Original Research Article

PREPARATION OF METHANOLIC CRUDE EXTRACTS OF *PADINA GYMNOSPORA* SEAWEED AND THEIR ANTICANCER ACTIVITIES AGAINST LUNG CANCER CELL LINE

Running title: Anticancer potential from *Padina gymnospora* Seaweed extract

ABSTRACT

Background: Cancer is a major health problem worldwide and still lacks fully effective treatments. As a result, natural-based alternative medicines have been developed. Marine algae are a vital part of the marine environment, with a high biodiversity and a diverse range of useful chemicals.

Aim: Aim of the study was to assess the anticancer activity of *Padina gymnospora* crude extract against lung cancer cell line

Materials and methods: The Seaweed *Padina gymnospora (P. gymnospora)* was evaluated for its anti- cancer activity via MTT assay, further, morphological study of the cells was done to check its efficacy. Finally, the results were analysed by Student's-t-test using MS-Excel, represented as mean ± SD for triplicates. The results were computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago) using one-way ANOVA. The level of statistical significance was set at $p<0.05$.

Results and Discussion: The results of this study indicate that *P. gymnospora* has concentration dependent significant anticancer activity. At highest concentration, 500µl, methanolic crude extracts of *P. gymnospora* showed the maximum anticancer activity, where the cell viability was only 16.41$\pm$7.15. Morphological study also revealed that maximum cell death had occured in the maximum concentration of the methanolic crude extract of *P. gymnospora*.

Conclusion: Despite the widespread use of alga-derived compounds and extracts in the food industry, there are still no anticancer drugs approved by the FDA based on these compounds. Thus, it is imperative that new drug discovery programs using seaweeds with a much more mechanistic approach are needed.

Key words: anti-cancer, crude-extract; lung cancer; seaweed

INTRODUCTION

Cancer is a group of disorders in which cells continue to develop uncontrollably, spread into adjacent tissues, and form tumours. Drug use, infectious organisms, a poor diet, environmental pollutants, inherited genetic mutations, hormones, and immunological disorders are all variables that might cause cancer. These factors can operate together or in sequence to produce cancer. According to the American Cancer Society, 1 685 210 new cancer cases were expected to be diagnosed worldwide in 2016, with 595690 patients in the USA expected to die of cancer, which translates to approximately 1630 people per day.

Cancer is frequently treated using a variety of therapies, depending on the characteristics and stage of the tumour, such as surgery, chemotherapy, radiation therapy, and immunotherapy. The goal of treatment in all circumstances is to remove the cells that make up the tumour in order to reduce it without harming healthy cells. Chemotherapy is a popular treatment option. Chemotherapy medications can cause anaemia, appetite loss, psychosis, baldness, peripheral neuropathy, and irreversible damage to essential organs, among other side effects. Drug tolerance is a problem in cancer treatment, in addition to these side effects of chemotherapy.

Despite decades of research, an effective treatment for cancer is still lacking; therefore, there is a need for new compounds with anticancer activity that are cell selective with fewer adverse effects, improving the quality of life of patients. Natural products provide a reliable alternative in the search for compounds that can help in the treatment of diseases. Over the past few decades, research attention has turned to natural products from marine organisms, mainly because of their large habitat (covering ~70% of the surface of the Earth), high biodiversity (95% of world biodiversity), and the specific conditions under which some species live (e.g., at extremes **of** salinity, pressure, and temperature). The anticancer potential of extracts and chemicals obtained from marine algae is particularly promising among the marine substances examined thus far.

Benthic marine algae or seaweeds, especially Padina gymnospora species, are plants that live either in marine or brackish water. They are a diverse group of autotrophic organisms that contain chlorophyll for oxygenic photosynthesis and have evolved adaptive osmoregulation mechanisms to regulate their internal osmotic pressure and prevent turgidity and flaccidity

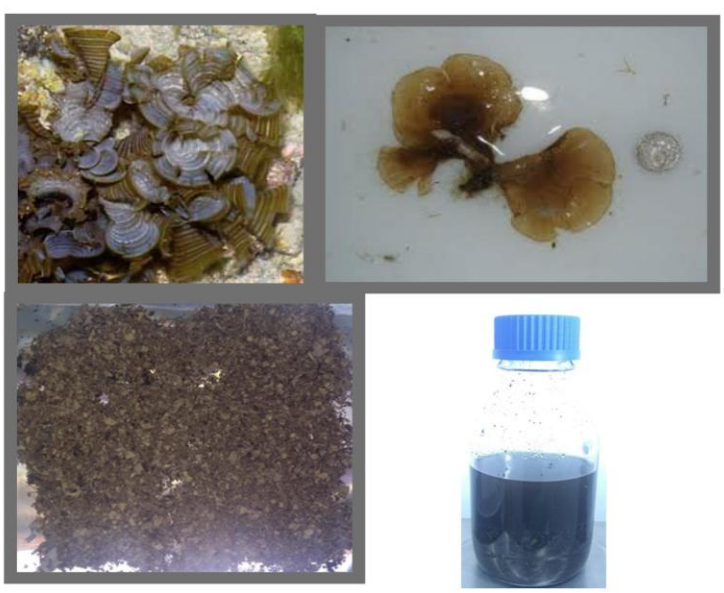
Comment [U1]: Scientific names should be itilized effects caused by changes in their habitat's salinity.. Marine algae are either unicellular (microalgae) or multicellular (macroalgae) vegetative organisms that vary in size, from 2 m to 30 m, and in their morphology. They are a diverse group of autotrophic organisms that contain chlorophyll for oxygenic photosynthesis and have evolved adaptive osmoregulation mechanisms to regulate their internal osmotic pressure and prevent turgidity and flaccidity effects caused by changes in their habitat's salinity. Hence, the aim of study was to evaluate the anticancer activity of *P.gymnospora* methanolic crude extracts.

Comment [U2]: Revisit all punctuation marks and correct appropriately

Previous studies show the various pharmacological actions of the plants such as anti-inflammatory activity (1) (2), antidiabetic activity (3) (4,5) (6) and anticancer activity (7) (8). Our team has extensive knowledge and research experience that has translated into high quality publications(9–13),(14),(15),(16)(17),(18),(19),((11,20,21),(22–26).,(27),(28)

This study was another such attempt at decoding the importance of one of the most appreciated seaweed, P. *gymnospora*.

Materials and Methods:



Comment [U3]: The images should have presented separately for clear view

Figure 1 : Images of *P. gymnospora* sample and their crude extract preparation

Sample collection and preparation:

The *P.gymnospora* seaweed was collected from Thoothukudi coastal area, Tamilnadu shown in figure 1. The sample was washed thoroughly with tap water then shade dried on table tissue paper for 4 weeks and turned into a fine power shown in figure 1.

Preparation of extraction: 25g of dried powdered *Padina gymnospora* seaweed samples were mixed with 100ml of Ethanol and allowed to place for 24 hours at ambient temperature. Then the mixture was passed through whatman filter paper (No.4) then the filtrate was centrifuged at 3000rpm for 10min and further filtered by 0.45µm syringe micro filter. At last, the solvents are evaporated via vacuum rotary evaporator until samples are obtained in powder form. Then the sample was stored in a shadowy aluminum container at 4ºC for further analysis shown in figure 1.

MTT Assay: The proliferation of MCF-7 cells was assessed by MTT assay Safadi et al., (2003). MCF-7 cells were plated in 48 well plates at a concentration of $2x10^4$ cells/well 24 hours after plating, cells were washed twice with 500µl of serum-free medium and starved by incubating the cells in serum-free medium for 3 hours at 37ºC. After starvation, cells were treated with *P. gymnospora* ethanol extract in different concentrations for 24 hours. At the end of treatment, the medium from control and *P. gymnospora* extract treated cells were discarded and 200µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated for 4h at 37ºC in the CO_2 incubator.

Comment [U4]: Citation should corrected to Safadi et al. (2003)

The MTT containing medium was then discarded and the cells were washed with 1x PBS. The crystals were then dissolved by adding 200µl of solubilization solution and this was mixed properly by pipetting up and down. Then the formazan crystals formed were dissolved in dimethylsulfoxide (200µl) and incubated in dark for an hour. The intensity of the colour created was then measured at 570 nm using a Micro ELISA plate reader. The percentage of control cells cultivated in serum-free medium was used to calculate the number of viable cells. Without any treatment, cell viability in the control media was indicated as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells]×100.

MORPHOLOGY STUDY: Based on MTT assay we selected the optimal doses (250µg/ml) for further studies. A phase contrast microscope was used to examine changes in cell morphology. 3104 cells were planted in six-well plates and given a 24-hour treatment with Padina gymnospora ethanol extract (250 g/ml for MCF-7 cells).. At the end of the incubation period, the medium was removed and cells were washed once with a phosphate buffer saline (PBS pH 7.4). The plates were observed under a phase contrast microscope.

STATISTICAL ANALYSIS: All data obtained were analyzed by Student's-t-test using MS-Excel, represented as mean ± SD for triplicates. The data were calculated statistically using one-way ANOVA (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA). The statistical significance level was set at 0.05.

RESULTS AND DISCUSSION:

Figure 2: This table indicates the cell viability of lung cancer cell lines tested in different concentrations of methanolic crude extract of *P. gymnospora*

Drug concentration(µl)	**24hrs**
Control	100
100	82.52+8.37
200	61.37+10.16
300	47.68+7.28
400	35.49+10.61
500	26.22+8.25
600	16.41+7.15

Comment [U5]: Double full stop correct please

Comment [U6]: Figure title should be transferred to the foot of the figure

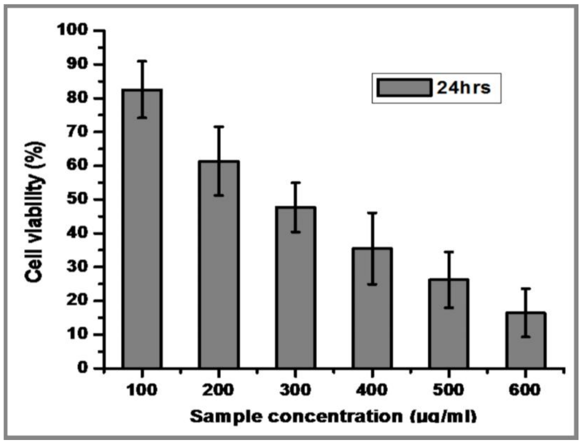


Figure 3 : This graph indicates the cell viability of lung cancer cell lines tested in different concentrations of methanolic crude extract of *P. gymnospora*

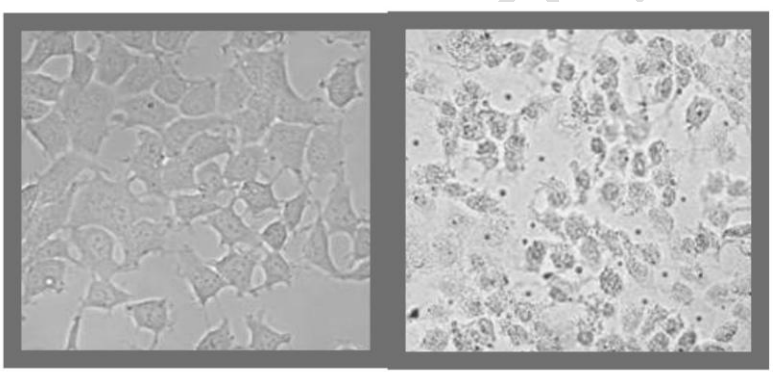


Figure 4: Microscopic image of lung cancer cell lines on being treated with control compared with the microscopic image of lung cancer cell lines on being treated with highest concentration of the prepared methanolic crude extract of *P .gymnospora*.

Figure 2 indicates the cell viability of lung cancer cell lines tested in different concentrations of methanolic crude extract of *P. gymnospora*. According to Figure II, in 100μl concentration of the methanolic crude extract of *P. gymnospora* showed 82.52% of the cells were viable. In 200μl concentration of the methanolic crude extract of *P. gymnospora* 61.37% of the cells were viable. In 300μl concentration of the methanolic crude extract of *P. gymnospora* 47.68% of the cells were viable and In 400μl concentration of the methanolic crude extract of *P. gymnospora* 35.49% of the cells were viable. Further, in 400μl concentration of the methanolic crude extract of *P. gymnospora* 61.37% and 500μl concentration of the methanolic crude extract of *P. gymnospora* 26.22% of the cells were viable. In 200μl concentration of the methanolic crude extract of *P. gymnospora* 16.41% of the cells were viable. Figure 3 indicates the graphical representation of the cell viability of lung cancer cell lines tested in different concentrations of methanolic crude extract of *P. gymnospora* showing concentration dependent inhibition in cell growth. Figure 4 compared the microscopic image of lung cancer cell lines being treated with control and the microscopic image of lung cancer cell lines being treated with highest concentration of the prepared methanolic crude extract of *P. gymnospora* .

Discussion :

Over the past few decades, articles in the literature suggests that the anticancer activity of extracts and compounds isolated from seaweeds has gained interest based on two main factors: (i) the need for new anticancer chemicals that are more effective, targeted, and have less side effects; and (ii) epidemiological evidence showing diets rich in marine seaweeds reduce the incidence of cancer (29). Additionally, several traditional medicinal systems, such as Chinese and Japanese approaches, have used seaweeds for centuries to treat neoplasms (29). A european study evaluated using in vitro and in vivo models in the three main groups of seaweeds: Rhodophyta, Chlorophyta, Phaeophyceae. Several studies investigating the in vivo anticancer activities of seaweed have focused on compounds from brown seaweeds, especially in leukemia, breast cancer, and Lewis sarcoma models (30). Brown algae are clearly the most investigated, not only for their anticancer properties, but also for their anti-inflammatory, hypoglycemic, anticoagulant, and antioxidant properties, as evidenced by the literature.Thus, it is possible that the importance of brown seaweeds in medicine is related to the fact that these have been the most studied so far (31). Organic extracts and chemicals extracted from seaweeds, such as polysaccharides, fucoidan, phloroglucinol, laminarian, pheophorbide, monoterpenes, and glycoproteins, have been employed in vitro studies to investigate the anticancer activity of seaweeds.. These studies have highlighted that the degree of sulfation and composition of polysaccharides from brown seaweeds appear to influence their antitumor activity (32). In

Comment [U7]: Double full stop ?

addition to that, crude extracts from marine microbes, plants and animals also showed the potential biological activities in terms of antimicrobial, antioxidant and cytotoxicity effects etc (33)). However, these studies also point out that the low yield obtained from alga- isolated compounds and extracts and the complexity of the compound structures represent a challenge to the use of these compounds in the drug discovery. The results obtained have also been heterogeneous, given that no one compound was able to achieve 100% cell death. Nevertheless, the distribution mechanism of the compounds of seaweed that exhibits anticancer activity within the body has not yet been demonstrated. However, it has been suggested that seaweed act by increasing the activity of the immune system. The heterogeneity of the experimental design within the studies in literature do not allow us to compare their results, because researchers used different cell types, concentrations, time of treatment, and the parameters evaluated (34). During the 1980s, the US National Cancer Institute (NCI) developed a protocol for the evaluation of the cytotoxicity of compounds with anticancer activity, testing the compounds against eight cell lines derived from the most common human malignancies. In 1990, the NCI introduced the NCI-60 Anti-cancer Drug Screen, which contains 60 different human cell lines against which dose-response curves were drawn up, along with biochemical pathways, and this screen is still in use. In addition, some companies now supply tumour cell panels for testing drugs and extracts. In this context, the use of cancer cell lines in the development and the design of new drugs has several advantages: low cost, repeatability of results, and high throughput. However, these models do not reflect the in vivo activity of a compound or extract (35). As a result, other factors such as absorption, distribution, prodrug activation, half-life, metabolism, elimination, side effects, and so on must be assessed. Compounds derived from seaweeds have been demonstrated to target signalling pathways that are resistant to traditional chemotherapy medications. Canadian research revealed that the p53 tumor suppressor protein is not functional in approximately 50% of tumors, causing resistance to chemotherapy. Therefore, the ability of compounds present in seaweed to effectively suppress proliferation independently of the p53 pathway is highly relevant. Moreover, triple-negative breast cancer (TNBC), which lacks estrogen, progesterone, and HER2 receptors, represents up to 20% of all breast cancers, has poor prognosis, and cannot be reduced with hormonal or anti-HER2 therapies, which are the standard therapy for breast cancers (36). Hence, seaweed-derived compounds that downregulate the PI3K/Akt pathway and act downstream of these receptors represent good chemotherapeutic agents against drug-resistant

Comment [U8]: Separate words

cancers .In addition to cell proliferation, other mechanisms, including the inhibition of angiogenesis, prevention of metastasis, and induction of differentiation, must be evaluated using animal models and specialized approaches, such as murine allografts and xenografts also needs to be studies more extensively as very articles in literature throws light on these concepts. Evaluation of the cell selectivity of anticancer drugs is another important issue, given that anticancer drugs must kill cancer cells without causing extensive damage to non-cancer cells(25) (37) (38) (39) (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) (50). Therefore, researchers usually select normal cells (primary cultures and non cancer cell lines) to evaluate the effect of extracts and compounds. Although some authors have used mouse epidermal JB6 Cl41 cell line, African green monkey kidney Vero cells, among others, these controls might not mimic the effect that drugs will have on7 normal cells because of the interactions within cell lineages and tissues. Furthermore, it is also essential to evaluate compounds against multidrug-resistant phenotype cell lines (e.g., the HCT-15 colon and renal cancer cell lines UO-31 and TK10) (29). Good effects, slight inhibition, and high inhibition have been used by authors to characterise the effects of their compounds, but there have been no measures created to estimate the effectiveness of an extract and/or compound. We believe that total growth inhibition (TGI) is a good metric to utilise when evaluating a compound's effects because it indicates its efficacy.

Conclusion:

From this study it could be concluded that *P. gymnospora* has concentration dependent significant anticancer activity. At highest concentration, 500µl, methanolic crude extracts of *P. gymnospora* showed the maximum anticancer activity, where the cell viability was only 16.41±7.15. Morphological study also revealed that maximum cell death had occured in the maximum concentration of the methanolic crude extract of *P. gymnospora* . Based on these results, further studies could be carried out as a search for new compounds from the family of brown algae to develop alternative therapeutic measures against diseases.

Ethics approval: Nil

Consent to participate: Nil

Consent for publication: Nil

REFERENCES

1. Anitha R, Prathoshni S, Lakshmi T. The effect of capsicum oleoresin on nitric oxide production and nitric oxide synthase gene expression in macrophage cell line [Internet]. Vol. 10, Pharmacognosy Research. 2018. p. 343. Available from: http://dx.doi.org/10.4103/pr.pr_46_18

2. Cinthura C, Thangavelu L, Rajeshkumar S, Gurunadhan D, Pradeep Kumar R, Roy A. COX2 Inhibitory activity of Abutilon indicum-An Invitro Study [Internet]. Vol. 10, Indian Journal of Public Health Research & Development. 2019. p. 3523. Available from: http://dx.doi.org/10.5958/0976-5506.2019.04131.7

3. Roy A, Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Nadu T, et al. Molecular docking analysis of compounds from Lycopersicon esculentum with the insulin receptor to combat type 2 diabetes [Internet]. Vol. 16, Bioinformation. 2020. p. 748–52. Available from: http://dx.doi.org/10.6026/97320630016748

4. Anitha R, Ashwini S. Antihyperglycemic activity of Caralluma fimbriata: An In vitro approach [Internet]. Vol. 13, Pharmacognosy Magazine. 2017. p. 499. Available from: http://dx.doi.org/10.4103/pm.pm_59_17

5. Leya MM, Anitha R. Anti-inflammatory Effect of the Aqueous Fruit Pulp Extract of Tamarindus indica Linn in Lipopolysaccharide-Stimulated Macrophages [Internet]. Vol. 11, Pharmacognosy Journal. 2019. p. 669–73. Available from: http://dx.doi.org/10.5530/pj.2019.11.105

6. Anitha R, Aneesa N, Varghese S. Antidiabetic activity of ajwain oil in different in vitro models [Internet]. Vol. 11, Journal of Pharmacy And Bioallied Sciences. 2019. p. 142. Available from: http://dx.doi.org/10.4103/jpbs.jpbs_128_18

7. Ashwini S, Ezhilarasan D, Anitha R. Cytotoxic effect of Caralluma fimbriata against human colon cancer cells. Pharmacognosy Journal [Internet]. 2017;9(2). Available from: https://www.phcogj.com/article/252

8. Suhasini SJ, Jennifer Suhasini S, Roy A, Sosa G, Lakshmi T. The Cytotoxic effect of Caralluma fimbriata on KB cell lines [Internet]. Vol. 12, Research Journal of Pharmacy and Technology. 2019. p. 4995. Available from: http://dx.doi.org/10.5958/0974-360x.2019.00865.5

9. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles usingMangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol [Internet]. 2018 Oct;117:91–5. Available from: http://dx.doi.org/10.1016/j.enzmictec.2018.06.009

10. Nandhini NT, Rajeshkumar S, Mythili S. The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation. Biocatal Agric Biotechnol [Internet]. 2019 May 1;19:101138. Available from: https://www.sciencedirect.com/science/article/pii/S1878818118308235

11. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.-mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. Environ Sci Pollut Res Int [Internet]. 2020 Mar;27(8):8166–75. Available from: http://dx.doi.org/10.1007/s11356-019-07511-x

12. Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, Anbarasan PM. Green synthesis of silver nanoparticles using Gymnema sylvestre leaf extract and evaluation of its antibacterial activity [Internet]. Vol. 32, South African Journal of Chemical Engineering. 2020. p. 1–4. Available from: http://dx.doi.org/10.1016/j.sajce.2019.11.005

13. Rajasekaran S, Damodharan D, Gopal K, Rajesh Kumar B, De Poures MV. Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends. Fuel [Internet]. 2020 Oct 1;277:118166. Available from: https://www.sciencedirect.com/science/article/pii/S0016236120311625

14. Santhoshkumar J, Sowmya B, Venkat Kumar S, Rajeshkumar S. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of Passiflora caerulea. S Afr J Chem Eng [Internet]. 2019 Jul;29:17–23. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1026918519300253

15. Raj R K, D E, S R. β-Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line. J Biomed Mater Res A [Internet]. 2020 Sep;108(9):1899–908. Available from: http://dx.doi.org/10.1002/jbm.a.36953

16. Saravanan M, Arokiyaraj S, Lakshmi T, Pugazhendhi A. Synthesis of silver nanoparticles from Phenerochaete chrysosporium (MTCC-787) and their antibacterial activity against human pathogenic bacteria. Microb Pathog [Internet]. 2018 Apr;117:68–72. Available from: http://dx.doi.org/10.1016/j.micpath.2018.02.008

17. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species–mediated cytotoxicity in HepG2 cells. Hum Exp Toxicol [Internet]. 2019 Jun 1;38(6):694–702. Available from: https://doi.org/10.1177/0960327119839173

18. Ezhilarasan D, Sokal E, Najimi M. Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets. Hepatobiliary Pancreat Dis Int [Internet]. 2018 Jun;17(3):192–7. Available from: http://dx.doi.org/10.1016/j.hbpd.2018.04.003

19. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. Arab J Gastroenterol [Internet]. 2018 Jun;19(2):56–64. Available from: http://dx.doi.org/10.1016/j.ajg.2018.03.002

20. Gomathi AC, Xavier Rajarathinam SR, Mohammed Sadiq A, Rajeshkumar S. Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line. J Drug Deliv Sci Technol [Internet]. 2020 Feb 1;55:101376. Available from: https://www.sciencedirect.com/science/article/pii/S1773224719313693

21. Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. Drug Dev Res [Internet]. 2019 Sep;80(6):714–30. Available from: http://dx.doi.org/10.1002/ddr.21571

22. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol [Internet]. 2018 Oct;89(10):1241–8. Available from: http://dx.doi.org/10.1002/JPER.17-0445

23. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. Arch Oral Biol [Internet]. 2021 Feb;122:105030. Available from: http://dx.doi.org/10.1016/j.archoralbio.2020.105030

24. Joseph B, Prasanth CS. Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry? Oral Surg Oral Med Oral Pathol Oral Radiol [Internet]. 2021 Jul;132(1):118–9. Available from: http://dx.doi.org/10.1016/j.oooo.2021.01.025

25. Ezhilarasan D, Apoorva VS, Ashok VN. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med [Internet]. 2019 Feb [cited 2021 Sep 15];48(2). Available from: https://pubmed.ncbi.nlm.nih.gov/30451321/

26. Duraisamy R, Krishnan CS, Ramasubramanian H, Sampathkumar J, Mariappan S, Navarasampatti Sivaprakasam A. Compatibility of Nonoriginal Abutments With Implants: Evaluation of Microgap at the Implant-Abutment Interface, With Original and Nonoriginal Abutments. Implant Dent [Internet]. 2019 Jun;28(3):289–95. Available from: http://dx.doi.org/10.1097/ID.0000000000000885

27. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products. Aquaculture [Internet]. 2019 May 30;507:1–6. Available from: https://www.sciencedirect.com/science/article/pii/S0044848618328072

28. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Res Ther [Internet]. 2021 Mar 18;12(1):192. Available from: http://dx.doi.org/10.1186/s13287-021-02265-1

29. Venkatesan J, Anil S, Kim S-K. Seaweed Polysaccharides: Isolation, Biological and Biomedical Applications [Internet]. Elsevier; 2017. 408 p. Available from: https://play.google.com/store/books/details?id=BZ2pDQAAQBAJ

30. Torres MD, Kraan S, Dominguez H. Sustainable Seaweed Technologies: Cultivation, Biorefinery, and Applications [Internet]. Elsevier; 2020. 750 p. Available from: https://play.google.com/store/books/details?id=nXLnDwAAQBAJ

31. Olas B, Skalski B, Ulanowska K. The Anticancer Activity of Sea Buckthorn [Elaeagnus rhamnoides (L.) A. Nelson] [Internet]. Vol. 9, Frontiers in Pharmacology. 2018. Available from: http://dx.doi.org/10.3389/fphar.2018.00232

32. Perumalsamy H, Sankarapandian K, Veerappan K, Natarajan S, Kandaswamy N, Thangavelu L, et al. In silico and in vitro analysis of coumarin derivative induced anticancer effects by undergoing intrinsic pathway mediated apoptosis in human stomach cancer. Phytomedicine [Internet]. 2018 Jul 15;46:119–30. Available from: http://dx.doi.org/10.1016/j.phymed.2018.04.021

33. Syed MH, Gnanakkan A, Pitchiah S. Exploration of acute toxicity, analgesic, anti-inflammatory, and anti-pyretic activities of the black tunicate, Phallusia nigra (Savigny, 1816) using mice model [Internet]. Vol. 28, Environmental Science and Pollution Research. 2021. p. 5809–21. Available from: http://dx.doi.org/10.1007/s11356-020-10938-2

34. Jeon K-H, Shrestha A, Jang HJ, Kim J-A, Sheen N, Seo M, et al. Anticancer Activity of Indeno[1,2-b]-Pyridinol Derivative as a New DNA Minor Groove Binding Catalytic Inhibitor of Topoisomerase IIα. Biomol Ther [Internet]. 2021 May 20; Available from: http://dx.doi.org/10.4062/biomolther.2020.231

35. Zeng B, Cheng Y, Zheng K, Liu S, Shen L, Hu J, et al. Design, synthesis and in vivo anticancer activity of novel parthenolide and micheliolide derivatives as NF-κB and STAT3 inhibitors. Bioorg Chem [Internet]. 2021 May 15;111:104973. Available from: http://dx.doi.org/10.1016/j.bioorg.2021.104973

36. Parthasarathi P. Synthesis and anticancer activity of phenylpropanoid sucrose esters [Internet]. Available from: http://dx.doi.org/10.32657/10356/62145

37. Danda AK, Krishna TM, Narayanan V, Siddareddi A. Influence of primary and secondary closure of surgical wound after impacted mandibular third molar removal on postoperative pain and swelling--a comparative and split mouth study. J Oral Maxillofac Surg [Internet]. 2010 Feb [cited 2021 Sep 15];68(2). Available from: https://pubmed.ncbi.nlm.nih.gov/20116700/

38. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. Clin Oral Investig [Internet]. 2019 Sep [cited 2021 Sep 15];23(9). Available from: https://pubmed.ncbi.nlm.nih.gov/30552590/

39. Sathivel A, Raghavendran HR, Srinivasan P, Devaki T. Anti-peroxidative and anti-hyperlipidemic nature of Ulva lactuca crude polysaccharide on D-galactosamine induced hepatitis in rats. Food Chem Toxicol [Internet]. 2008 Oct [cited 2021 Sep 15];46(10). Available from: https://pubmed.ncbi.nlm.nih.gov/18706469/

40. Panda S, Doraiswamy J, Malaiappan S, Varghese SS, Del Fabbro M. Additive effect of autologous platelet concentrates in treatment of intrabony defects: a systematic review and meta-analysis. J Investig Clin Dent [Internet]. 2016 Feb [cited 2021 Sep 15];7(1). Available from: https://pubmed.ncbi.nlm.nih.gov/25048153/

41. Neelakantan P, Varughese AA, Sharma S, Subbarao CV, Zehnder M, De-Deus G. Continuous chelation irrigation improves the adhesion of epoxy resin-based root canal sealer to root dentine. Int Endod J [Internet]. 2012 Dec [cited 2021 Sep 15];45(12). Available from: https://pubmed.ncbi.nlm.nih.gov/22612994/

42. Govindaraju L, Neelakantan P, Gutmann JL. Effect of root canal irrigating solutions on the compressive strength of tricalcium silicate cements. Clin Oral Investig [Internet]. 2017 Mar [cited 2021 Sep 15];21(2). Available from: https://pubmed.ncbi.nlm.nih.gov/27469101/

43. Sekhar CH, Narayanan V, Baig MF. Role of antimicrobials in third molar surgery: prospective, double blind,randomized, placebo-controlled clinical study. Br J Oral Maxillofac Surg [Internet]. 2001 Apr [cited 2021 Sep 15];39(2). Available from: https://pubmed.ncbi.nlm.nih.gov/11286448/

44. DeSouza SI, Rashmi MR, Vasanthi AP, Joseph SM, Rodrigues R. Mobile phones: the next step towards healthcare delivery in rural India? PLoS One [Internet]. 2014 Aug 18 [cited 2021 Sep 15];9(8). Available from: https://pubmed.ncbi.nlm.nih.gov/25133610/

45. Nasim I, Neelakantan P, Sujeer R, Subbarao CV. Color stability of microfilled, microhybrid and nanocomposite resins--an in vitro study. J Dent [Internet]. 2010 [cited 2021 Sep 15];38 Suppl 2. Available from: https://pubmed.ncbi.nlm.nih.gov/20553993/

46. Danda AK, Muthusekhar MR, Narayanan V, Baig MF, Siddareddi A. Open versus closed treatment of unilateral subcondylar and condylar neck fractures: a prospective, randomized clinical study. J Oral Maxillofac Surg [Internet]. 2010 Jun [cited 2021 Sep 15];68(6). Available from: https://pubmed.ncbi.nlm.nih.gov/20303209/

47. Molecular structure and vibrational spectra of 2,6-bis(benzylidene)cyclohexanone: A density functional theoretical study. Spectrochim Acta A Mol Biomol Spectrosc [Internet]. 2011 Jan 1 [cited 2021 Sep 15];78(1):113–21. Available from: http://dx.doi.org/10.1016/j.saa.2010.09.007

48. Putchala MC, Ramani P, Herald J. Sherlin, Premkumar P, Natesan A. Ascorbic acid and its pro-oxidant activity as a therapy for tumours of oral cavity – A systematic review [Internet]. Vol. 58,

Archives of Oral Biology. 2013. p. 563–74. Available from: http://dx.doi.org/10.1016/j.archoralbio.2013.01.016

49. Neelakantan P, Grotra D, Sharma S. Retreatability of 2 mineral trioxide aggregate-based root canal sealers: a cone-beam computed tomography analysis. J Endod [Internet]. 2013 Jul;39(7):893–6. Available from: http://dx.doi.org/10.1016/j.joen.2013.04.022

50. Suresh P, Marimuthu K, Ranganathan S, Rajmohan T. Optimization of machining parameters in turning of Al-SiC-Gr hybrid metal matrix composites using grey-fuzzy algorithm [Internet]. Vol. 24, Transactions of Nonferrous Metals Society of China. 2014. p. 2805–14. Available from: http://dx.doi.org/10.1016/s1003-6326(14)63412-9