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Full Length Research Article

In vitro Antitumor Activity of *Sargassum wightii* Greville (Sargassaceae) Chloroform Extract against Human Cancer Cells

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Abstract

Seaweeds are exploited for their rich source of pharmacologically bioactive secondary metabolites and bioactive compounds for anticancer properties. In the present study, the chloroform extract of *Sargassum wightii* was tested for antitumor activity against human liver, colon and breast cancer cells. MTT assay was adopted, and the percentage of cell viability was calculated. The inhibitory activity of *S. wightii* chloroform extract tested at five different concentrations of treatments (25, 50, 100, 250 and 500µg/mL) with control against three different cancer cells showed significant inhibition at higher concentrations. The dose-dependent chloroform extract of *Sargassum wightii* inhibited cell growth inhibitions *in vitro* 85% of liver cancer cells (hepG2), 70% of colon cancer cells (HT-29), and 55% of breast cancer cells (MCF7) when compared with control. Cell morphology studies undertaken exhibited the presence of dead cancer cells of liver, colon and breast at the highest concentration (500µg/mL). Detailed examination is needed on the mechanisms involved in cell death, as *Sargassum wightii* chloroform extract was found to exhibit promising results towards anticancer property.

***Key words*:** *Sargassum wightii*, Chloroform extract, Antitumor, Liver, Colon, Breast

Cancer is a leading cause of death worldwide, accounting for nearly one in six deaths [1], and is estimated, that approximately 26 million new cases will be enrolled by 2030, and the quantum would increase globally in future [1-3]. Cancer, characterized by the uncontrolled proliferation and spread of abnormal cells is indicated by unrestrained cell division, which has the propensity to permeate into other tissues, by growing directly into adjoining tissues via invasion or by getting implanted to a distant site by metastasis via circulatory or lymphatic system [4]. Anticancer drugs currently used in chemotherapy are cytotoxic to normal cells, and causes immune toxicity which affects not only the tumor development, but also aggravates patient's recovery. Identification of anticancer agents that arrest the cancer cell proliferation/growth without inducing toxicity to the normal cells have become essential in the field of cancer research [5], and is a major challenge in cancer treatment. Advocating of natural products for cancer treatment has increased owing to its availability, affordability and relatively lesser side effects when compared to chemotherapeutic agents [6-7]. It is well established that the marine products from seaweeds possess antitumor activity [8].

Seaweeds are exploited for their rich source of pharmacologically active metabolites for anticancer properties. Bioactive compounds from seaweeds possess cytotoxic effects on several human cancers (pancreatic, breast, bladder and lung) [4], [9], and the mechanisms for their anticancer activity are related to their ability to suppress the growth of cancer cells [10]. Genus *Sargassum* (brown macroalgae), the most diverse genus of seaweeds widely distributed in the temperate and tropical oceans of the world [11], are reported for anticancer property. *Sargassum wightii*, distributed in abundance on the southern coasts of Tamil Nadu, India, is known for its medicinal and biological properties [12], [13], and possesses antibacterial, anti-Alzheimer, antiasthmatic, antidiabetic, antioxidant and antitumor properties [14-20]. Though literature speaks on the miscellaneous bioactivity property of *Sargassum wightii*, information remained lacking about the antitumor activity of its chloroform extract. This paucity of knowledge initiated the current investigation on *in vitro* antitumor activity on human liver, colon, and breast carcinoma cells.

MATERIALS AND METHODS

Collection of seaweed

Sargassum wightii was collected by hand picking from the intertidal zone of Rameswaram, Tamil Nadu, India (8° 46

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N, 78° 9 E and 9° 14 N, 79° 14°E). The collected macroalgae was immediately rinsed in water to remove all kinds of epiphytes and other particles (sand, molluscs, and sea grasses), and kept in sterilized ziplock bags, and transferred to laboratory for further studies. Taxonomical identification and confirmation of the collected seaweed was done at the Marine Algal Research Station, Mandapam, Tamil Nadu, India with the aid of morphological key characters and identification manual [21-22].

