liver, lungs or brain and stops them from functioning properly. Lung cancer is one of the most common cancers to be diagnosed in the World, but many patients are only diagnosed when the condition is at an advanced stage. The most common cause of lung cancer is smoking.

Material and Methods

Preparation of extracts: The seaweeds were rinsed with sterile sea water to remove any adherents and necrotic parts and then dried in the shade at room temperature. The dried seaweeds were then powdered in an electric grinder or using sterile mortar and pestle. Each powdered sample of seaweeds (2g) was suspended in 3 different solvents (methanol, acetone and distilled water) in 50ml respectively. After 72 hrs, the mixture was kept in shaker at 25°C and 100 RPM for 9 hrs. The filtrate was filtered through Whatmann no. 1 filter paper fitted with a buchner funnel using suction presser funnel or glass funnel. The process was repeated once more and the two filtrates were combined. The collected filtrates were stored in the refrigerator for future further activity.

Laurencia karachiana the newly discovered seaweed specimen, was preserved in the Herbarium of Centre of Excellence in Marine Biology at Karachi University. *Laurencia karachiana* species was jointly discovered by Pakistani and Brazilian scientists.

MTT assay: MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570 nm^2.

Materials required:

1. Cell lines:

a) A549-Human Lung adenocarcinoma cell lines (NCCS, Pune)
b) L929–Mouse embryo fibroblast cell line (NCCS, Pune)
c) Cell culture medium: DMEM- High Glucose - (AL111, Himedia)

