RESEARCH PAPER

Evaluating the Flavonoid-Enriched fraction of *Padina tetrastromatica* a brown Seaweed as a Marine Chemotherapeutic Prospect Integrated MTT Cytotoxic Profiling in A375 Melanoma Cells.

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ABSTRACT:
Background: Marine algae are emerging as valuable sources of bioactive compounds with significant therapeutic potential. Brown seaweeds possess diverse secondary metabolites, particularly flavonoids, which are known for their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. This study aimed to evaluate the biological potential of a flavonoid-enriched fraction obtained from *Padina tetrastromatica*, a marine brown seaweed. **Methods:** A colorimetric cell viability assay was used to determine the cytotoxic activity of the flavonoid-enriched fraction of *Padina tetrastromatica* in a human melanoma cell line. **Results:** The extract showed dose-dependent cytotoxicity ranging from 3.12 to 100 µg/mL, with an IC_{50} of 50.15 µg/mL compared to 4.82 µg/mL of cisplatin. Cellular changes were supported by morphological observations associated with apoptosis. **Conclusion:** The flavonoid-enriched fraction exhibited moderate cytotoxic effects, suggesting its potential as a marine-based anticancer candidate. Further research, including normal cell line evaluation and comprehensive phytochemical characterization, is needed.

KEYWORD: *Padina tetrastromatica*, Extraction, Flavonoidal fraction, MTT assay, Cell viability.

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INTRODUCTION:

Cancer is a significant global health issue and one of the leading causes of death worldwide (1,2). One of the most violent types of skin cancer is melanoma which is a malignant tumour of melanocytes and is closely related to long-term exposure to ultraviolet radiation and genetic mutations impacting the tumour suppressor pathways (3). Nevertheless, melanoma remains a serious clinical problem since it has a metastatic potential and resistance to conventional treatments, regardless of the developments in the therapeutic strategies (4). Natural products have traditionally been considered as rich sources of anticancer agents. Phytochemicals, including flavonoids and phenolic compounds, have shown a great potential in the reduction of oxidative stress and preventing tumour progression. There is evidence that diets rich in antioxidants and plant-derived compounds have been shown to help in the prevention of cancer (5–10). Nevertheless, the compounds of terrestrial plants are usually restricted by such factors as low stability and bioavailability (11-14). Marine macroalgae (seaweeds) have attracted growing attention as alternative sources of bioactive compounds because of their special metabolic adaptations in marine ecosystem (15,16). Such organisms synthesize structurally diverse secondary metabolites, such as polysaccharides, polyunsaturated fatty acids, pigments, and phenolic

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compounds, that have antioxidant, anti-inflammatory, and anticancer effects (17-19). *Padina tetrastromatica* is a brown macroalga, which has been reported to have a wide range of bioactive constituents that possess antioxidant and therapeutic properties (18,20). Previously, most research has concentrated on its polysaccharide, rather than its antioxidant properties, and there is little information on its cytotoxic properties, especially against melanoma cell lines (20,21). Thus, the objective of the current research was to determine the cytotoxic potential of the flavonoid-enriched part of *Padina tetrastromatica* against a human melanoma cell line. This study gives a preliminary insight of its anticancer activity, but additional confirmation by normal cell line research and chemical characterization is needed.

MATERIALS AND METHODS:

Collection and Identification:

The marine macroalgae *Padina tetrastromatica* samples were collected from coastline of Mandapam (9˚16'37.34"N; 79˚7'30.78"E), Ramanathapuram district, India. The sample were authenticated by marine botanist at Annamalai university, Portonova.

Extraction:

This alga does not have any rooting system but they had an attachment portion by which they attached at rocks. Samples were collected, and extraneous materials were removed by rigorous washing with seawater, followed by a final wash with tap water. The samples were then shade-dried and powdered using an electric mixer (22–24). The resulting powder was stored in an airtight container for later use. The fine powder of *Padina tetrastromatica* was macerated with 150 ml of methanol. The extract was enriched with flavonoids by partitioning the organic and aqueous layers in a separating funnel. The filtrate was initially separated using petroleum ether and allowed to stand for 30 minutes; the aqueous layer was then collected and separated using n-hexane to obtain the final fraction for further processing (21).

