International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Anti-mitotic and Cytotoxic Potential of Methanolic Extract from *Lingula anatina*

Shraddha Hatangadi[1], Shashank More[2], Bhagyashree Ghag[3], Nirav Bhatia[4], Madhavi Indap[1]*

Central Research Laboratory, Life Sciences, D.G. Ruparel College, Mumbai, Maharashtra, India

Abstract: ***Natural products (NP) have been always preferred over the synthetic products due to various side effects it brings along. Natural products have been a source of new drugs since few decades now. The current focus of NP research has been shifted towards marine ecosystem. Secondary metabolites of marine organisms have been found to possess antibiotic, antiviral and anti-cancer activity. Lingula anatina commonly known as "lamp shell" has been found to tolerate high salinities, which might be an indication of presence of some potential bioactive compounds. Hence, our present study is based on Cytotoxic and genotoxic potential. Chromosomal aberration and mitotic index test were applied to evaluate genotoxic potential of the extract using Allium cepa. root model. Cell viability was assessed on tumor and non-tumor cell lines such as MCF-7, A549 and CHO by MTT assay. Our study revealed that mitotic index decreases with increase in concentration from 100µg/ml to 400µg/ml. Maximum breaks were observed at 400µg/ml conc. Spindle abnormalities along with C-Mitosis, Vagrant and Lagging Chromosome, Stickiness, breaks and disoriented, fragmented chromosomes were also observed. Extract exhibited cytotoxicity against Breast Carcinoma cell line (MCF-7) and the IC-50 value was 605.60µg/ml. The Methanolic extract of Lingula anatina possesses cytotoxic and genotoxic potential which could be considered as a future drug in medicine.***

Keywords: Natural Products, *Lingula*, *Allium cepa*., Cytotoxicity, MTT. Genotoxicity, Aberrations

1. Introduction

Nature has provided us with a variety of compounds which are addressed as natural products. Natural products are produced in nature in the form of secondary metabolites which have certain bioactive properties [1,2]. This compound is produced in the form of defense mechanism and has been found to possess anti-microbial, anti-cancer and anti-oxidant properties [3]. Natural products and its research have been now inclined towards marine ecosystem as they are one of the major untapped resources for screening of their bioactive compounds, as they have been found to live in a pool of diverse conditions which prove to be quite powerful and have strong bioactivity which has pertained to its increased applications in medical science [4].

The majority of marine natural product studies have been conducted on marine algae, sponges and other soft-bodied organisms for search of bioactive compounds, as most of these organisms reside in the intertidal zone of the ocean. The intertidal zone is always exposed to extreme conditions like salinity, temperature, moisture and dryness which gives us a scope to study their novel compounds which could be a potential discovery in pharmacology [5]. Chaudhari et al. has mentioned about three potential marine sponges *Xestospongia carbonaria, Sarcotragus foetidus* and *Spongia obscura* component which enhances IL-1β and IL-6 in lipopolysaccharide induced RAW 264.7 macrophages (murine) and carrageenan-induced edema in rats in their study [6].

Eghianruw et al. has also mentioned in his studies about two marine molluscs: *Tympanatonus fuscatus var radula* and *Pachymelania aurita,* which possess anti-mitotic, anti-proliferative and selective cytotoxic activity [7]. In the pool of intertidal animals, there are a group of animals belonging to the minor phylum of Brachiopoda which have not been explored much.

Brachiopoda is considered as a separate phylum due to its affinity with Molluscs, Annelida, Ectoprocta, Chaetognatha and Phoronida, which makes it a potential group for biological studies [8].

Lingula is a genus of brachiopods, within the class *lingulata* and is commonly, known as "lampshell". It is a rare and primitive animal, dating to Cambrian period which occurs as a patchy distribution in Indian coasts and estuaries. They stay in vertical burrows in the bottom sand or mud [9]. Till now researchers have been studying about *Lingula* with respect to its behavior, phylogeny and distribution [10]. Samantaet et al. has presented his work on morphological and microanatomical features of *Lingula anatina* [11]. Based on the previous findings, *Lingula* has been found to tolerate high salinities which gives an indication of the presence of potential bioactive compounds which might be an asset to medical science [12]. This interested us to work on this organism and to evaluate its bioactive potential. As researchers have mentioned in their previous studies about the cytotoxic and genotoxic property of a marine animal extract to be a potential anti- cancer agent, [13,14] we have also based our present study on investigating anti-mitotic and cytotoxic potential of *Lingula anatina.*

2. Materials and methods

2.1 Animal Collection and Extraction

The animal *Lingula anatina* was handpicked and collected from the coast of Ratnagiri, Maharashtra. The organism was deshelled and weighed. The deshelled mass was cold

Licensed Under Creative Commons Attribution CC BY

percolated in methanol for 2 days, filtered and concentrated under reduced pressure giving rise to gummy methanolic extract which was named as LME.

The cold percolation in methanol was repeated until colorless methanol was obtained.

2.2 Qualitative detection of biochemical compounds

The detection of the biochemical constituents was carried out for the extract using standard qualitative tests [15]. Majorly, the active secondary metabolites were tested for their presence in LME as follows:

1. **Detection of Alkaloids -** Dragendorf's test,
   Mayer's test
   Wagner's test
2. **Detection of Flavonoids -** Ethyl acetate test
3. **Detection of Phenolic compounds –** Ferric Chloride test
4. **Detection of Saponins-** Foam test
5. **Detection of Terpenoids** – Chloroform and acetic anhydride test

2.3 Genotoxicity: *Allium cepa*. Assay

Allium cepa bulbs have been suggested by various researchers as a suitable model to study and detect general toxicity, genotoxicity, chromosomal aberrations and environmental effect. Tedesco. et al. has mentioned Allium cepa as cost effective and the bioindicator of genotoxicity at pre-liminary levels [16]. Considering the reliability of Allium cepa test, it has been used as a model in our study to evaluate the genotoxic potential of LME.