Preparation of seaweed extract

The cleaned macroalgae was shade dried for a week, and powdered with the aid of a mixer grinder. The powdered sample (250g) was suspended in chloroform (750mL), and transferred to Soxhlet apparatus for extraction [23]. Thereafter, the extracted sample was filtered using Whatman No.1 filter paper, and the filtered sample was centrifuged at 5000rpm for 10 minutes at 4°C, and the supernatant was collected in a separate flask. The chloroform extract was then concentrated using a rotary vacuum evaporator (Puchi RII, Switzerland). The final concentrated chloroform crude extract was stored in sterile air tight amber coloured bottle, and kept in a refrigerator until further use. Prior to this, the percentage of yield of extraction of this crude extract was calculated.

Analysis of chemical compounds

Sargassum wightii chloroform extract was subjected to qualitative tests for the identification of various chemical constituents, viz., alkaloids, carbohydrates, flavonoids, phenols, proteins, saponins, steroids, and tannins as per standard procedures [24].

Cell line and cell culture

Human carcinoma cell lines were used to evaluate the antitumor property of *Sargassum wightii* chloroform extract. Three cell lines, viz., liver cancer cells (hepG2), colon cancer cells (HT-29) and breast cancer cells (MCF-7) used for the present study were procured from the National Centre for Cell Science (NCCS), Pune, Maharashtra, India. The three cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS) and streptomycin and penicillin (100µg/mL and 100 IU/ mL respectively). Cells were then cultured in carbon dioxide (5%) and humidified atmosphere (37°C) until confluence. The entire processes was carried out in a vertical laminar air flow chamber.

In vitro cytotoxicity assay

The *in vitro* analysis was carried at the Biological Research Centre in Pondicherry University, Puducherry, India. Antitumor activity was carried by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay technique [25]. MTT cell proliferation and viability assay is a safe, sensitive, *in vitro* assay for the measurement of cell proliferation, because metabolic events lead to apoptosis or necrosis, resulting in reduction in the cell viability. The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions, and viable cells were counted using a haemocytometer and diluted with medium containing 5% FBS to give a final density of 1x105 cell mL^{-1}. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells $well^{-1}$, and incubated to allow for cell attachment at 37°C, 5% carbon dioxide, 95% air and 100% relative humidity. After 24 hours, the cells were treated with serial concentrations of *Sargassum wightii* chloroform crude extract. They were initially dissolved in dimethyl sulfoxide (DMSO), and an aliquot of the sample solution was diluted twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five concentrations. Aliquots of 100µL of each sample dilutions were added to the appropriate wells already containing 100µL of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 hours at 37°C, 5% carbon dioxide, 95% air and 100% relative humidity. The medium containing 5% FBS without treated samples served as control, and triplicates were maintained for all concentrations. After 72 hours of incubation, 20µL of MTT dye at 5mg mL^{-1} phosphate buffered saline (PBS) was added to every well. The cells were again incubated at 37°C and 5% carbon dioxide for four hours, followed by addition of DMSO (100µL) to each well. Absorbance was read at 570nm using ELISA micro plate reader to assess the cytotoxicity [26], and percentage of cell viability was calculated.

Cell morphology studies

The cell lines and numbers of cells used were same as in the MTT assay. After 24 hours of seeding in a 96-well plate, the cells were treated with *Sargassum wightii* chloroform extract, and incubated for another 24 hours. Micrographs were taken at 20x magnification to observe any changes in the cell morphology, viz., unattached rounded cells, membrane blebbing, apoptotic bodies, and enlarged cells undergoing necrosis.