ANTICANCER ACTIVITY:

The antioxidant activity of this flavonoidal fraction, along with its total flavonoid and phenolic concentrations, was previously reported, providing a basis for this cytotoxicity assessment (21). The cytotoxic activity of the flavonoid-rich fraction of *Padina tetrastromatica* was evaluated using the MTT assay in human melanoma cells (A375 cell line). The MTT assay is a straightforward method for demonstrating anticancer activity with reproducible results; cell viability is indicated by the formation of a purple formazan product, which is measured spectrophotometrically. This measurement reflects the number of viable cells and indicates the extent of cytotoxicity induced by the test sample

Preparation of test solution

For cytotoxic evaluation, the flavonoidal fraction was prepared in a series of concentration from 3.125-100µg/mL using serial dilution method.

Cell culture preparation:

For cell line preparation, human melanoma cells were procured from the National Centre for Cell Science. Cells were cultured in growth medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 IU/mL), streptomycin (100 µ g/mL). Cultures were maintained at 37ºC in a humidified incubator with 5% CO_2. Upon reaching adequate confluency, cells were detached using a trypsin- EDTA solution and evaluated for viability. Subsequently, 50,000 viable cells per well were seeded into 96 well plates and incubated for 1 day under standard culture condition (25–27).

Procedure

The monolayer cells were detached using trypsin, and the cell density was adjusted to 1.0×10^5 cells/mL using complete growth medium supplemented with 10% fetal bovine serum. Aliquots of 100 µL of the cell suspension were seeded into each well of a 96-well microplate, resulting in approximately 50,000 cells per well. After 24 h of incubation, a partial monolayer was observed. The supernatant was discarded, and the cell layer was gently washed with fresh medium. Subsequently, 100 µL of test drug solutions at varying concentrations were added to the respective wells. The plates were incubation at temperature of 37°C for a day in an environment having 5% CO_2. Following incubation, all test solution were removed from wells and 100 µL of a MTT solution (which consists of 5 mg per 10 ml of MTT added in PBS) was added into each well. The plates underwent another four h incubation at 37^0C in condition of 5 % CO_2 (28–31). After four hours, supernatant fluid was taken out and 100 µL of the DMSO were added to each well so plates could be moved slightly to help the formazan become dissolved. The microplate reader was used to get the absorbance at 570 nm wavelength. The inhibition percentage was found with a specific calculation and the drug concentration needed to stop cell growth by a factor of 50 (IC_{50}) was figured by dose-response graph generated for each treatment group.

IC_{50} Value:

The half maximal inhibitory concentration (IC_{50}) is quantitative parameter used to describe the potency of a test compound in suppressing a defined biological or chemical reaction. It represents the concentration at which drug decrease cell viability by 50% compared with untreated controls. In this study, IC_{50} values were determined by plotting compound concentration against percentage growth inhibition, followed by non-linear regression analysis to generate a sigmoidal dose-response curve. This method enables reliable comparison of cytotoxicity efficacy among test samples (22,23,32).

RESULTS AND DISCUSSION:

The cytotoxic potential of the flavonoidal fraction of *Padina tetrastromatica* (FRPD) was evaluated against A375 human melanoma cells using the MTT colorimetric assay, with cisplatin as a reference standard. This assay measures cell viability based on mitochondrial dehydrogenase activity, which enzymatically converts MTT into insoluble formazan crystals in active cells. The intensity of the formazan color, measured spectrophotometrically at 570 nm, provide a direct measure of the number of viable cells remaining after treatment.

Quantitative Evaluation of Cytotoxicity

Cells were exposed to increasing concentrations of FRPD (3.12-100 µg/ml) and cisplatin for 1 day, and mean optical density (OD) values were used to calculate cell viability percentages shown in Table.1 & Fig 1. Both compounds demonstrated a concentration-dependent decrease in viability. The flavonoidal fraction exhibited a gradual reduction in cell survival from 95.96% at 3.12µg/mL to 27.92% at 100 µg/mL, whereas cisplatin showed a much sharper decline from 66.22% at 3.26% at 100µg/mL. The calculated IC_{50} values were 50.15µg/mL for flavonoidal fraction and 4.82µg/mL for cisplatin, respectively. These findings indicate that cisplatin remains significantly more potent than FRPD shown in Table 2 & Fig 2; however, FRPD demonstrated measurable dose-dependent cytotoxic effects, suggesting its potential as a promising anticancer compound.