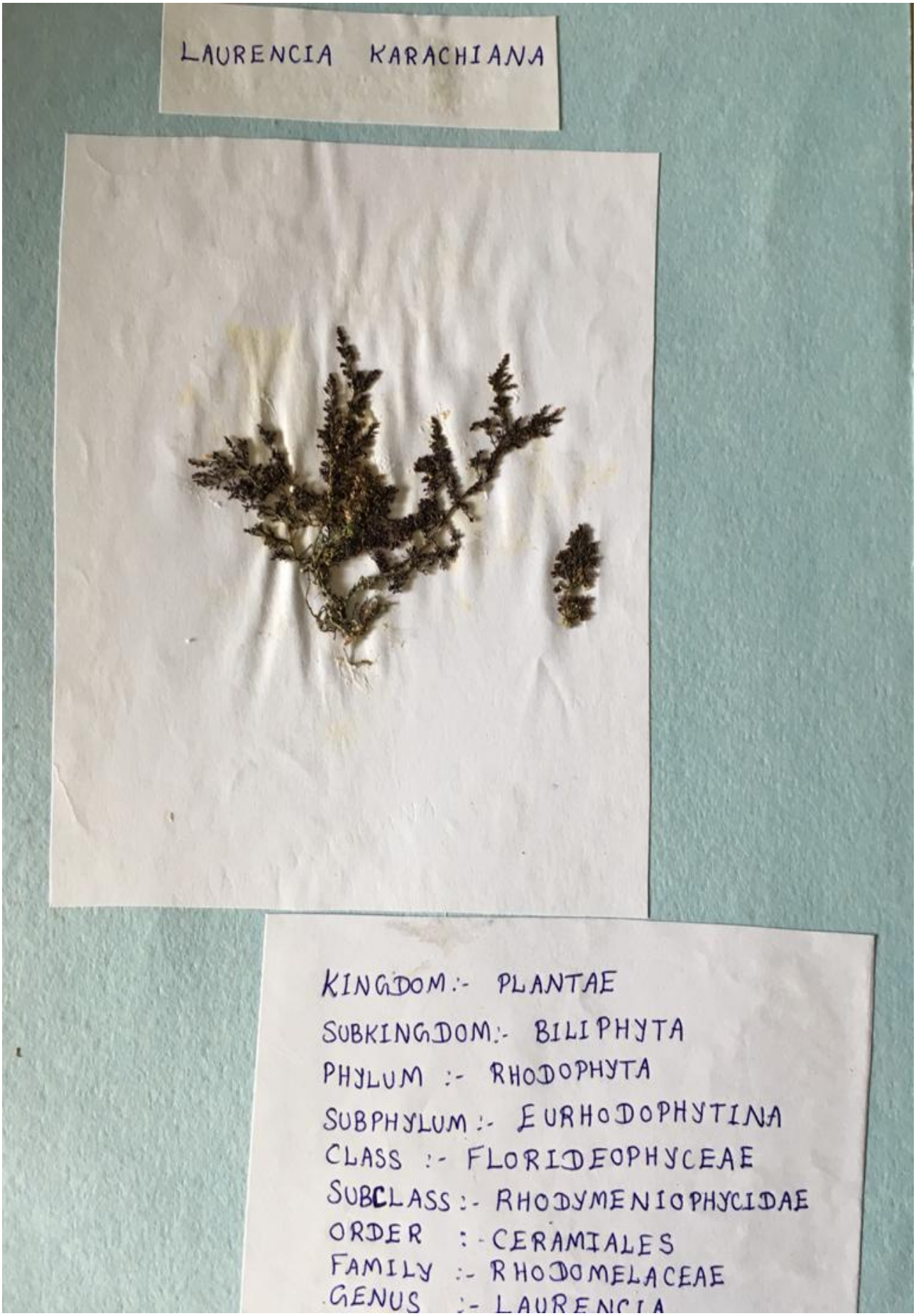

Fig. 1: Herbarium preparation of *Laurencia karachiana* bibi

Table 1
Phytochemical analysis of *Laurencia karachiana*

S.N.	Test	Experiment	Observation	Inference
01.	Test for Alkaloids	Extract+Mercuric chloride and Potassium iodide	White turbidity or precipitate develops.	Presence of Alkaloids.
02.	Test for steroids	1ml test solution+chloroform+2 drops of conc. H_2SO_4	Red colour develops	Presence of steroids
03.	Test for Tannins	1ml extract+1ml Distilled water+few drops of 1%lead acetate	White ppt present	Presence of Tannins
04.	Test for pholobatanins	10ml extract+few drops1%Hcl	-	Pholobatanins absent
05.	Test for Saponins	1ml extract +1ml distilled water &mix well	Foamy lather develops	Presence of Saponins
06.	Test for Flavonoids	1ml extract+few drops 1%ammonia solution	Appearance of yellow colour	Flavonoids present
07	Test for Terpenoids	5ml extract+2ml chloroform+3ml conc. H_2SO_4	Reddish brown colour formed	Terpenoids present
08	Test for Cardiac Glycosides	5ml test solution +2ml glacial acetic acid+1ml ferric chloride+add 1ml of conc. H_2SO_4	Brown ring appears	Presence of cardiac glycosides
09	Test for phenolic compound	1ml seaweed solution+few drops of Ferric Chloride	Intense colour develops	Presence of phenol compound.
10.	Test for Aromatic acids	1ml of seaweed solution+add a pinch of Sodium bicarbonate	Brick effervescence absent	Aromatic compound absent
11.	Test for Xanthoprotein	1ml of seaweed solution+add a few drops of H_2SO_4+1ml Ammonia solution.	Yellow ppt	Xanthoprotein present

2. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
3. Fetal Bovine Serum (RM10432, Himedia)
4. MTT Reagent (5 mg/ml) (4060 Himedia)
5. DMSO (PHR1309, Sigma)
6. D-PBS (TL1006, Himedia)
7. Cisplatin (Cat No: PHR1624, Sigma)
8. 96-well plate for culturing the cells (From Corning, USA)
9. T25 flask (12556009, Biolite - Thermo)
10. 50 ml centrifuge tubes (546043 TORSON)
11. 1.5 ml centrifuge tubes (TORSON)
12. 10 ml serological pipettes (TORSON)
13. 10 to 1000 ul tips (TORSON)

Equipment's:
1. Centrifuge (Remi: R-8ºC).
2. Pipettes: 2-10µl, 10-100µl and 100-1000µl.
3. Inverted binocular biological microscope (Biolinkz, India)
4. Biosafety hood (Biobase, China)
5. 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China)

Assay controls:
(i) Medium control (medium without cells)
(ii) Negative control (medium with cells but without the experimental drug/compound)
(iii) Positive control (medium with cells treated with 15ug/ml of Cisplatin)

Extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as in test wells.

Maintenance of cell lines: The A549 (Human lung adenocarcinoma cell line) and L929 (Mouse embryo fibroblast cell line) were purchased from NCCS, Pune, India. The A549 and L929 cells were maintained in DMEM high glucose media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^0C temperature in the CO_2 incubator and sub-cultured for every 2days.

Steps followed
1. Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours.
2. Add appropriate concentrations of the test agent.
3. Incubate the plate for 24hrs at 37°C in a 5% CO_2 atmosphere.
4. After the incubation period, takeout the plates from incubator and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.
5. Wrap the plate with aluminium foil to avoid exposure to light.
6. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within

one experiment, incubation time should be kept constant while making comparisons).
7. Remove the MTT reagent and then add 100 µl of solubilisation solution (DMSO).
8. Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures.
9. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference wavelength.
10. % cell viability is calculated using below formula:
% cell viability = [Mean abs of treated cells/Mean abs of Untreated cells] x 100
11. The IC_{50} value was determined by using linear regression equation:

$Y = Mx + C$

where Y = 50, M and C values were derived from the viability graph.