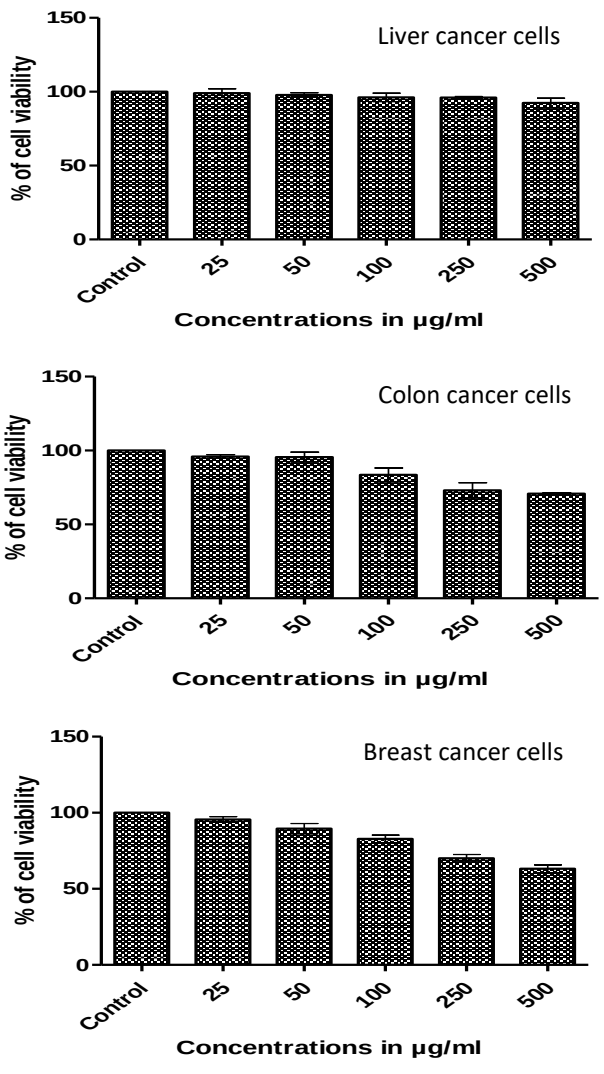

Fig 1 Inhibitory activity of *Sargassum wightii* chloroform extract on human cancer cells