Table 1 Cytotoxic effect of FRPD on human melanoma cell line assessed by MTT assay

MTT Assay of FRPD Human melanoma cell line								
Parameters	**Blank**	**Untreated**	**Concentration µg/mL**					
			3.12	**6.25**	**12.5**	**25**	**50**	**100**
Reading 1	0.004	1.214	1.154	1.022	0.960	0.814	0.686	0.329
Reading 2	0.004	1.203	1.168	1.01	0.927	0.816	0.704	0.352
Reading 3	0.003	1.211	1.160	1.018	0.955	0.833	0.690	0.340
Mean	0.003	1.209	1.1606	1.0166	0.947	0.821	0.693	0.340
Blank Mean OD		1.205	1.157	1.013	0.9436	0.817	0.6896	0.337
Standard deviation		0.0056	0.0070	0.0061	0.017	0.0104	0.0094	0.0115
% Viability		**100**	95.96	84.01	78.26	67.79	57.20	27.92
IC_{50} Value								**50.15**

Values represent triplicate readings (Reading 1–3) expressed as mean ± standard deviation. Blank represents media without cells, and untreated cells serve as the control (100% viability)

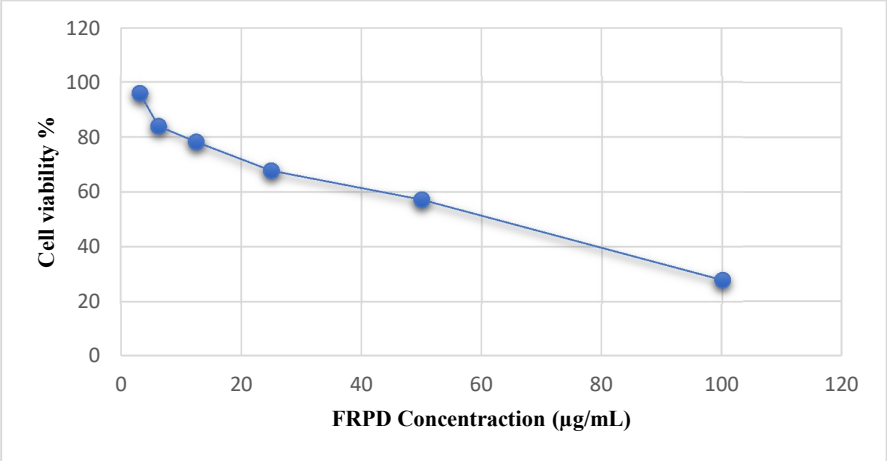

Figure 1 Effect of FRPD on Cell Viability of Human Melanoma Cells.

Table 2 Cytotoxic effect of Cisplatin on human melanoma cell line assessed by MTT assay

MTT Assay of Standard (Cisplatin) Human melanoma cell line								
Parameters	**Blank**	**Untreated**	**Concentration µg/mL**					
			3.12	**6.25**	**12.5**	**25**	**50**	**100**
Reading 1	0.004	1.214	0.815	0.553	0.273	0.182	0.088	0.042
Reading 2	0.004	1.203	0.788	0.568	0.296	0.16	0.103	0.026
Reading 3	0.003	1.211	0.803	0.537	0.27	0.176	0.095	0.061
Mean	0.003	1.209	0.802	0.5526	0.2796	0.1726	0.0953	0.040
Mean OD		1.205	0.798	0.549	0.276	0.169	0.091	0.039

Standard deviation		0.00561	0.013	0.0155	0.0142	0.0113	0.007	0.017
% Viability		**100**	66.21	45.53	22.89	14.01	7.60	3.26
IC_{50} Value								**4.82**

Values represent triplicate readings (Reading 1–3) expressed as mean ± standard deviation. Blank represents media without cells, and untreated cells serve as the control (100% viability)

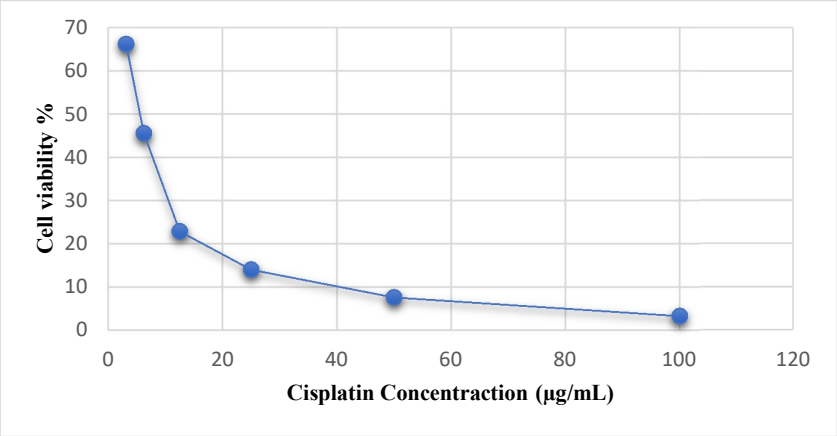

Figure 2 Dose-dependent effect of cisplatin on cell viability in human melanoma cells determined by MTT assay.