Equal sized bulbs of commercial variety of *Allium cepa* were used in this assay. The onions were purchased from a local market and stored in dry and well aerated conditions till the time they were utilized for the experiment. The assay was performed as per O. Timothy et.al with slight modifications [17].

2.3.1 Growth Inhibition Assay and Morphological observations

The onion bulbs were directly grown in the test concentration of LME and control. The base of each bulb was dipped into test solutions of 100µg/ml, 200µg/ml and 400 µg/ml in a 15 ml plastic cup, which was then kept in dark for 96 hours. The test samples were replaced after every 24 hours. The roots of these bulbs were observed for morphological changes such as; root hooks, twists, colour and swelling. Along with these observations, root length was also measured for each concentration, as an indicator of general toxicity. General toxicity was calculated by:

$$\%\,\text{Root Growth of Control} = \frac{\text{Overall mean root length of the Test Soln}}{\text{Overall mean root length of Control}} \times 100$$

2.3.2 Microscopic Analysis

Allium cepa bulbs were suspended in the test samples of 100µg/ml,200 µg/ml and 400 µg/ml along with control for 48 hours as well as 96 hours. Post exposure, the roots were cut and fixed in Carnoy's solution (ethanol: glacial acetic acid) to maintain its integrity. The roots were picked at random and placed on a clean slide containing distilled water to remove traces of the fixative and then hydrolyzed it with 1N HCl at 60°C for few minutes to soften the tissue. The hydrolyzed roots were rinsed in distilled water to remove the residues of acid. Root tips were excised and stained with acetocarmine, mounted with 45%acetic acid. A clean coverslip was placed over it. Roots were macerated by gently tapping the coverslip for the cells to spread out distinctly. The slides were then observed under Optical Microscope (KYOWA M No. 1015) at 40X magnification. Around 1000 cells were scored and observed for different mitotic phases and chromosomal aberrations including control and treated ones [18]. The experiments were conducted in triplicates to ensure the reproducibility of the results. The genotoxic potential of LME was determined by calculating mitotic index and % aberrant cells using the following formulae:

$$MI = \frac{\text{No. of dividing cells}}{\text{Total no. of cells}} \times 100$$

$$\%\,\text{Aberrant Cells} = \frac{\text{No. of Aberrant cells}}{\text{Total no. of cells}} \times 100$$

2.4 Cytotoxicity

2.4.1 Cell culture

Human Breast Adenocarcinoma (MCF-7), Adenocarcinoma Human Alveolar Basal epithelial cells (A-549), Murine Macrophage (RAW 264.7) and Chinese Hamster Ovary cells (CHO) were procured from National Centre for Cell Science (NCCS), Pune. The cell line was maintained in HAM's F12 medium, supplemented with 10% Fetal Bovine Serum, 1% penicillin-streptomycin and 2% L-Glutamine and was grown at 37ºC in a humified incubator with 5% CO_2.

2.4.2 MTT Assay

The cytotoxicity of the different concentrations of LME was tested by performing MTT assay with minor changes and modifications [20]. Cells were seeded in a 96 well plate with a density of $3x10^5$ and incubated for 24 hours. Post incubation, the used media was decanted and replaced with fresh batch along with LME concentrations which ranged from 100 to 800µg/ml (100µg/ml, 200µg/ml, 400µg/ml and 800µg/ml), followed by 24 hours incubation.

After 24 hours, post treatment, the medium was carefully removed and 20µl of MTT ((3-(4, 5-dimethyl thiazol-2yl)-2, 5diphenyl tetrazolium bromide) and 80µl of complete media was added to each well and the plate was then incubated at 37ºC in 5% CO_2 incubator for 4 hours. The well plate contents were then replaced with 100µl DMSO and further read at 570 nm of ELISA plate reader (BIORAD Microplate reader) [20]. This Assay was performed in triplicates by using a blank and a normal cell line (CHO- Chinese

Licensed Under Creative Commons Attribution CC BY

Hamster Ovary). CHO cells were used to check whether the extract had any cytotoxic effect on control cell line. The results were determined by using the formula of

$$\% \text{ Viability} = \frac{\text{Mean OD Treated}}{\text{Mean OD Control}} \text{ X } 100$$

3. Result

3.1 Secondary metabolite chemical screening

The results in Table 1 below indicate the presence of alkaloids and terpenoids in abundant amount, presence of Saponins in moderate concentration and absence of flavonoids and phenolic compounds.

Table 1: Results of Qualitative analysis of LME

Test for Alkaloids	+ +
Test for Flavonoids	-
Test for Phenolic compounds	-
Test for Saponins	+
Test for Terpenoids	+ +

+ indicates presence, - indicates absence of compounds

3.2 Genotoxic effect of LME

The macroscopic observations of the *Allium cepa* roots after been treated to different concentrations, showed a decrease in root length with a gradual increase in concentrations of LME as shown in Figure 1. Morphology of the roots was also noted for both control and treated roots. The control roots showed healthy growth, while the roots treated with highest concentration (400µg/ml) of LME showed stunted root growth along with mushy appearance, hooks, twists and slight decolorization as shown in Figure 6.

The microscopic analysis of *Allium cepa* root cells shows different stages of mitosis along with chromosomal aberrations at 48 and 96hrs of exposure to LME. At 48hrs we perceived that Anaphase is much more than other dividing stages at highest concentration of LME and moreover, at 96hrs we could spot metaphase much more than that of other dividing stages at 400µg/ml of LME as depicted in Figure 2a and 2b. The normal stages of mitosis have been represented in Figure 7.

The effect of control and different concentrations of LME have appeared to show us that, with a gradual increase in concentration there has been a decline in the mitotic phases at 48 as well as 96hrs of exposure as per represented graphically in figure 3a and 3b. Concentration – dependent decrease in mitotic index was observed at 48hrs as well as 96hrs of treatment with LME which has been depicted in figure 4.

Various visible aberrations were also analyzed, noticed, and observed which symbolizes LME as a remarkable genotoxic agent as tabulated in table 2. The effect of different concentrations LME on the no. of aberrations in *Allium cepa* root cells have been found to be highest at 400µg/ml when treated for 48hrs, and to be increasing in a dose-dependent manner when treated for 96hrs as represented graphically in figure 5. Several Chromosomal anomalies like Clumped chromosomes and disoriented chromosomes were observed in metaphase, along with laggard chromosomes and chromosomal bridges in anaphase at 48hrs of treatment with LME as portrayed in figure 8, thereby suggesting higher score of Anaphase in figure 2a. In addition to this, 96hrs of treatment with LME showed us numerous abnormalities namely, sticky chromosomes in metaphase, laggard and vagrant chromosomes in anaphase and the most striking feature observed was c-mitosis and vacuolation of cells in interphase at 400 µg/ml of LME as indicated in fig. 9 which could be exhibiting the anti-mitotic potential of LME.

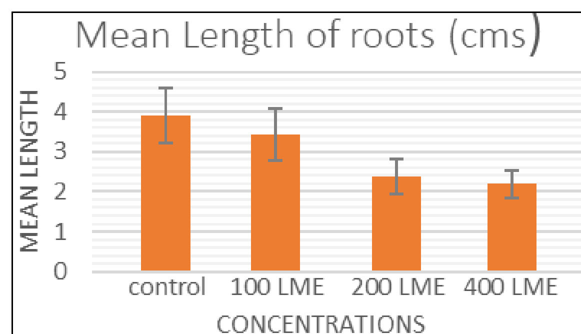

Figure 1: Effect of control and different concentrations of LME on the root length of *Allium cepa*. Data is expressed as ±SD.

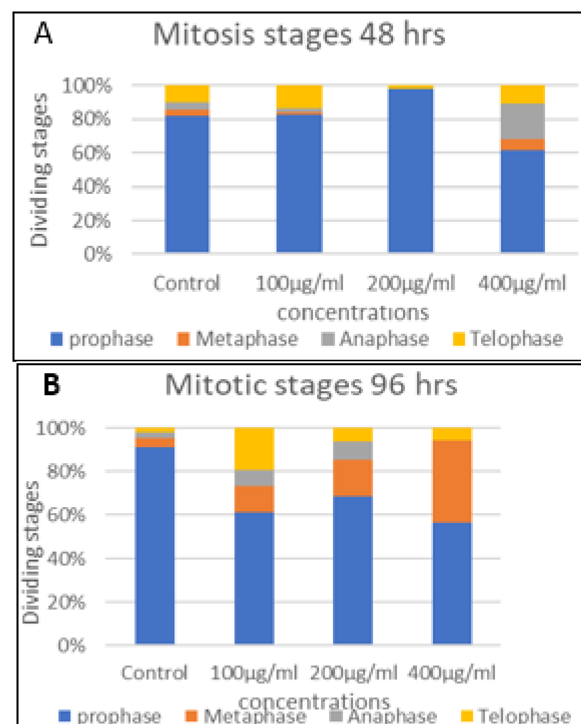

Figure 2: Effect of Control and LME on Mitotic stages (a) 48hrs (b) 96hrs

Licensed Under Creative Commons Attribution CC BY

International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

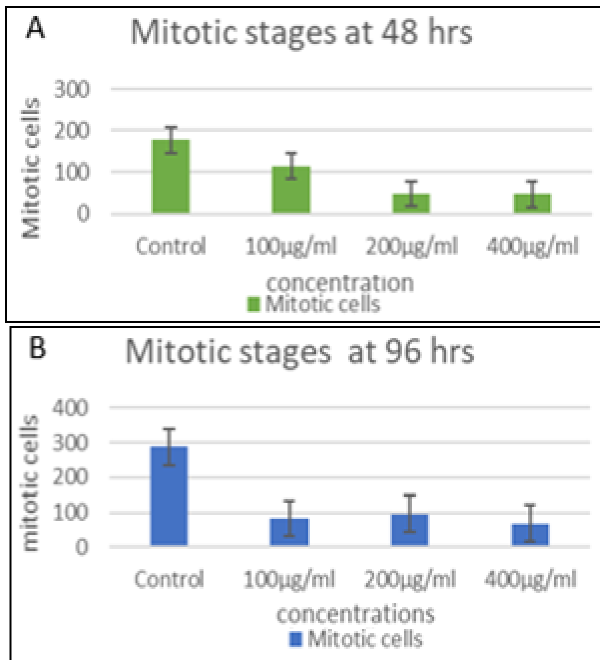

Figure 3: Effect of LME on Mitotic cells of *Allium cepa* roots (a) 48hrs (b) 96hrs. Data is expressed as ±SD.