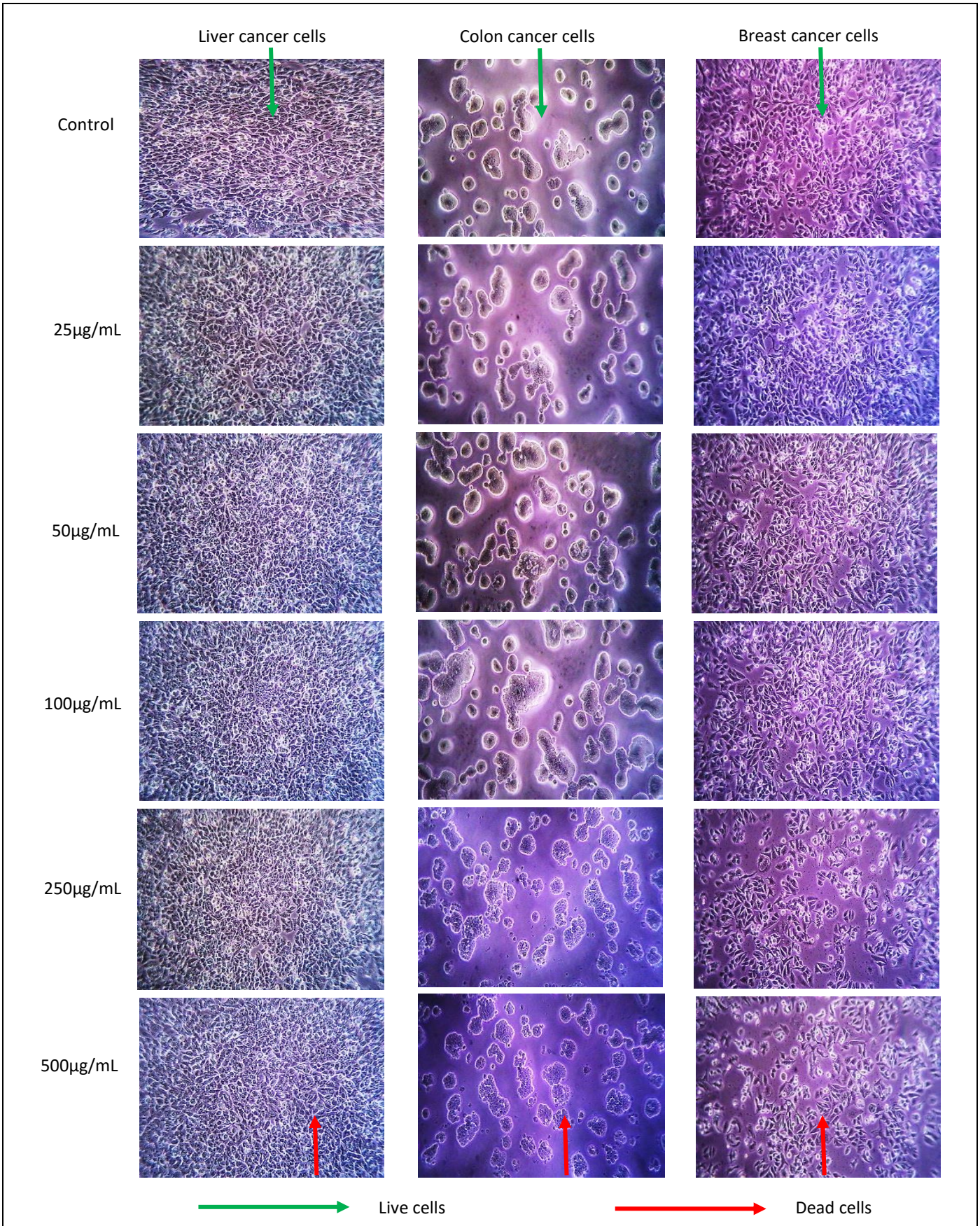

Fig 2 Micrographs of human cancer cells treated with *Sargassum wightii* chloroform extract

RESULTS AND DISCUSSION

The percentage yield of *Sargassum wightii* chloroform extract was 2.35%, and its qualitative chemical analysis revealed presence of alkaloids, carbohydrates, flavonoids, phenols, saponins, and tannins. The inhibitory activity of *Sargassum wightii* chloroform extract tested at five different concentrations of treatments (25, 50, 100, 250 and 500µg/mL) with control against three different human cancer cells showed significant inhibition at higher concentrations. The dose-dependent chloroform extract of *Sargassum wightii* inhibited 85% liver cancer cells (hepG2), 70% colon cancer cells (HT-29), and 55% breast cancer cells (MCF7) growth inhibitions *in vitro* at the highest concentration (Fig 1). Cell morphology

studies exhibited the presence of dead cancer cells of liver, colon and breast at the highest concentration (500µg/mL). Cancerous cells were stressed with blebbed membranes, shrunken appearance, and were enlarged which could be considered necrotic (Fig 2).

Cancer is characterized by excessive and abnormal proliferation of cells [27]. During the cancer multi-stage cascade, normal cells undergo initiation, promotion, and progression processes [28]. Extensive researches on the cellular and molecular basis of the carcinogenesis cascade provide a targeted approach for cancer chemoprevention, which aims to reverse the development and progression of precancerous cells through pharmacological agents. Chemotherapy are prone to cause several side effects, and to overcome this, the research thirst of searching new natural compounds from seaweeds to treat cancer is on the rise. Antiproliferative screening is a technique to determine anticancer activities [29]. It is mandatory to determine the potential of cytotoxicity against human cancer cell lines, because the agents which display specific activity on cell lines are recommended for *in vivo* studies. Therefore, it is necessary to screen the agents with the common methods like tryphan blue and MTT assay [30], and for the present study MTT assay was adopted. *Sargassum* species have been reported for anticancer property, as its extracts induced cytotoxicity against cancer cells[31], since they contain a glycoprotein with anticancer effects [12].

Sargassum wightii seaweeds reduces the progression of cancer [32]. The present investigation showed that the chloroform extract of *Sargassum wightii* showed significant anti-proliferative activities (in three different human cancer cell lines). The ability of a cell to survive toxicity has been the basis of most cytotoxic assays. Living cells did not allow the entry of dye due to cells permeability. A dead cell loses its permeability and allows dye to enter in to the cells. Hence, living cells are colourless and dead cells are blue to violet in colour. Cytotoxicity of this study was found to be time-dependent, and cell viability was also found to be diminishing with increasing concentrations of extracts. Further, the cell morphology studies also indicated the death of cancerous cells. The same was reported by Murugan and Iyer [33] too by *Sargassum wightii* chloroform and ethyl acetate extracts against cancer cells. Theisen [34] reported that *Sargassum wightii* possessed cancer chemopreventive activity that prevented different stages of carcinogenesis process, and this was due to polysaccharides present in it. Anjana *et al*. [35] reported that the decrease in the cancer cell number observed in *Sargassum wightii* ethanolic extract indicated significant inhibitory effect on the tumor cell proliferation. *Sargassum wightii* exhibited inhibitory effect against on HeLa, K-562 and MDA-MB cell lines [36]. The cytotoxicity of *Sargassum wightii* aqueous extract based synthesized silver nanoparticles showed profound effect on PC-3 cells due to chemical compounds, viz., tannins, phenols, alkaloids, flavonoids and saponins [37]. *Sargassum wightii* ethanol extract possessed novel polysaccharides which exhibited anti proliferative activity against AGS, HeLa, MCF 7 and PC 12 cell lines [30]. Taskin *et al.* [38] also stated that polysacchardies and terpenoids from brown algae are considered as promising bioactive molecules with anticancer activity. Zhang *et al*. [39] stated that brown seaweeds with low molecular weight fucoidan, mediated the broad-spectrum growth inhibition of human carcinoma cells like HeLa, HT1080, K562, U937, A549 and HL-60.

Organic solvents provide higher efficiency in extracting bioactive compounds than aqueous methods. Reports confirm that bioactive compounds, viz., carbohydrates, steroids, tannins, saponins, fats, gums and proteins are extracted from *Sargassum wightii* [40]. Seenivasan *et al*. [41] reported high flavonoid and phenol content in *Sargassum wightii*, and the same was observed in the present study also. The cytotoxic potential of seaweeds via the inhibition of the proliferation of human cancer cell lines are likely to be related to bioactive compounds, viz., alkaloids, flavonoids, phenols, and saponins which were reported in the present study too. Alkaloids cytotoxic activity is due to the presence of microtubule interfering agents that can bind to β-tubulin, inhibiting the formation of the mitotic spindle fibre required for cell division [42-43]. Flavonoids affect the development of hormones, and prevent aromatase to avoid the proliferation of cancer cells, while polyphenolic compounds modify carcinogens metabolic activation and inhibit cancer cells [44], and interact with main enzymes in the cell cycle, cellular signaling pathways, metastasis, and apoptosis [45]. Saponins protect cells from being cancerous, by improving immune function, delaying the growth of cancer cells, reducing the development of cancer-related hormones, and in addition serve as antioxidants [46]. A close relationship exists between anticancer and antioxidant bioactive compounds, viz., polyphenols, flavonoids, saponins, tannins, alkaloids, and fatty acids, and the present study reported these bioactive compounds for the anticancer activity. Hence, prevalence of both cytotoxic and antioxidant properties in a single compound could be beneficial in terms of rational, preventive or therapeutic purposes. Hence, additional studies are needed to prove this dual property of *Sargassum wightii* chloroform extract.

CONCLUSION

Detailed examination on the mechanisms involved in cell death, impact on cell cycle, and induced mitochondrial and DNA damages, as *Sargassum wightii* chloroform extract was found to exhibit promising results towards anticancer properties.

LITERATURE CITED

1. WHO. 2022. Cancer key facts. World Health Organization.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. 2015. Global cancer statistics 2012. *CA: A Cancer Journal for Clinicians* 65(2): 87-108.
3. WHO. 2017. Cancer fact sheet. World Health Organization.
4. Cragg GM, Newman DJ. 2005. Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology* 100: 72-79.
5. Xu H, Yao L, Sung H, Wu L. 2009. Chemical composition and antitumor activity of different polysaccharides from the roots *Actinidia eriantha*. *Carbohydrate Polymers* 78: 316-322.
6. Engel N, Oppermanne C, Falodunb A, Kragle U. 2011. Proliferative effects of five traditional Nigerian medicinal plant extracts on human breast and bone cancer cell lines. *Journal of Ethnopharmacology* 137(2): 1003-1010.
7. Manosroi J, Sainakham M, Manosroi W, Manosroi A. 2012. Anti-proliferative and apoptosis induction activities of extract from Thai medicinal plant recipes selected from MANOSROI II database. *Journal of Ethnopharmacology* 141(1): 451-459.
8. Ayesha, Hira, Sultana V, Ara J, Ehteshamul-Haque S. 2010. *In vitro* cytotoxicity of seaweeds from Karachi coast on brine shrimp. *Pakistan Journal of Botany* 42: 3555-3560.