Table 2: Cell viability (Concentration versus % of cell viability)

Concentration (µg/mL)	**Flavonoidal fraction – Mean OD (after blank)**	**Viability %**	**Cisplatin – Mean OD (after blank)**	**Viability %**
Untreated control	1.2057	100.00	1.2057	100.00
3.12	1.1570	95.96	0.7983	66.22
6.25	1.0130	84.02	0.5490	45.53
12.5	0.9437	78.27	0.2760	22.89
25	0.8173	67.79	0.1690	14.02
50	0.6897	57.20	0.0917	7.60
100	0.3367	27.92	0.0393	3.26

The resulting dose-dependent curve (Fig. 3) clearly illustrates a concentration-dependent reduction in cell viability for both compounds. Cisplatin exhibited a pronounced inhibition of cell proliferation, with viability decreasing to below 10% at a concentration of 50 µg/mL. In contrast, the flavonoidal fractions demonstrated a more gradual decline, reaching approximately 28% viability at 100 µg/mL. The calculated IC_{50} values were 50.15 µg/mL for FRPD and 4.82 µg/mL for cisplatin, indicating that while cisplatin is more potent, the flavonoidal fraction exhibits significant cytotoxic activity.

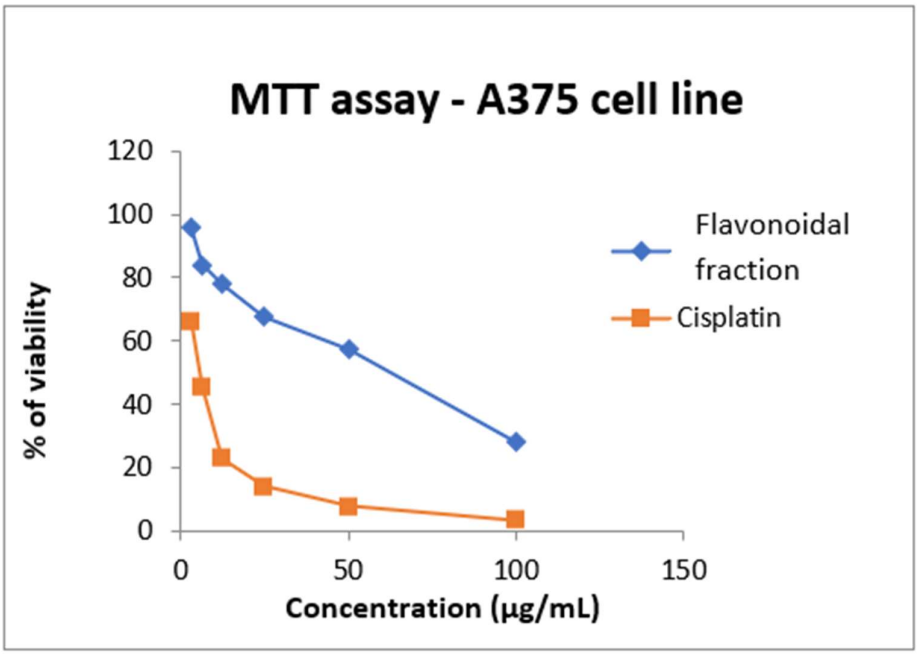

Figure 3: MTT assay of A375 cell line treated with Flavonoidal fraction of Padina tetrastromatica and Cisplatin for 24 hours, showing percentage cell viability versus concentration (µg/mL).

Morphological Analysis (Based on 24 hours microscopic Observation)

Direct microscopic imaging (Fig.4 & 5) from the experimental data revealed progression morphological damage with increasing drug concentration when compared with control cells in Fig.4.