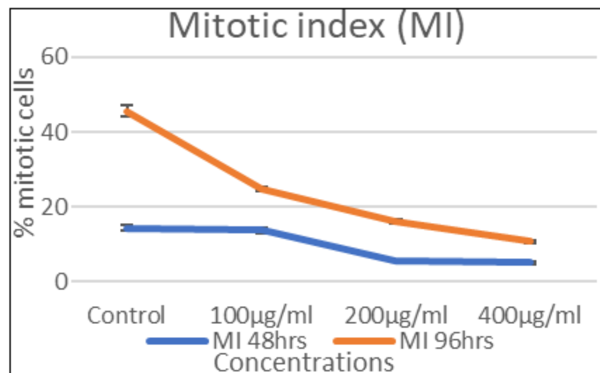

Figure 4: Effect of LME on Mitotic Index of *Allium cepa* root cells after 48 and 96hrs of treatment

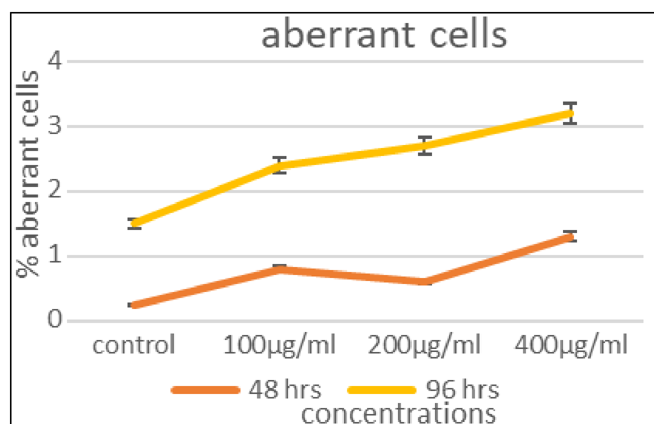

Figure 5: Effect of LME on %aberrant cells induced in *Allium cepa* root cells after 48 and 96hrs of treatment

Table 2: Types of Chromosomal aberrations induced by LME in *Allium cepa* root cells

48hrs	control	100µg/ml	200µg/ml	400µg/ml
No. of aberrations	2	4	10	12
Spindle abnormality	1	3	1	1
Vagrant chromosomes		1	1	1
Laggard chromosomes		1	1	5
c-mitosis			3	3
fragments	1		4	2
96hrs	control	100µg/ml	200µg/ml	400µg/ml
No. of aberrations	13	18	25	30
Spindle abnormality	9	4	3	1
Vagrant Chromosomes		1	3	
Laggard chromosomes	3	2	1	
c-mitosis	1	2	13	28
bridges		1		
stickiness		5	3	
clumped		1		
fragments		1		1
Non-oriented chromosomes		1	2	

Figure 6: Effect of LME on morphology of *Allium cepa* roots (a) Control (b) treated

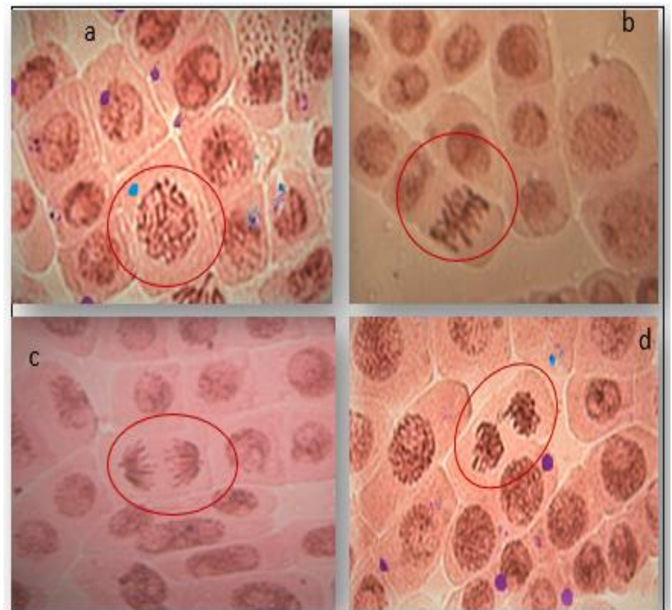

Figure 7: Normal Stages of Mitosis in *Allium cepa* root cells (a) Prophase (b) Metaphase (c) Anaphase (d) Telophase

Licensed Under Creative Commons Attribution CC BY

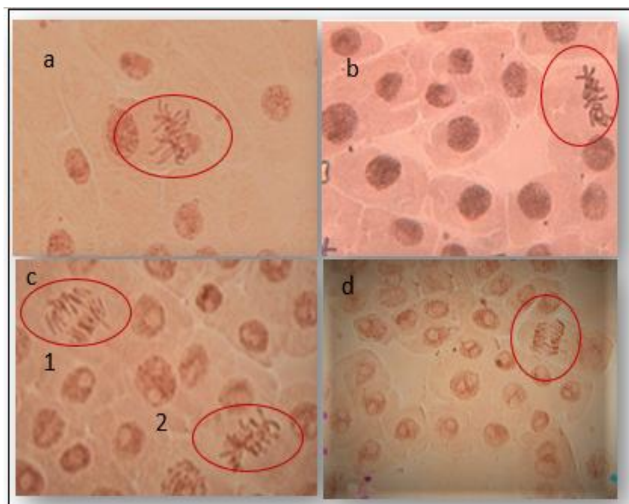

Figure 8: Types of aberrations induced by different concentrations of LME (100µg/ml, 200 µg/ml and 400 µg/ml) at 48hrs of exposure (a) clumped chromosomes (b) disoriented chromosomes (c) laggard chromosomes (1) dis-oriented chromosomes (2) (d) Anaphase bridges

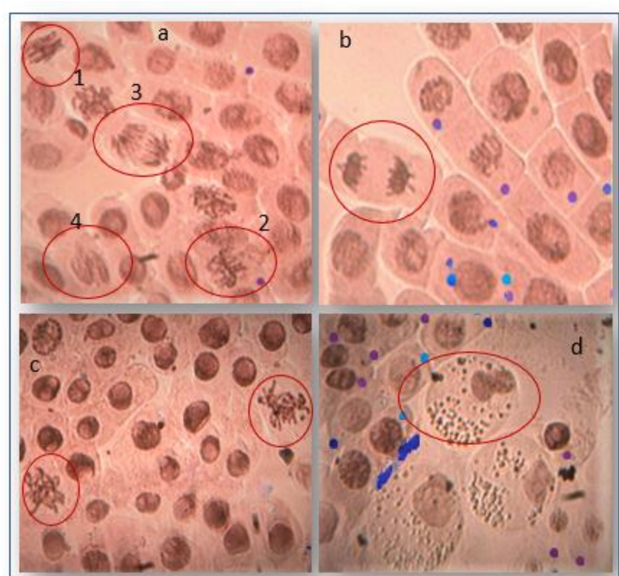

Figure 9: Types of aberrations induced by different concentrations of LME (100µg/ml, 200 µg/ml and 400 µg/ml) at 96hrs of exposure. (a) Sticky chromosomes (1) non-oriented chromosomes (2) Laggard chromosomes (3) Chromosomal fragments (4) (b) Vagrant chromosomes (c) C-mitosis/metaphase arrest (1 and 2) (d) Nuclear vacuolation

3.3 Cytotoxic effect of LME

The cytotoxic potential of LME and its activity were tested on A549, RAW 264.7, CHO and MCF-7 (Human Breast Adenocarcinoma), but the cytotoxic effect was notably observed in MCF-7 cell lines which have been depicted in Figure 10. LME represented concentration dependent activity on MCF-7 cell line with IC_{50} value being 200µg/ml. The number of viable cells decreased with subsequent increase in the concentration of LME when compared with control. CHO (Chinese Hamster Ovary) cells, however did not show much of a difference in viability of cells between control and concentrations, proving the extract does not affect normal cell lines(data not shown).

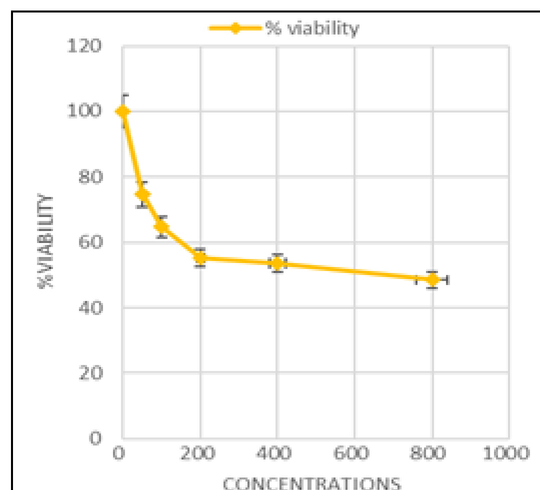

Figure 10: Effect of different concentrations of LME (100 µg/ml, 200 µg/ml and 400 µg/ml) against MCF-7 cell lines.

4. Discussion

Natural Products and their usage in manufacture of drugs have been preferred over synthetic ones. It has been a boon to treat cancer and other life – threatening diseases as they have the least of side effects. Natural products contain formulations of organic secondary metabolites which have been proven to possess bioactive potential against various types of cancer and other deadly diseases [20]. With reference to natural products and their bioactive potential, Ortiz et.al has mentioned about the cytotoxic and genotoxic potential of Sandalwood essential oil against MCF- 7 cell lines [21]. Even Sarvameili et. al talks about cytotoxic potential of *Pinus elderica* essential oil and extract on HeLa and MCF-7 cell lines [22].

Even though, these terrestrial sources have been proved to be a gold chest for various forms of drugs, for the past few decades marine biodiversity has been a field of keen interest for researchers to explore. Many Active compounds were discovered in the marine realm which could hail as a potential anti-cancer drug and play a major role in cancer research [23]. Beedesse and Ramanjooloo et. al have spoken about cytotoxic activities of marine sponges obtained from Mauritius waters on various human cancer cell lines [24]. Similarly, Mary and Vinotha et. al have effectively indicated in their study about cytotoxic activity of Seaweed, *Sargassum Sps* against Hep-2 and MCF-7 cancer cell lines [25]. Although several marine organisms have been studied and scrutinized previously for their bioactive potential [26], brachiopods have remained untouched with respect to studying and evaluating their bioactive potential. Thereby, our current study concentrates on a brachiopod; *Lingula anatina*. Our extract; LME (*Lingula* methanolic extract) has shown noted cytotoxic effect on Human Breast Adenocarcinoma cell lines (MCF-7) by decrease in cell viability with a gradual increase in concentration and the IC_{50} value being 605.60µg/ml. LME concentrations had negligible effect on CHO (Chinese Hamster Ovary) cell

Licensed Under Creative Commons Attribution CC BY

lines, proving our extract to be harmless to normal cells. This presents LME to be a potential cytotoxic agent. Many researchers have also got remarkable results against MCF-7 cell lines by utilizing marine natural products. Renata Pinheiro Chaves et.al has mentioned about anti-cancer property of two iso-lectins obtained from marine red alga *Solieria filiformis* against MCF-7 cells [27]. In another study by Luiz Gonzaga do Nascimento-Neto, Halilectin-3, a lectin isolated from marine sponge *Haliclona* caerulea has been found to induce autophagy and apoptosis in MCF-7 cell lines [28].

Genotoxic agents have been found to arrest cell division which could be used as a possible cancer drug in future.[29]. Allium cepa model was used to study the genotoxic property of LME. Many research scholars have also mentioned in their work about the use of Allium cepa to test the genotoxicity of various natural products. In a recent work by Pesnya et. al it has been seen that *Heracleum sosnowstyi* juice has genotoxic effects at conc. 10-300 ml^{-1} on *Allium cepa* root cells [30]. In a similar study Chukwejukwo et. al also provided an insight to the genotoxic effects of aqueous extract of *Distephanus angulifolius* using the *Allium cepa* model and found that it had mito-depressive effect on cell division and that it also induced various chromosomal aberrations [31]. Our study based on the genotoxic potential of LME also provides similar results, whereby mitotic index (MI) has been seen to drop with an increase in the dose and exposure time of LME. The highest MI has been found to be 13.7% at 100µg/ml and the lowest being 5% at 400µg/ml, when the roots were exposed for 48 hours to concentrations of LME (100µg/ml, 200µg/ml and 400µg/ml) as compared to the MI of control being 14.3 %. Another set of the roots which were exposed to same concentrations of LME for 96 hours the MI was highest at 100µg/ml being 11.1% and lowest at 400µg/ml being 5.7% when compared with the MI of control being 31.3%. Diverse chromosomal abnormalities were also profoundly observed. Chromosomal anomalies like laggard chromosomes and vagrant chromosomes in Anaphase and clumped chromosomes, disoriented chromosomes and sticky chromosomes in Metaphase and c-mitosis have been occurred quite frequently. The disoriented chromosomes formed during metaphase was due to defective linearity of the DNA molecule. The formation of sticky chromosomes is due to linking of sub-chromatids strands along with extravagant genesis of nucleoproteins and improper protein-protein interaction [32], it is basically due to increased condensation Vagrant chromosome is a feature, where the chromosomes move ahead of its group towards the poles and divides subsequently causing separation of unequal no. of chromosomes. The formation of laggard chromosomes which are basically the lagging chromosomes were formed due to failure of chromosomes to get attached to the spindle fibers and move to either poles. Chromosomal bridges formed is usually due to chromosomal fusion [33]. Significantly at 400µg/ml concentration, when the roots were treated for 96hrs, it exhibited two peculiar features of Nuclear vacuolation which confirms the toxic effect of LME and mitotic arrest/c-mitosis. Similar results of mitotic arrest/c-mitosis have been demonstrated by Kwan Yuet Ping et. al 's research work [34]. Mitotic Arrest/ C-Mitosis is a characteristic very close to Colchicine's anti-mitotic effect on *Allium cepa* root cells as colchicine has been found to block the formation of spindle microtubules by cleaving the disulfide bonds [35][36][37]. Colchicine is an alkaloid which have been found to have wonderful anti-mitotic effect. Anna Krahulcava has mentioned in her work about the mito-depressive effects of Alkaloids on root tip cells of *Allium cepa* [38]. Presence of alkaloids in our extract have been confirmed by biochemical screening, which could be indicated as a cause of mitotic arrest of *Allium cepa* root cells and thereby making LME a possible agent against cancer.

Overall, it can be concluded that LME pertains to have cytotoxic and anti-mitotic potential which could be further used as a possible cancer drug.

5. Conclusion

The results of our study have reported LME to possess cytotoxic activity on MCF-7 cells with IC_{50} value being 605.60µg/ml, which is non-toxic to normal cell lines. LME also showed concentration-dependent decrease in root length and mitotic index. The positive relation of the morphological and microscopical analysis proves LME to be a potential genotoxic agent. Dose- dependent rise in chromosomal aberrations along with Mitotic arrest at 400µg/ml in the *Allium cepa* root tip cells which were treated for 96hrs were also provides a broad insight into LME as a prospective anti-mitotic agent. The Qualitative detection of biochemical constituents in LME confirmed the presence of alkaloids, saponins and terpenoids. These active constituents can be further isolated and purified to have a deeper insight at the molecular level.

6. Declaration

Authors state that there are no disagreements of interests.

7. Acknowledgements

The authors are grateful to Dr. Tushar Desai, Principal, D.G. Ruparel College for providing us with the laboratory facilities to carry out our research work.

References

[1] Vaishnav, P., & Demain, A. L. (2011). Unexpected applications of secondary metabolites. *Biotechnology Advances*, *29*(2), 223–229. https://doi.org/10.1016/j.biotechadv.2010.11.006
[2] Demain, A. L., & Fang, A. (2000). The natural functions of secondary metabolites. *Advances in Biochemical Engineering/Biotechnology*, *69*, 1–39.
[3] Cavanagh, J., Rance, M., Maplestone, R. A., Stone, M. J., Williams, D. H., Xia, J., Bjorndahl, T. C., Tang, P.,

Licensed Under Creative Commons Attribution CC BY

Wishart, D. S., Dal Poggetto, G., Castañar, L., Adams, R. W., Morris, G. A., Nilsson, M., Johnson, C. S., Du Penhoat Hervé, C., Imberty, A., Roques, N., Michon, V., … Pérez, S. (1999). The evolutionary role of secondary metabolites-a review* (Antibiotic resistance; pleiotropic switching; gene clusters; spores; natural products; biosynthesis). *Journal of Magnetic Resonance (1969)*, *115*(54), 151–157. https://doi.org/10.1039/c7cc03150e

[4] Sivanandham, Vignesh & A, Raja & James, R. Arthur. (2011). Marine Drugs: Implication and Future Studies. International Journal of Pharmacology. 7. 10.3923/ijp.2011.22.30.

[5] Babar, A. G., Pande, A., & Kulkarni, B. G. (2012). Bioactive potential of some intertidal molluscs collected from Mumbai coast, West coast of India. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2 SUPPL.), S1060–S1063. https://doi.org/10.1016/S2221-1691(12)60361-X

[6] Chaudhari, S., & Kumar, M. S. (2020). Marine sponges Sarcotragus foetidus, Xestospongia carbonaria and Spongia obscura constituents ameliorate IL-1 β and IL-6 in lipopolysaccharide-induced RAW 264.7 macrophages and carrageenan-induced oedema in rats. *Inflammopharmacology*, *0123456789*. https://doi.org/10.1007/s10787-020-00699-2

[7] Eghianruwa, Q., Osoniyi, O., Wachira, S., Maina, N., R., & Imbuga, M. (2019). In vitro antiproliferative studies of extracts of the marine molluscs: Tympanatonus fuscatus Var radula (linnaeus) and Pachymelania aurita (muller). *International Journal of Biochemistry and Molecular Biology*, *10*(1), 1–8. http://www.ncbi.nlm.nih.gov/pubmed/31149366

[8] De Rosa, R. (2001). Molecular data indicate the protostome affinity of brachiopods. *Systematic Biology*, *50*(6), 848–859. https://doi.org/10.1080/106351501753462830

[9] Jordan, E. L., & Verma, P. S. (2009). *Invertebrate zoology*. S. Chand & Company.

[10] Savazzi, E. (1986). Burrowing sculptures and life habits in Paleozoic lingulacean brachiopods. *Paleobiology*, *12*(1), 46–63. https://doi.org/10.1017/S0094837300002979

[11] S., S., A., C., & S.K, C. (2014). Morpho-anatomical study of Lingula anatina Lamarck, 1801 from West Bengal-Odisha coast, India. *Journal of the Marine Biological Association of India*, *56*(2), 26–33. https://doi.org/10.6024/jmbai.2014.56.2.01775-04

[12] Society, S., & Geology, S. (2020). *Experimental Studies of Salinity Tolerance,Burrowing Behavior and Pedicle Regeneration in Lingula anatina (Brachiopoda , Inarticulata) Author (s): L . S. Hammond Published by : SEPM Society for Sedimentary Geology Stable URL : https://www.jstor.org/stable/1304816 REFERENCES Linked references are available on JSTOR for this article : You may need to log in to JSTOR to access the linked references* . *57*(6), 1311–1316.

[13] Suarez-Jimenez, G. M., Burgos-Hernandez, A., & Ezquerra-Brauer, J. M. (2012). Bioactive peptides and depsipeptides with anticancer potential: Sources from marine animals. *Marine Drugs*, *10*(5), 963–986. https://doi.org/10.3390/md10050963

[14] Bhavnani, N., More, S., Ghag, B., & Indap, M. (2017). Evaluation of Anti-mitotic and Cytotoxic Potential of Methanolic Extract of Balanus amphitrite. *International Journal of Science and Research*, *6*(1015), 2567–2571.

[15] Nwodo, O. F. C., & Nkwocha, P. E. (2015). *Qualitative and Quantitative Phytochemical Screening of the Aqueous Leaf Extract of Senna mimosoides : Its Effect in in vivo Leukocyte mobilization induced by inflammatory stimulus*. *4*(5), 1176–1188.

[16] Bosio, S., & Laughinghouse IV, H. D. (2012). Bioindicator of Genotoxicity: The Allium cepa Test. *EnvironmentalContamination*. https://doi.org/10.5772/31371

[17] Timothy, O., Idu, M., Olorunfemi, D. I., & Ovuakporie-Uvo, O. (2014). Cytotoxic and genotoxic properties of leaf extract of icacina trichantha oliv. *South African Journal of Botany*, *91*, 71–74. https://doi.org/10.1016/j.sajb.2013.11.008

[18] Pakrashi, S., Jain, N., Dalai, S., Jayakumar, J., Chandrasekaran, P. T., Raichur, A. M., Chandrasekaran, N., & Mukherjee, A. (2014). In vivo genotoxicity assessment of titanium dioxide nanoparticles by Allium cepa root tip assay at high exposure concentrations. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0087789

[19] Ali, M. A., & Farah, M. A. (2014). *In vitro cytotoxicity screening of wild plant extracts from Saudi Arabia on human breast adenocarcinoma cells*. *13*(2), 3981–3990.

[20] Martin-Cordero, C., Jose Leon-Gonzalez, A., Manuel Calderon-Montano, J., Burgos-Moron, E., & Lopez-Lazaro, M. (2012). Pro-Oxidant Natural Products as Anticancer Agents. *Current Drug Targets*, *13*(8), 1006–1028. https://doi.org/10.2174/138945012802009044

[21] Ortiz, C., Morales, L., Sastre, M., Haskins, W. E., & Matta, J. (2016). Cytotoxicity and Genotoxicity Assessment of Sandalwood Essential Oil in Human Breast Cell Lines MCF-7 and MCF-10A. *Evidence-Based Complementary and Alternative Medicine*, *2016*. https://doi.org/10.1155/2016/3696232

[22] Sarvmeili, N., Jafarian-Dehkordi, A., & Zolfaghari, B. (2016). Cytotoxic effects of Pinus eldarica essential oil and extracts on HeLa and MCF-7 cell lines. *Research in Pharmaceutical Sciences*, *11*(6), 476–483. https://doi.org/10.4103/1735-5362.194887

[23] Cherigo, L., Lopez, D., & Martinez-Luis, S. (2015). Marine natural products as breast cancer resistance protein inhibitors. *Marine Drugs*, *13*(4), 2010–2029. https://doi.org/10.3390/md13042010

[24] Beedessee, G., Ramanjooloo, A., Aubert, G., Eloy, L., Surnam-Boodhun, R., Van Soest, R. W. M., Cresteil, T., & Marie, D. E. P. (2012). Cytotoxic activities of hexane, ethyl acetate and butanol extracts of marine sponges from Mauritian Waters on human cancer cell lines. *Environmental Toxicology and Pharmacology*,

Licensed Under Creative Commons Attribution CC BY

34(2), 397–408. https://doi.org/10.1016/j.etap.2012.05.013

[25] Mary, J. S., Vinotha, P., & Pradeep, A. M. (2012). *Screening for in vitro Cytotoxic Activity of Seaweed, Sargassum sp. Against Hep-2 and MCF-7 Cancer Cell Lines*. *13*, 6073–6076.

[26] Chaugule, S., & More, A. (2010, August). WDM and optical amplifier. In *2nd International Conference on Mechanical and Electronics Engineering (ICMEE 2010), Kyoto, Japan* (pp. 1-3).

[27] Chaves, R. P., Silva, S. R. da, Nascimento Neto, L. G., Carneiro, R. F., Silva, A. L. C. da, Sampaio, A. H., Sousa, B. L. de, Cabral, M. G., Videira, P. A., Teixeira, E. H., & Nagano, C. S. (2018). Structural characterization of two isolectins from the marine red alga Solieria filiformis (Kützing) P.W. Gabrielson and their anticancer effect on MCF-7 breast cancer cells. *International Journal of Biological Macromolecules*, *107*(PartA), 1320–1329. https://doi.org/10.1016/j.ijbiomac.2017.09.116

[28] do Nascimento-Neto, L. G., Cabral, M. G., Carneiro, R. F., Silva, Z., Arruda, F. V. S., Nagano, C. S., Fernandes, A. R., Sampaio, A. H., Teixeira, E. H., & Videira, P. A. (2018). Halilectin-3, a Lectin from the Marine Sponge Haliclona caerulea, Induces Apoptosis and Autophagy in Human Breast Cancer MCF7 Cells Through Caspase-9 Pathway and LC3-II Protein Expression. *Anti-Cancer Agents in Medicinal Chemistry*, *18*(4), 521–528. https://doi.org/10.2174/1871520617666171114094847

[29] Temel, A., & Gözükirmizi, N. (2015). Genotoxicity of metaphase-arresting methods in barley. *Turkish Journal of Biology*, *39*(1), 139–146. https://doi.org/10.3906/biy-1405-58

[30] Pesnya, D. S., Romanovsky, A. V., Serov, D. A., & Poddubnaya, N. Y. (2017). Genotoxic effects of Heracleum sosnowskyi in the Allium cepa test. *Caryologia*, *70*(1), 55–61. https://doi.org/10.1080/00087114.2016.1272313

[31] Chukwujekwu, J. C., & Van Staden, J. (2014). Cytotoxic and genotoxic effects of water extract of Distephanus angulifolius on Allium cepa Linn. *South African Journal of Botany*, *92*(1), 147–150. https://doi.org/10.1016/j.sajb.2014.03.001

[32] Nefic, H., Musanovic, J., Metovic, A., & Kurteshi, K. (2013). Chromosomal and nuclear alterations in root tip cells of allium cepa L. Induced by alprazolam. *Medical Archives (Sarajevo, Bosnia and Herzegovina)*, *67*(6), 388–392. https://doi.org/10.5455/medarh.2013.67.388-392

[33] Khanna, N., & Sharma, S. (2013). Allium Cepa Root Chromosomal Aberration Assay: A Review. *Indian Journal of Pharmaceutical and Biological Research*, *1*(03), 105–119. https://doi.org/10.30750/ijpbr.1.3.15

[34] Ping, K. Y., Darah, I., Yusuf, U. K., Yeng, C., & Sasidharan, S. (2012). *Genotoxicity of Euphorbia hirta: An Allium cepa Assay*. 7782–7791. https://doi.org/10.3390/molecules17077782

[35] Bharathi, P., Philomina, D., & Chakkaravarthi, S. (2006). Antimitotic effect of colchicine from six different species of Gloriosa in onion roots (Allium cepa). In *Journal of Medical Sciences* (Vol. 6, Issue 3, pp. 420–425). https://doi.org/10.3923/jms.2006.420.425

[36] Soodan, R. K., Katnoria, J. K., & Nagpal, A. (2014). Allium cepa Root Chromosomal Aberration Assay : An Efficient Test System for Evaluating Genotoxicity of Agricultural Soil. *International Journal of Science and Research*, *3*(8), 245–250.

[37] Levan, A. (1938). The Effect of Immunosuppressive. *Hereditas*, 1058–1069.

[38] Krahulcová, A. (1979). Mitodepressive effects of Chelidonium alkaloids on root tip cells of Allium cepa L. *Biologia Plantarum*, *21*(1), 15–21. https://doi.org/10.1007/BF02888713

Author Profile

Shraddha Hatangadi (Research Intern): Completed MSc in Zoology (Oceanography) from Mumbai University in 2018. Currently working as a Research Intern in Life Science Department, at Central Research Laboratory, D.G. Ruparel College, University of Mumbai.

Shashank More (PhD student): Completed MSc in Zoology (Oceanography) from Mumbai University in 2012. Currently working as a Research Scholar for PhD work in Life Science Department, at Central Research Laboratory, D.G. Ruparel College, University of Mumbai.

Bhagyashree Ghag (JRF): Completed MSc in Zoology (Animal Physiology) from Mumbai University in 2018. Currently working as a Junior Research Fellow under a DBT project in Life Science Department, at Central Research Laboratory, D.G. Ruparel College, University of Mumbai.

Nirav Bhatia (Research Trainee): Completed B. Pharm from College of Pharmacy Pratapbhai Patel School of Pharmacy and Technology Management, SKVM's NMIMS (Deemed University). Currently Working as a Research Trainee in Life Science Department, at Central Research Laboratory, D.G. Ruparel, University of Mumbai.

Dr. Madhavi Indap (Principal investigator) is currently working as Professor Emeritus at Life Science Lab, Central Research Laboratory, D.G. Ruparel College.

Licensed Under Creative Commons Attribution CC BY