9. Malaker A, Ahmad SAI. 2013. Therapeutic potency of anticancer peptides derived from marine organism. *International Journal of Engineering and Applied Science* 2(4): 1-13.
10. Wang YY, Khoo KH, Chen ST, Lin CC, Wong CH, Lin CH. 2002. Studies on the immune modulating and antitumor activities of *Ganoderma lucidum* (Reishi) polysaccharides: Functional and proteomic analyses of a fucose-containing glycoprotein fraction responsible for the activities. *Bioorganic and Medicinal Chemistry* 10: 1057-1062.
11. Budhiyanti SA, Raharjo S, Marseno DW, Lelana IYB. 2012. Antioxidant activity of brown algae *Sargassum* species extract from the coastline of Java Island. *American Journal of Agricultural and Biological Sciences* 7(3): 337-346.
12. Ranahewa TH, Premarathna AD, Wijesundara RM, Wijewardana V, Jayasooriya AP, Rajapakse RP. 2019. Biochemical composition and anticancer effect of different seaweed species (*In-vitro* and *in-vivo* studies). *Sustainable Marine Structures* 1(2): 5-11.
13. Lim S, Cheung P, Ooi V, Ang P. 2002. Evaluation of antioxidative activity of extracts from a brown seaweed *Sargassum siliquastrum*. *Journal of Agricultural and Food Chemistry* 50(13): 3862-3866.
14. Xue M, Ge Y, Zhang J, Liu Y, Wang Q, Hou L, Zheng Z. 2013. Fucoidan inhibited 4T1 mouse breast cancer cell growth *in vivo* and *in vitro* via down regulation of Wnt/β-catenin signaling. *Nutrition and Cancer* 65(3): 460-468.
15. Meenakshi S, Manicka GD, Tamilmozhi S, Arumugam M, Balasubramanian T. 2009. Total flavonoid and *in vitro* antioxidant activity of two seaweeds of Rameshwaram coast. *Global Journal of Pharmacology* 3(2): 59-62.
16. Kumar K, Anbu J, Sumithra MA, Sathish R. 2012. Influence of ethanolic leaf extract of *Sargassum wightii* and *Adiantum capillus* on histamine induced asthma in animal model. *International Journal of Pharmacy and Pharmaceutical Sciences* 4(4): 121-123.
17. Syad AN, Shunmugiah KP, Kasi PD. 2013. Antioxidant and anti-cholinesterase activity of *Sargassum wightii*. *Pharmaceutical Biology* 51(11): 1401-1410.
18. Chandrasekaran M, Venkatesalu V, Raj G, Krishnamoorthy S. 2014. Antibacterial properties of various extracts of *Sargassum wightii* against multidrug resistant bacterial strains. *Phykos* 44(2): 17-28.
19. Mohapatra L, Bhattamishra S, Panigrahy R, Parida S, Pati P. 2016. Antidiabetic effect of *Sargassum wightii* and *Ulva fasciata* in high fat diet and multi low dose streptozotocin induced Type 2 diabetic mice. *UK Jr. of Pharm. and Biosci.* 4(2): 13-23.
20. Rinu KA, Joseph D, Venugopal A. 2017. Therapeutic uses of *Sargassum* species: A review. *Int. Jr. of Phar. Pharm. Res.* 9(3): 53-59.
21. Dhargalkar VK, Kavlekar D. 2004. Seaweeds - a field manual. (1st Edn). National Institute of Oceanography, Goa.
22. Rao SG. 2012. Common seaweeds and seagrasses of India. Herbarium Vol. 1, Central Marine Fisheries Research Institute, Kerala.
23. Vogel AL. 1978. Text book of practical organic chemistry. London: The English Language Book Society and Longman.
24. Harborne JB. 1984. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd Ed. Chapman and Hall, London, NY.
25 Mosmann T. 1983. Rapid colorimeteric assay for cellular growth and survival: applications to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65(1-2): 55-63.
26. Talarico LB, Zibetti RG, Faria PC, Scolaro LA, Duarte ME, Noseda MD, Pujol CA, Damonte EB. 2004. Anti-herpes simplex virus activity of sulphated galactans from the red seaweed *Gymnogongrus grifithsiae* and *Cryptonemia crenulata*. *International Journal of Biological Macromolecules* 34(1-2): 63-71.
27. Zandi K, Ahmadzadeh S, Tajbakhsh S, Rastian Z, Yousefi F, Farshadpour F, Sartavi K. 2010. Anticancer activity of *Sargassum oligocystum* water extract against human cancer cell lines. *Eur. Rev. for Medical and Pharmacological Sciences* 14: 669-673.
28. Manojkumar K, Seema. 2013. *In vitro* cancer chemopreventive properties of polysaccharide extract from the brown alga, *Sargassum wightii*. *IOSR Journal of Pharmacy and Biological Sciences* 8(3): 6-11.
29. Donapaneedi SN, Suresh K, Chakraborty AK, Valluru L. 2018. *In vitro* antiproliferative effect of marine macroalgae on different human cancer cell lines. *International Journal of Pharmaceutics & Drug Analysis* 6(2): 342-351.
30. Gunasekaran S, Thirumalairaj VK, Shanmugasokan LS, Pitchai S, Yesudas R, Chacko A. 2017. Screening of *in vitro* cytotoxic activity of brown seaweeds against hepatocellular carcinoma. *Journal of Applied Pharmaceutical Science* 7(5): 51-55.
31. Khanavi M, Nabavi M, Sadati N, Ardekani MS, Sohrabipour J, Nabavi SMB, Ghaeli P, Ostad SN. 2010. Cytotoxic activity of some marine brown algae against cancer cell lines. *Biological Research* 43: 31-37.
32. Krishnan S, Suveena S, Aswathy P. 2017. *In silico* study on anticancerous impact of compounds isolated from *Sargassum wightii* by blocking Bcl-xL. *Journal of Advances in Biological Science* 4(2): 45-47.
33. Murugan K, Iyer VV. 2013. Differential growth inhibition of cancer cell lines and antioxidant activity of extracts of red, brown and green marine algae. *In Vitro Cellular & Developmental Biology - Animal* 49: 324-334.
34. Theisen C. 2001. Chemoprevention: What's in a name? *Journal of the National Cancer Institute* 93(10): 743-774.
35. Anjana A, Ahamed KFHN, Ravichandiran V, Sumithra M, Anbu J. 2014. Anticancer activity of *Sargassum wightii* Greville on Dalton's ascitic lymphoma. *Chinese Journal of Natural Medicines* 12(2): 114-120.
36. Ilahi S, Shameem A, Sasi BRP. 2014. Anti-cancer activity of selected seaweeds against HeLa, K-562 and MDA-MB cell lines. In: Biotechnology and Bioforensics New Trends, Springer Verlag, 35-42.
37. Jayaprakash P, Sivakumari K, Ashok K, Rajesh S, Prabhu D, Chandrasekar D. 2017. Anticancer potential of green synthesized silver nanoparticles of *Sargassum wightii* against human prostate cancer (PC-3) cell line. *Eur. Jr. of Pharma. Medical Res.* 4(3): 275-287.
38. Taskin E, Caki Z, Ozturk M, Taskin E. 2010. Assessment of *in vitro* antitumoral and antimicrobial activities of marine algae harvested from the eastern Mediterranean Sea. *African Journal of Biotechnology* 9: 4272-4277.
39. Zhang Z, Teruya K, Eto H and Shirahata S. 2011. Fucoidan extract induces apoptosis in MCF-7 cells via a mechanism involving the ROS-dependent JNK activation and mitochondria-mediated pathways. *PLoS One* 6(11); e27441.
40. Mansuya P, Aruna P, Sridhar S, Kumar JS, Babu S. 2010. Antibacterial activity and qualitative phytochemical analysis of selected seaweeds from Gulf of Mannar region. *Journal of Experimental Sciences* 1(8): 23-26.
41. Seenivasan R, Rekha M, Indu H, Geetha S. 2012. Antibacterial activity and phytochemical analysis of selected seaweeds from Mandapam coast, India. *Journal of Applied Pharmaceutical Science* 2(10): 159-169.
42. Solanki R, Khanna M, Lal R. 2008. Bioactive compounds from marine actinomycetes. *Ind. Jr. of Microbiology* 48: 410-431.
43. Kasım CG, Percot A, Sezik E. 2010. Alkaloids in marine algae. *Marine Drugs* 8: 269-284.
44. Zhao M, Yang B, Wang J, Liu Y, Yu L, Jiang Y. 2007. Immunomodulatory and anticancer activities of flavonoids extracted from litchi (*Litchi chinensis* Sonn.) pericarp. *International Immunopharmacology* 7(2): 162-166.
45. Lamoral-Theys D, Pottier L, Dufrasne F, Neve J, Dubois J, Kornienko A, Kiss R, Ingrassia L. 2010. Natural polyphenols that display anticancer properties through inhibition of kinase activity. *Current Medicinal Chemistry* 17(9): 812-825.
46. Shree TJ, Poompavai S, Begum S, Gowrisree V, Hemalatha S. 2019. Cancer-fighting phytochemicals: another look. *Journal of Nanomedicine & Biotherapeutic Discovery* 8: 162.