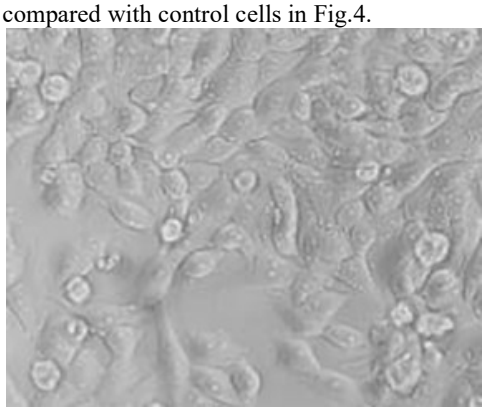

Figure 4: Control cells (untreated) appeared healthy, polygonal, and tightly attached to the culture surface.

Cells exposed to the flavonoidal fraction exhibited distinct cytoplasmic shrinkage and rounding at concentrations ranging from 12.5 to 50 µg/mL. At the highest concentration tested (100 µg/mL), the majority of cells detached, lost membrane integrity, and displayed hallmark features of apoptosis, including membrane blebbing (Fig. 5 A). In comparison, cisplatin-treated cells showed more severe morphological deterioration even at lower concentrations (6.25 µg/mL), with near-complete detachment observed between 25 and 100 µg/mL, underscoring its potent cytotoxic effects (Fig. 5 B).

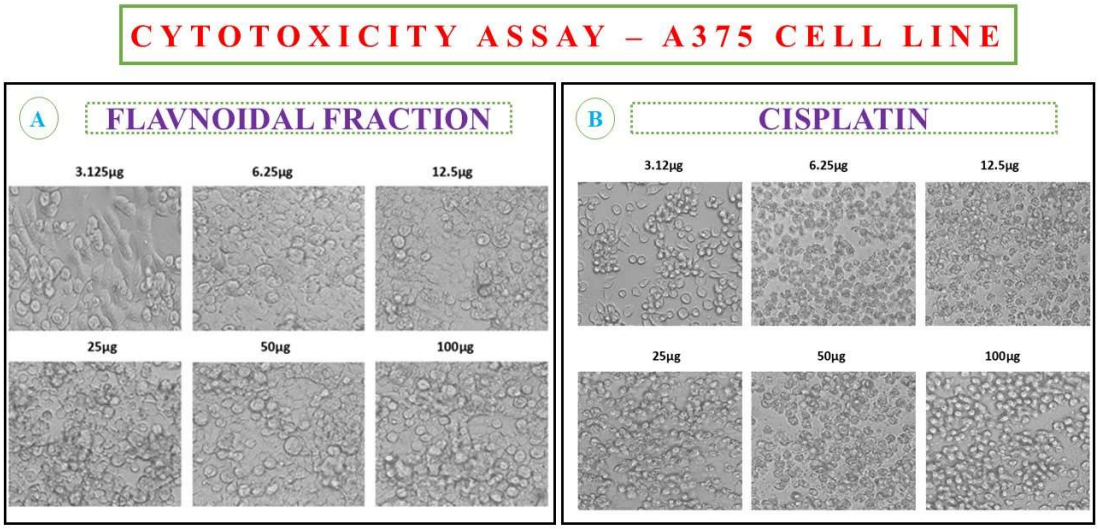

Figure 5 The cell morphology of A375 cells after treating with various concentration of A) FRPD & B) Cisplatin for 24 h

Interpretation and Literature Correlation

The cytotoxicity of FRPD could be attributed to its interaction with mitochondrial enzymes, which results in decreased energy metabolism and the initiation of apoptotic signalling. The characteristic cellular shrinkage and membrane blebbing observed in microscopy support this hypothesis. Similar findings have demonstrated that MTT reduction is a valid indicator of mitochondrial function and cell viability (24,33,34).

Although the cytotoxic activity of the flavonoidal fraction is lower than that of standard cisplatin, the results indicate a clear dose-dependent antiproliferative effects. FRPD stands as a promising lead molecule for further optimisation, potentially offering reduced toxicity compared with conventional chemotherapeutics.

Conclusion

In conclusion, the present study confirms that flavonoidal fraction of *Padina tetrastromatica* exerts significant cytotoxic effects on A375 melanoma cells, as evidenced by both the MTT assay and morphological alteration. With an IC_{50} value of 50.15 µg/mL, FRPD demonstrated measurable anticancer potential. The combination of quantitative and qualitative evidence supports the hypothesis that FRPD induces apoptosis-mediated cell death. Further studies should aim to characterize the underlying molecular mechanism and assess *in vivo* efficacy.