Results

In this study, 1 test compound is evaluated to analyse the cytotoxicity effect on A549 and L929 cells. The results of cytotoxicity study performed by MTT assay suggest that the test compound is cytotoxic in nature on human lung cancer (A549) cells with IC_{50} value of 56.85ug/ml and same compound is non-cytotoxic in nature on Mouse embryo fibroblast (L929) cells. Hence, test compound is showing significant anti-cancer (anti-lung cancer) potency on human lung cancer cells due to its low IC_{50} value.

The absorbance readings with calculations are enclosed in MS excel format. The direct microscopic observations of drug treated images of test compound after 24 hours of incubation were enclosed in the separate folder along with this report.

Discussion

Chemotherapy in cancer treatment often suffers from the issue of resistance towards the cancerous cells and toxicity towards normal cells. Such issues have encouraged researchers to look for an alternative medicine, particularly from natural sources, that are considered to be safe. In the present study, Laurencia karachiana seaweed extract has the ability to kill the cancerous growth. Phenolic compounds are a large family of natural organic compounds found in seaweeds. Poly phenols are known as radical scavengers that improve endogenous antioxidant properties.

Table 2
Details of drug treatment to respective cell lines used for the study

S.N.	Test Compound	Cell Line	Concentration treated to cells
1	Untreated	A549 & L929	No treatment
2	Blank	-	Only Media without cells
3	Std control	A549 & L929	15µg/ml
4	Test	A549 & L929	5 (12.5, 25, 50,100 and 200µg/ml)

Table 3
IC_{50} values of Test treated on A549 cells after the treatment period of 24hrs.

Cell line	IC50 conc (µg/ml)
A549	99.78
L929	219.05

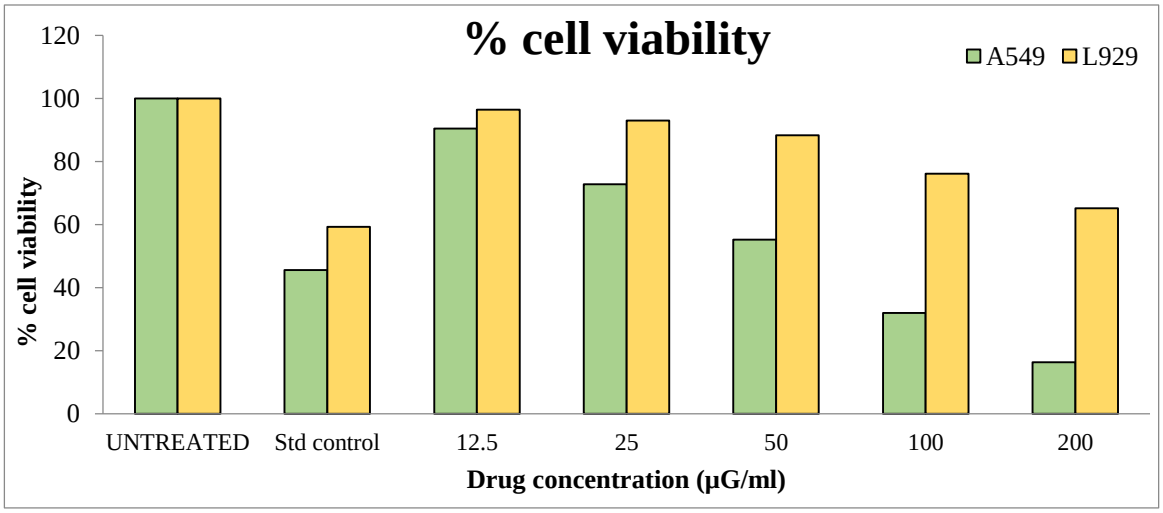

Fig. 2: % cell viability of Test treated A549and L929cells after the incubation period of 24hrs

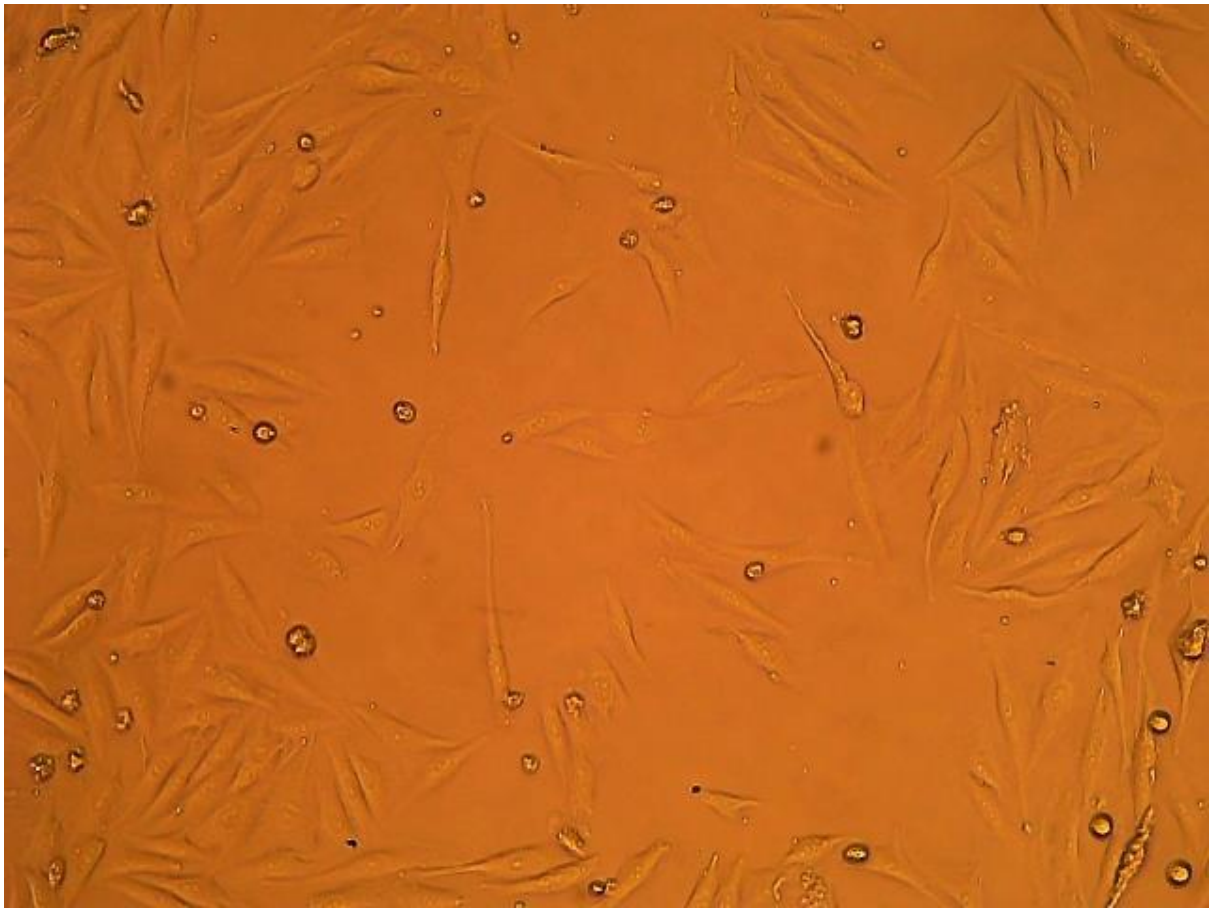

Fig. 3: Cell viability test (12.5µg) L929 -Mouse embryo fibroblast cell line (NCCS,Pune)

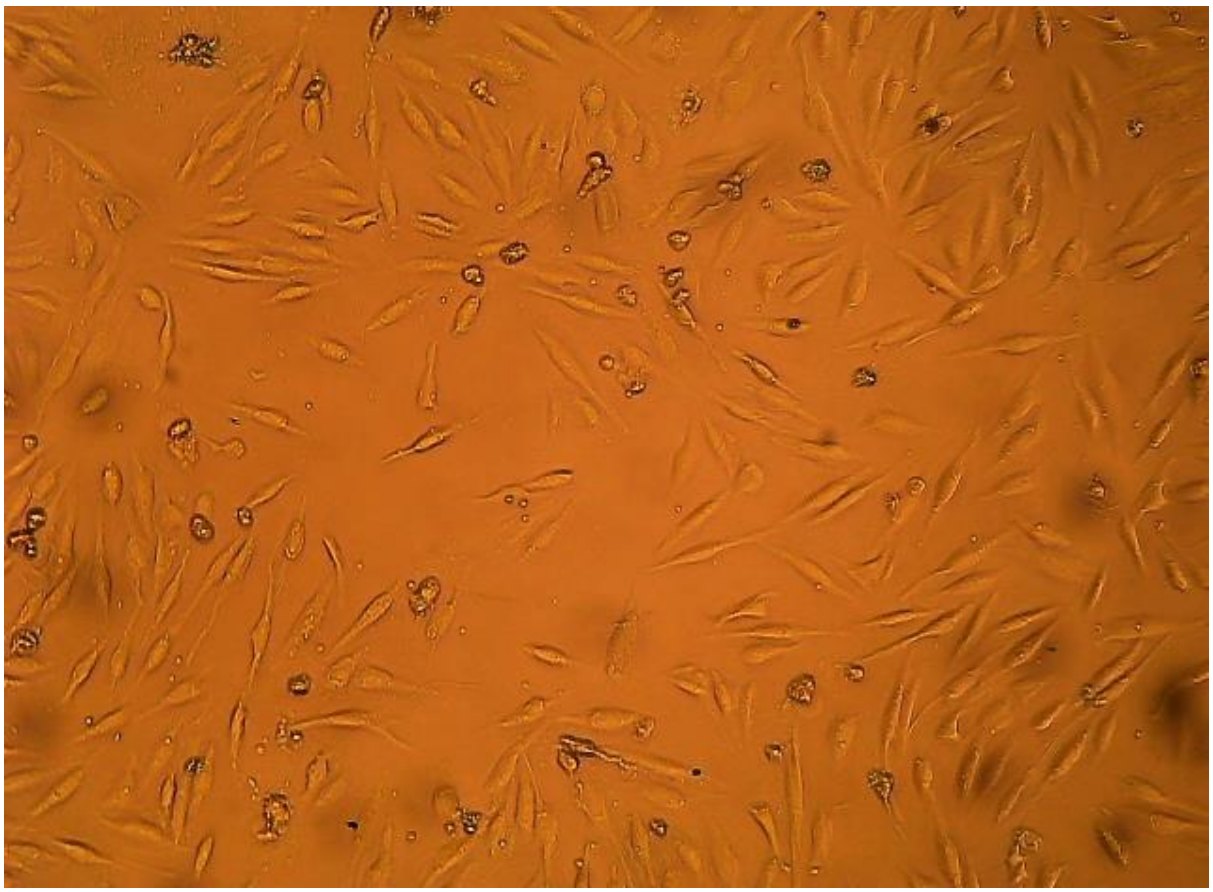

Fig. 3: (a) Cell viability test (25µg) L929 -Mouse embryo fibroblast cell line (NCCS, Pune)

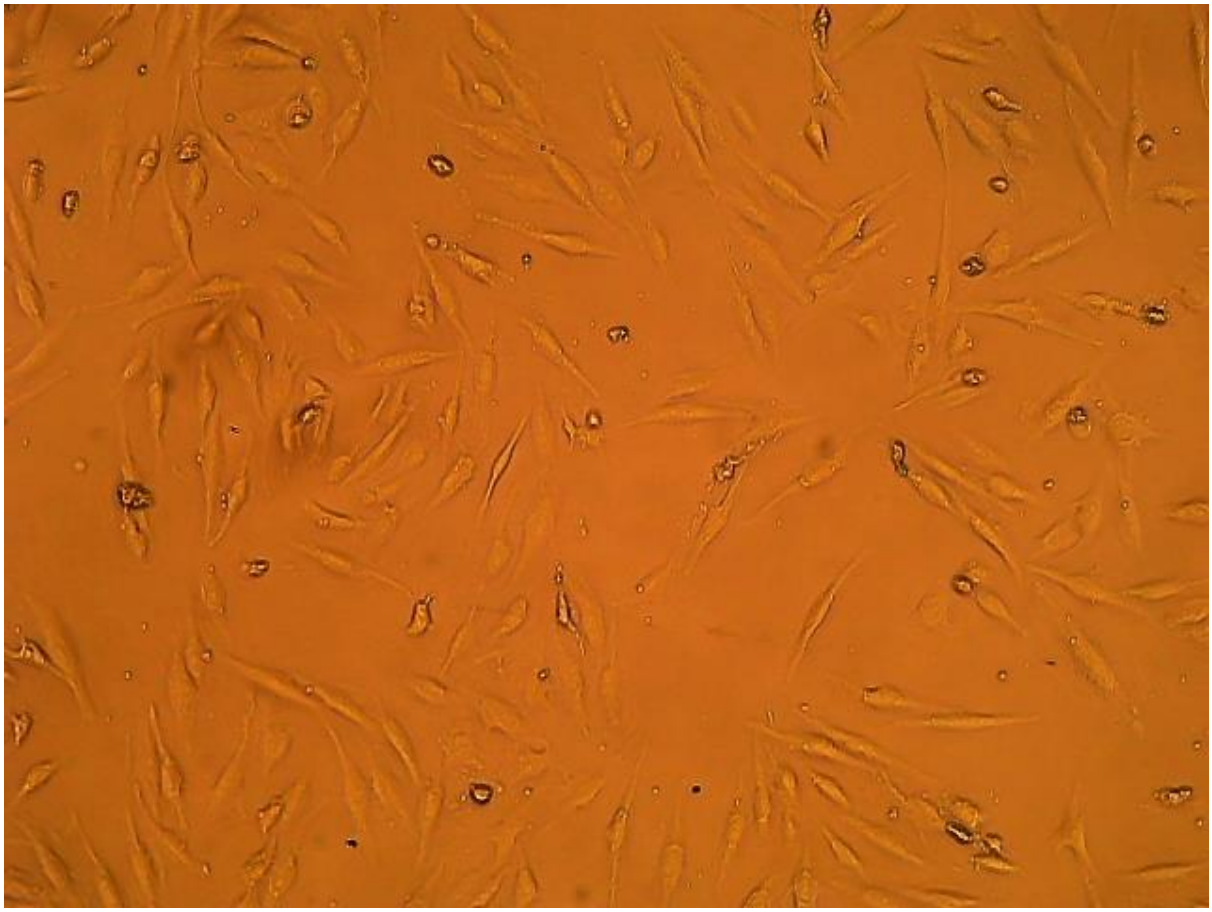

Fig. 3: (b) Cell viability test (50µg) L929 -Mouse embryo fibroblast cell line (NCCS, Pune)

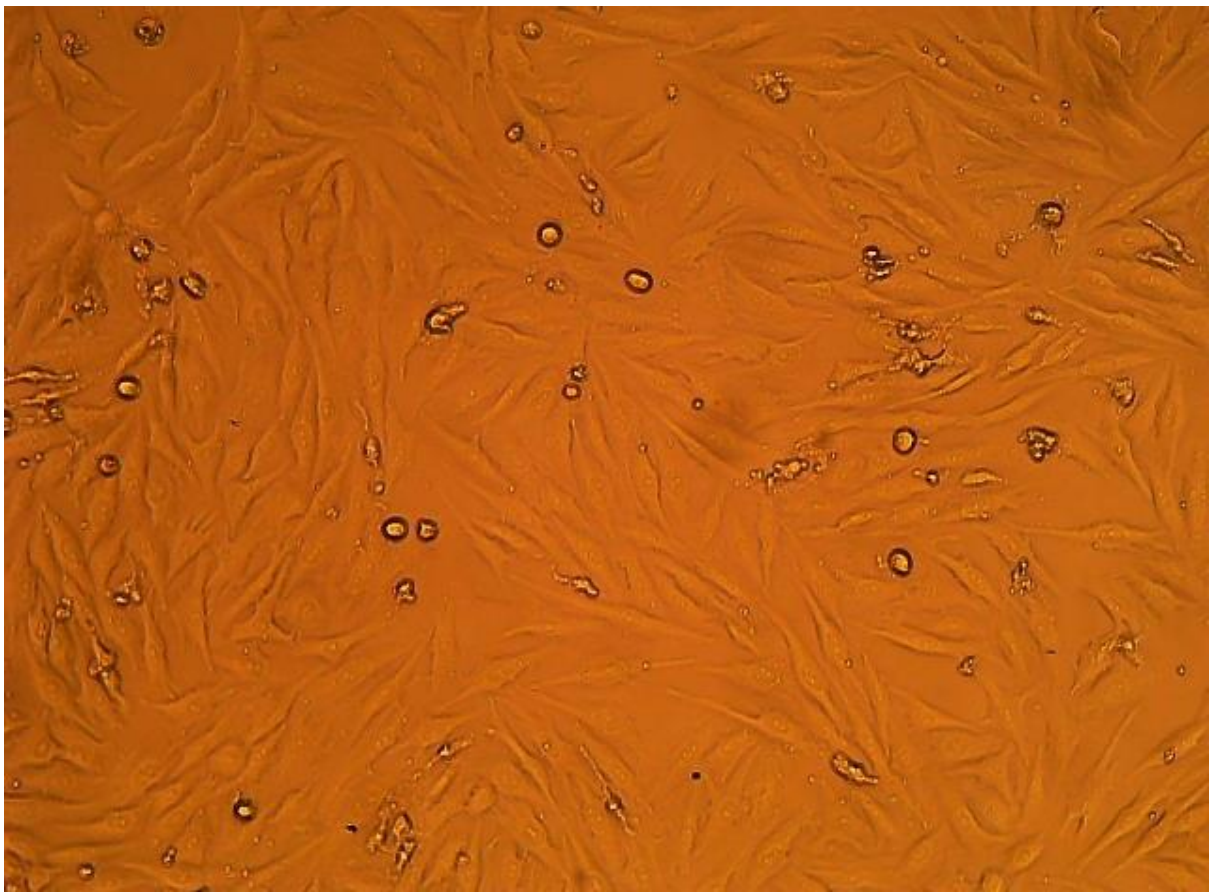

Fig. 3: (c) Cell viability test (100µg) L929 -Mouse embryo fibroblast cell line (NCCS, Pune)

Seaweeds generate poly phenols as secondary metabolites and poly phenols have beneficial effects including antioxidant, antiviral, antimicrobial and anticancereffect[25]. The seaweed diterpenoid showed cytotoxic effect on cancer cell line and this compound exhibited a clear mechanism of microtubule assembly inhibition, which can cause mitotic arrest and inhibitory effects on cell invasion[5].

Conclusion

The results of this study suggest that the therapeutic benefits of the seaweeds extract in the treatment of cancer could be improved effectively. The MTT assay results suggest us that the given test compound is cytotoxic as well as anti-cancer in nature on human lung cancer cells without affecting any toxic potency on normal mouse fibroblast cells. Further studies have to be conducted to determine the molecular mechanism behind cytotoxicity properties of the test compound at *in vitro* level.

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References

1. Alley M.C. et al, Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay, *Cancer Res*., **48**, 589-601 **(1988)**

2. Alley M.C., Scudiere D.A., Monks A., Czerwinski M., Shoemaker R. II. and Boyd M.R., Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines, *Proc. Am. Assoc. Cancer Res*., **27**, 389 **(1986)**

3. Beauchamp C. and Fridovich I., Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels, *Analytical Biochemistry*, **44(1)**, 276–287 **(1971)**

4. Damu Amooru G. et al, Isolation, Structute and Structure - Cytotoxic Activity Relationships of Withanolides and Physalins from Physalis angulate, *J. Nat. Prod*., **70(7)**, 1146-1152 **(2007)**

5. Depix M.S., Martinez J., Santibanez F., Rovirosa J., San Martin A. and Maccioni R.B., The compound 14-keto-Stypodiol diacetate from the algae Stypopodium flabelliforme inhibits microtubules and cell proliferation in DU-145 human prostatic cells, *Mol Cell Biochem*., **187**, 191-199 **(1998)**

6. Edeoga H.O., Okwu D.E. and Mbaebie B.O., Phytochemical constituents of some Nigerian medicinal plants, *African Journal of Biotechnology*, **4(7)**, 685-688 **(2005)**

7. Ferlay J., Steliarova-Foucher E., Lortet-Tieulent J., Rosso S., Coebergh J.W.W., Comber H., Forman D. and Bray F., Cancer incidence and mortality patterns in Europe:estimates for countries in 2012, *Eur J Cancer*, **49(6)**, 1374-403 **(2012)**

8. Gerlier D. and Thomasset N., Use of MTT colorimetric assay to measure cell activation, *J. Immunol. Methods*, **94**, 57-63 **(1986)**

9. Guide – ATCC, VA, USA, www.atcc.org

10. Gosta Rutger Persson and Rigmor Elisabeth Persson, Cardiovascular disease and periodontitis: an update on the associations and risk, *Journal of Clinical Periodontology*, **35**, 362-379 **(2008)**

11. Halliwell B. and Arnoma O.L., The Deoxyribose method: A simple test tube assay for the determination of rate constant for reaction of hydroxyl radical, *Anal Biochem*., **165(1)**, 215-219 **(1987)**

12. Halliwell B. and Gutteridge J.M.C., The antioxidants of Human Extracellular fluids, *Archives of Biochemistry and Biophysics*, **280(1)**, 1-8 **(1998)**

13. Hoyert D.L., Kung H.C. and Smith B.L., Deaths preliminary data for 2003, *Natl. Vital. Stat. Rep*., **53**, 1-48 **(2005)**

14. John M. Pezzuto, Plant derived anticancer agents, *Biochemical Pharmacology*, **53(2)**, 121-133 **(1997)**

15. Kawashima T., Sumamal W., Pholsen P., Chaithiang R. and Terada F., Comparative study on energy and nitrogen metabolism of Brahman cattle and sheep given ruzi grass hay with different levels of soybean meal, *Japan Agric. Res. Quarterly*, **41(3)**, 253-260 **(2007)**

16. http://himedialabs.com/TD/CCK003.pdf

17. Morten Hoyer, Henrik Roed, Lisasengelov, Anders Traberg, Lars Ohlhuis, Jorgen Pedersen, Hanne Nellemann, Annekiil, Berthelsen, Frey Eberholst, Svend Aage Engelholm and Hans Vonder Maase, Phase- II study on Stereotactic Radiotheraphy of locally advanced Pancreatic Carcinoma, *Radiotheraphy and oncology: Journal of the European Society for Therapeutic Radiology and Oncology*, **76(1)**, 48-53 **(2005)**

18. Mosmann T.J., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *Immunol. Methods*, **65**, 55-63 **(1983)**

19. Natesan S., Badami S., Dongre S.H. and Godavrthi A., Antitumor activity and antioxidant status of the methanol extract of Careya arborea bark against Dalton's lymphoma ascites-induced ascetic and solid tumor in Mice, *J Pharmacol Sci*.**,103(12)**, 12-23 **(2007)**

20. Nabi S.M. et al, Free radical scavenging activity and antioxidant capacity of Eryngiumcaucasicum trautv and Froripiasubpinnata, *Pharmacolognosy Magazine*, **4(18)**, 123127 **(2008)**

21. Paul Zarogoulids, Haidong Huang, Zhenli Hu, Ning Wu, Jiannan Wang, Dimitris Petridis, Kosmas Tsakiridis, Dimitris Matthaios, Christoforos Kosmidis, Wolfgang Hohenforst Schmidt, Christos Tolis, Ioannis Boukovinas, Nikolaos Courcoutsakis, George Nikolaidis, Chrysanthi Sardeli, Chong Bai and Chrysanthi Karapantzou, Priority of PET-CTvs CT Thorax for EBUS-TBNA 22Gvs 19G: Mesothorax Lymphadenopathy, *Journal of Cancer*, **12(19)**, 5874-5878 **(2021)**

22. Paola Antonia Corsetto P.A., Gigliola Montorfano, Stefania Zava, Irma Colombo, Bergros Ingadottir, Rosa Jonsdottir, Kolbrun

Sveinsdottir and Angela Maria Rizzo, Characterization of Antioxidant potential of Seaweed Extracts for Enrichment of Convenience Food, *Antioxidants*, **9(3)**, 249 **(2020)**

23. Persson S., Torpdahl M. and Olsen K.E.P., New multiplex PCR method for the detection of Clostridium difficile toxin A (tcd A and toxin B (tcdB) and the binary toxin (cdtA/cdtB) genes applied to Danish Strain collection, *Clinical Microbiology and Infection*, **14(11)**, 1057-1064 **(2008)**

24. Persson C., Sasazuki S., Inoue M., Kurahashi N., Iwasaki M., Miura T., Ye W. and Tsugame S., Plasma levels of carotenoids,retinol and tocopherol and the risk of gastric cancer in Japan, A nested case- control study, *Carcinogenesis*, **29**, 1042-1048 **(2008)**

25. Rosa G.P., Tavares W.R., Sousa P.M.C., Pages A.K., Seca A. and Pinto D.C.G.A., Seaweed secondary metabolites with beneficial health effects: An overview of successes in *in vitro* studies and clinical trials, *Mar. Drugs*, **18**, 8 **(2020)**

26. Rose P., Ong C.N. and Whiteman M., Protective effects of Asian green vegetables against oxidant induced cytotoxicity, *World J. Gastroenterol.*, **11**, 7607-7614 **(2005)**

27. Sharma Abhishek and Garg Pankaj, Analytical Method Development and Validation: Quantitative Estimation for Assay of Revefenacin in Pharmaceutical Dosage form by RP-HPLC method, *Res. J. Chem. Environ.*, **28(3)**, 32-39 **(2024)**

28. Takashi Sugimura, Food and cancer, *Toxicology*, **27(181-182)**, 17-21 **(2002)**

29. Van Dinh Thuy, Chinh Pham The, Tham Pham Thi, Nga Mai Thanh and Ounkuea Thongsaving, Chemical components as a potential resource for synthesis of organic compounds and anti-inflammatory activity of essential bark oil of Illicium verum, *Res. J. Chem. Environ.*, **28(1)**, 68-72 **(2024)**

30. Yoshio Iwashima M.D., Takeshi Horio M.D., Yoichi Takami M.D. and Takashi Inenaga M.D., Effects of the creation of arteriovenous fistula for haemodialysis on cardiac function and natriuretic peptide levels in CRF, *American Journal of Kidney Diseases*, **40(5)**, 974-982 **(2002)**

31. Zeman M.N., Green C. and Akin E.A., Spectrum of [F] FDG-PET/CT Finding in Benign Lymph Node Pathology, *Molecular Imaging and Biology*, **23(4)**, 469-480 **(2021)**.

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