Author Statements:

- **Ethical approval:** The conducted research is not related to either human or animal use.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper
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- **Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declaration

- **Competing interests:** There are no competing interests to declare
- **Clinical trial number**: Not applicable
- **Ethics approval and Consent to participate declaration**: Not applicable
- **Consent to publish declaration:** Not applicable

Reference:

1. What Is Melanoma Skin Cancer? | What Is Melanoma? Available from: https://www.cancer.org/cancer/types/melanoma-skin-cancer/about/what-is-melanoma.html
2. Tattoos and risk of cutaneous melanoma and non-melanoma skin cancer in France. Available from: https://www.iarc.who.int/cancer-type/skin-cancer
3. Raimondi S, Suppa M, Gandini S. Melanoma Epidemiology and Sun Exposure. Acta Derm Venereol. 2020;100(11):adv00136.
4. Vicente ALSA, Novoloaca A, Cahais V, Awada Z, Cuenin C, Spitz N, et al. Cutaneous and acral melanoma cross-OMICs reveals prognostic cancer drivers associated with pathobiology and ultraviolet exposure. Nat Commun. 2022 July 15;13(1):4115.

5. PEM D, JEEWON R. Fruit and Vegetable Intake: Benefits and Progress of Nutrition Education Interventions- Narrative Review Article. Iran J Public Health. 2015 Oct;44(10):1309–21.
6. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, et al. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. Mol Basel Switz. 2015 Nov 27;20(12):21138–56.
7. Kapinova A, Stefanicka P, Kubatka P, Zubor P, Uramova S, Kello M, et al. Are plant-based functional foods better choice against cancer than single phytochemicals? A critical review of current breast cancer research. Biomed Pharmacother Biomedecine Pharmacother. 2017 Dec;96:1465–77.
8. Kuphal S, Bosserhoff A. Recent progress in understanding the pathology of malignant melanoma. J Pathol. 2009 Dec;219(4):400–9.
9. Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochim Biophys Acta BBA - Gen Subj. 2013 June;1830(6):3670–95.
10. George VC, Kumar DRN, Suresh PK, Kumar RA. Antioxidant, DNA protective efficacy and HPLC analysis of Annona muricata (soursop) extracts. J Food Sci Technol. 2015 Apr;52(4):2328–35.
11. Iqbal J, Abbasi BA, Mahmood T, Kanwal S, Ali B, Shah SA, et al. Plant-derived anticancer agents: A green anticancer approach. Asian Pac J Trop Biomed. 2017 Dec;7(12):1129–50.
12. Mao QQ, Xu XY, Shang A, Gan RY, Wu DT, Atanasov AG, et al. Phytochemicals for the Prevention and Treatment of Gastric Cancer: Effects and Mechanisms. Int J Mol Sci. 2020 Jan 16;21(2):570.
13. Redondo-Blanco S, Fernández J, Gutiérrez-Del-Río I, Villar CJ, Lombó F. New Insights toward Colorectal Cancer Chemotherapy Using Natural Bioactive Compounds. Front Pharmacol. 2017;8:109.
14. Clardy J, Walsh C. Lessons from natural molecules. Nature. 2004 Dec;432(7019):829–37.
15. Gomez-Zavaglia A, Prieto Lage MA, Jimenez-Lopez C, Mejuto JC, Simal-Gandara J. The Potential of Seaweeds as a Source of Functional Ingredients of Prebiotic and Antioxidant Value. Antioxid Basel Switz. 2019 Sept 17;8(9):406.
16. Da Rocha A. Natural products in anticancer therapy. Curr Opin Pharmacol. 2001 Aug 1;1(4):364–9.
17. Senthilkumar K, Manivasagan P, Venkatesan J, Kim SK. Brown seaweed fucoidan: Biological activity and apoptosis, growth signaling mechanism in cancer. Int J Biol Macromol. 2013 Sept;60:366–74.
18. Jose GM, Radhakrishnan A, Muraleedhara Kurup G. Antioxidant and antimitotic activities of sulfated polysaccharide from marine brown algae *Padina tetrastromatica*. J Phytol. 2015 Dec 23 ;7. Available from: https://updatepublishing.com/journal/index.php/jp/article/view/2921
19. Sivakumar M, Chandrasekaran VS, Kumari BP. In vitro and in vivo Anti-arthritic and Antioxidant potential of Glycosmis pentaphylla in Complete Freund's Adjuvant model. Res J Pharm Technol. 2023 Feb 28;621–6.
20. Gowrishankar J. An Abridged Review of Beneficial Potentials of a Brown Seaweed, *Padina tetrastromatica*, (Dictyotaceae). Int J Pharm Pharm Res. 2020 Mar;17(4):711–22.
21. J Gowrishankar, Yoganandam P. Assessment of phytochemical constituents and invitro antioxidant properties of flavonoid rich fractions of *padina tetrastromatica*: a marine brown sea weed; 2020. Available from: https://www.researchgate.net/doi/10.13140/RG.2.2.33742.57924
22. Abosedera DA, Emara SA, Tamam OAS, Badr OM, Khalifa SAM, El-Seedi HR, et al. Metabolomic profile and in vitro evaluation of the cytotoxic activity of Asphodelus microcarpus against human malignant melanoma cells A375. Arab J Chem. 2022 Oct;15(10):104174.
23. Looi CY, Moharram B, Paydar M, Wong YL, Leong KH, Mohamad K, et al. Induction of apoptosis in melanoma A375 cells by a chloroform fraction of Centratherum anthelminticum (L.) seeds involves NF-kappaB, p53 and Bcl-2-controlled mitochondrial signaling pathways. BMC Complement Altern Med. 2013 July 10;13:166.
24. Eldera SS, Alkhtaby LA, Al-Wafi R, El-Nour MA. Biosynthesis of Zinc Oxide Nanoparticles by Origanum majorana Aqueous Leaves Extracts, Characterization and Evaluated Against to Schistosoma haematobium. J Biomed Mater Res B Appl Biomater. 2025 Feb;113(2):e35538.
25. Ramezanpour Z, Ghanbari Pirbasti F, Waaland JR. Marine Algae Extract Effects on Cell Proliferation in a Malignant Melanoma Cell Line and an Immortalized Fibroblast Cell Line. Plant Algae Environ. 2025 Mar [cited 2025 Dec 8];9(1). Available from: https://doi.org/10.48308/pae.2025.238286.1101
26. Suganya S, Dhanalakshmi B, Dinesh Kumar S, Santhanam P. Cytotoxic Effect of Silver Nanoparticles Synthesized from Sargassum wightii on Cervical Cancer Cell Line. Proc Natl Acad Sci India Sect B Biol Sci. 2020 Oct 1;90(4):811–8.
27. Abdallah S, Abdel-Halim KY, Alm-Eldeen A. Anticancer potency of Egyptian venom snakes on MCF-7 and HepG2 carcinoma cells. Environ Anal Health Toxicol. 2024 Jan 10;39:e2024001.
28. Crouch SPM, Kozlowski R, Slater KJ, Fletcher J. The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. J Immunol Methods. 1993 Jan;160(1):81–8.
29. Gonzalez RJ, Tarloff JB. Evaluation of hepatic subcellular fractions for Alamar blue and MTT reductase activity. Toxicol Vitro Int J Publ Assoc BIBRA. 2001 June;15(3):257–9.
30. Hattori N, Sakakibara T, Kajiyama N, Igarashi T, Maeda M, Murakami S. Enhanced microbial

biomass assay using mutant luciferase resistant to benzalkonium chloride. Anal Biochem. 2003 Aug;319(2):287–95.
31. Kangas L, Grönroos M, Nieminen AL. Bioluminescence of cellular ATP: a new method for evaluating cytotoxic agents in vitro. Med Biol. 1984;62(6):338–43.
32. Tuzimski T, Petruczynik A, Plech T, Kaproń B, Makuch-Kocka A, Szultka-Młyńska M, et al. Determination of Cytotoxic Activity of Sanguinaria canadensis Extracts against Human Melanoma Cells and Comparison of Their Cytotoxicity with Cytotoxicity of Some Anticancer Drugs. Molecules. 2021 Mar 20;26(6):1738.
33. Mustafa M, Ahmad R, Tantry IQ, Ahmad W, Siddiqui S, Alam M, et al. Apoptosis: A Comprehensive Overview of Signaling Pathways, Morphological Changes, and Physiological Significance and Therapeutic Implications. Cells. 2024 Nov 6;13(22):1838.
34. Shao L, González-Cardenete MA, Prieto-Garcia JM. In Vitro Cytotoxic Effects of Ferruginol Analogues in Sk-MEL28 Human Melanoma Cells. Int J Mol Sci. 2023 Nov 14;24(22):16322.

Graphical Abstract: