'Nori' (*Porphyra* sp.), a Functional Food Showing Anticancer Activity against Ovarian Cancer

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Abstract: Marine seaweeds are nature's largesse resource in human well-being. Seaweed's contribution to the food industry is being widened for its rich source of essential vitamins, minerals, and PUFA. 'Nori' is a dried sheet of red seaweed *Porphyra* sp. used to make 'sushi' a renowned cuisine in Japan, and other Southeast Asian countries. In this present study, commercial-grade 'Nori' was extracted and fractionated with different solvents, and subjected to evaluate the cytotoxic activity on human ovarian tetracarcinoma cells (PA1). As a result, the ethyl acetate fraction was found effective in inhibiting the proliferation of human ovarian cancer cells with an IC_{50} value of 41.1 μg/mL. The main component responsible for anticancer activity was determined as a sesquiterpene lactone Tatridin B (3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-). Moreover, it was found effective in inhibiting NSD2, an important and recently proposed biomarker for several types of cancer. This is a *hitherto* report on the presence of this compound in 'Nori' showing potent anticancer activity on human ovarian tetracarcinoma (PA1).

Keywords: Nori; human ovarian tetracarcinoma (PA1); Tatridin B; NSD2 inhibitor

1. Introduction

Other than antioxidants, polyphenols, flavonoids, alkaloids, vitamins, and minerals, polysaccharides in seaweeds are natural fibers that make them prebiotic for gut microbiota and health (Choudhary et al., 2021). However, the utilization of seaweed as food is being practiced in Southeast Asian countries. In recent decades, the exploration of bioactive compounds from marine seaweeds has attained much attention due to their wide range of applications in pharmaceuticals, and neutraceuticals (Amorim et al., 2012). Marine seaweeds are a rich source of natural pigments as food-coloring agents without any toxic effects as synthetic food colorants (Manivasagan et al., 2018). Polysaccharides, polyphenols, and terpenes are the major seaweed biochemical constituents that have interest for their anti-cancer potential (Atashrazm et al., 2015; Peng et al., 2011; Senthilkumar et al., 2013).

Other than polysaccharides, terpenes and their derivatives are synthesized under environmental stress conditions and have higher anticancer activity. For example, halogenated monoterpene halomon [6(R)-bromo-3(S)-bromomethyl)-7-methyl-2,3,7-trichloro-1-octene] isolated from a red seaweed *Portieria hornemanii* have potent anticancer activity with an IC50 value of 0.9 μM (Fuller et al., 1992; Rocha et al., 2018). Similarly, monoterpenes from red seaweeds *Plocamium cornutum*, and *P. suhrii* had shown more potent inhibitory activity on esophageal cancer (WHCO1) than that of positive control cisplatin (Antunes et al., 2011).

Sesquiterpene lactones are plant-based secondary metabolites, most commonly found in the angiosperms family Asteraceae with a wide range of bioactivities including anti-inflammatory, antimicrobial, phytotoxic, antifungal, and anticancer potential. This kind of metabolite induces oxidative stress in cancer cells by disrupting the cellular redox balance (Gach et al., 2015). Simultaneously, sesquiterpene lactone inhibits inflammatory responses, hampers metastasis in cancer progression, and induces apoptosis.

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Additionally, sesquiterpene lactone is a potent inhibitor of nuclear transcription factor-kappa B (NF-Kb) (S. Zhang et al., 2005).

'Nori' (*Porphyra* sp.) is a red seaweed farmed like square sheets and press-dried and packed and used in renowned cuisine like Sushi in Southeast Asian countries. Since 'Nori' is a rich source of nutrients with anti-anemic, anti-inflammatory, anticancer, anti-hyperlipidemic, and antioxidative properties (Bito et al., 2017). Therefore, for this present investigation, 'Nori' (*Porphhyra* sp.) was commercially obtained from Taipei, Taiwan, and evaluated for its cytotoxic activity on human ovarian tetracarcinoma cancer (PA1).

2. Materials and methods

2.1. Extraction

The food grade 'Nori' (red seaweed: *Porphyra* sp.) imported from Taipei, Taiwan was cut into 5 cm^2 pieces approximately and subjected to homogenization using a mortar and pestle to a fine powder. Then about 100 g of the homogenized biomass was soaked in 700 mL of Methanol in a 1 L glass conical flask overnight at room temperature (28^oC). The extract was filtered through a Whatman No.1 filter paper, and the residual content was again re-extracted using 100 mL of methanol. Both the filtrate was pooled together, freeze-thawed to obtain a crude extract, and subjected to fractionation using different solvents.

2.2. Column fractionation

For column fractionation, a 1 metre length glass column (Borosil Pvt. Ltd) with a 2 cm diameter was used and packed with 60 g of Silica Gel 60 -120 mesh size (Sigma-Aldrich). First, the gel was immersed and soaked with hexane (Merck) in a beaker, glass wool was used initially to avoid loss of silica gel, and the slurry was loaded onto the column and packed to obtain a gel-pack length of 60 cm. After washing the packed column with hexane three times, the crude extract (filtrate) was loaded onto the column and eluted with different solvent fractions including hexane, dichloromethane, ethyl acetate, ethanol, and methanol, and simultaneously the fractions for the respective solvents were collected F1 (hexane fraction), F2 (dichloromethane fraction), F3 (ethyl acetate fraction), F4 (ethanol fraction), and F5 (methanol fraction). The obtained solvent fractions were evaporated to yield the crude fractions and subjected to cytotoxicity activity assay.

2.3. Anticancer activity assay

Human ovarian tetracarcinoma cell line PA1 was retrieved from the National Centre for Cell Sciences (NCCS), Pune, India, and cultured in DMEM-high glucose medium supplemented with 10% inactivated FBS (fetal bovine serum) and penicillin (100 IU/mL) and streptomycin (100 µg/ml) in a CO_2 (5 %) incubator at 37^oC until confluence. All the five different solvent fractions F1 to F5 were taken at different concentrations as 25 µg/ml, 50 µg/ml, and 100 µg/ml concentrations with Dimethyl sulfoxide (DMSO) for MTT cytotoxicity activity assay.

2.4. MTT cytotoxicity assay (Mosmann, 1983)

It is a cell viability check assay, in which MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium is reduced to purple-coloured formazan by mitochondria during cell respiration when cells are viable. Therefore, the rate of reduction of MTT to formazan is directly proportional to the number of viable cells. The MTT is a substrate in this 96-well plate high throughput cytotoxicity screening assay. The test samples were first treated with human ovarian tetracarcinoma cells and finally supplemented with MTT to check whether the test sample has cell cytotoxicity.

The MTT was first diluted in Dulbecco's phosphate-buffered saline, pH – 7.4 at 5 mg/ml concentration and filtered through a 0.2 µm filter in a sterile, amber container, and stored at 4^oC under dark (avoid photoreduction). The solubilisation solution was prepared to contain 40 % dimethyl formamide (DMF), 2 % glacial acetic acid, and 16 % sodium dodecyl sulphate (SDS) with pH of 4.7. The human ovarian tetracarcinoma cells

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(PA1) and the test compounds at three different concentrations (25, 50, and 100 µg/mL) were prepared in 96-well microplates (100 µL/well) and incubated for four hours. Then 10 µL of MTT solution was added to all the wells and again incubated for four hours at 37°C in a CO_2 incubator. After adding 100 µL of solubilisation solution to all the wells, the absorbance values were measured at 570 nm. The cell viability is inversely proportional to the inhibition by the test compound. Thus, the percentage of cell inhibition (cytotoxicity) of human ovarian tetracarcinoma cells was calculated for different concentrations of all the fractions (F1 to F5) and the inhibitory concentration of 50% (IC_{50}) values were also calculated and recorded. Cisplatin was used as a positive control. Microscopic images were taken by 40X magnification using an inverted microscope.

2.5. DNA (deoxyribonucleic acid) fragmentation assay

The human ovarian tetracarcinoma cells (PA-1) were treated with 25, 50, and 100 µg/ml of test sample F3 fraction for 24 hrs. Lysis buffer (500 µl of 100 mM Tris pH-8.5, 5 M NaCl, 0.5 M EDTA, 0.05% TritonX-100, 10 µg/ml proteinase K and 10% SDS) was used to lyse the cells by incubating for 30 min on ice. The supernatant collected was added with a 25:24:1 mixture of phenol: chloroform: isoamyl alcohol then precipitated with two equivalents of ice-cold ethanol plus the one-tenth equivalent of sodium acetate. This was followed by centrifugation at 12,000×g for 20 min. The pellet was re-suspended in 30 µl of sterile water–RNase solution (15 µg/ml RNase in sterile water) and subjected to electrophoresis in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) on a 1% agarose gel.

2.6. Gas chromatography-mass spectrometry (GC-MS) analysis

The gas chromatography was performed by using Perkin Elmer Clarus 680 instrument equipped with a fused silica column with Elite -5MS (30 m x 0.25 mm ID x 250 µm df). The fatty acid components were separated by using a carrier gas Helium (He) at a constant flow rate of 1 ml m^{-1}. About 260°C was set as the injector temperature during gas chromatography. A 1 µl of the extracted sample was injected into the instrument at 60°C for 2 min. Then the temperature was raised up to 300°C at the rate of 10°C per minute.

The mass spectrometry was done by using Perkin Elmer Clarus 600 (EI), the conditions were 240°C for transfer line temperature the same temperature was followed as ion source temperature. The ionization mode was electron impact at 70 eV, a scan time of 0.2 sec and a scanning interval of 0.1 sec. The fragments with a mass between 40 to 600 Da were determined and spectra were retrieved. The spectrums of the compounds were compared with the mass spectrum database of known compounds deposited in the GC-MS NIST (2008) library using the software TurboMass ver. 5.4.2.

3. Results

3.1. Extraction and fractionation

Figure 1 shows the workflow chart of extraction and fractionation of the edible seaweed 'Nori' (*Porphyra* sp.). The total crude extract obtained from the methanolic extraction was about 9.08 %. Among different solvent fractionation, F1 (hexane) yielded about 0.031 g; F2 (dichloromethane) fractionated 0.030 g; F3 (ethyl acetate) engulfed 0.436 g; F4 (ethanol) segregated 0.350 g; and F5 (methanol) obtained 3.42 g of fractions respectively (**Table 1**), which is 0.72 % for F1, 0.70 % for F2, 10.21 % for F3, 8.20 % for F4, and 80.14 % for F5 fractions. Fraction 3 (ethyl acetate) scored top second in terms of yield (10 %) next to fraction 5 (methanol).

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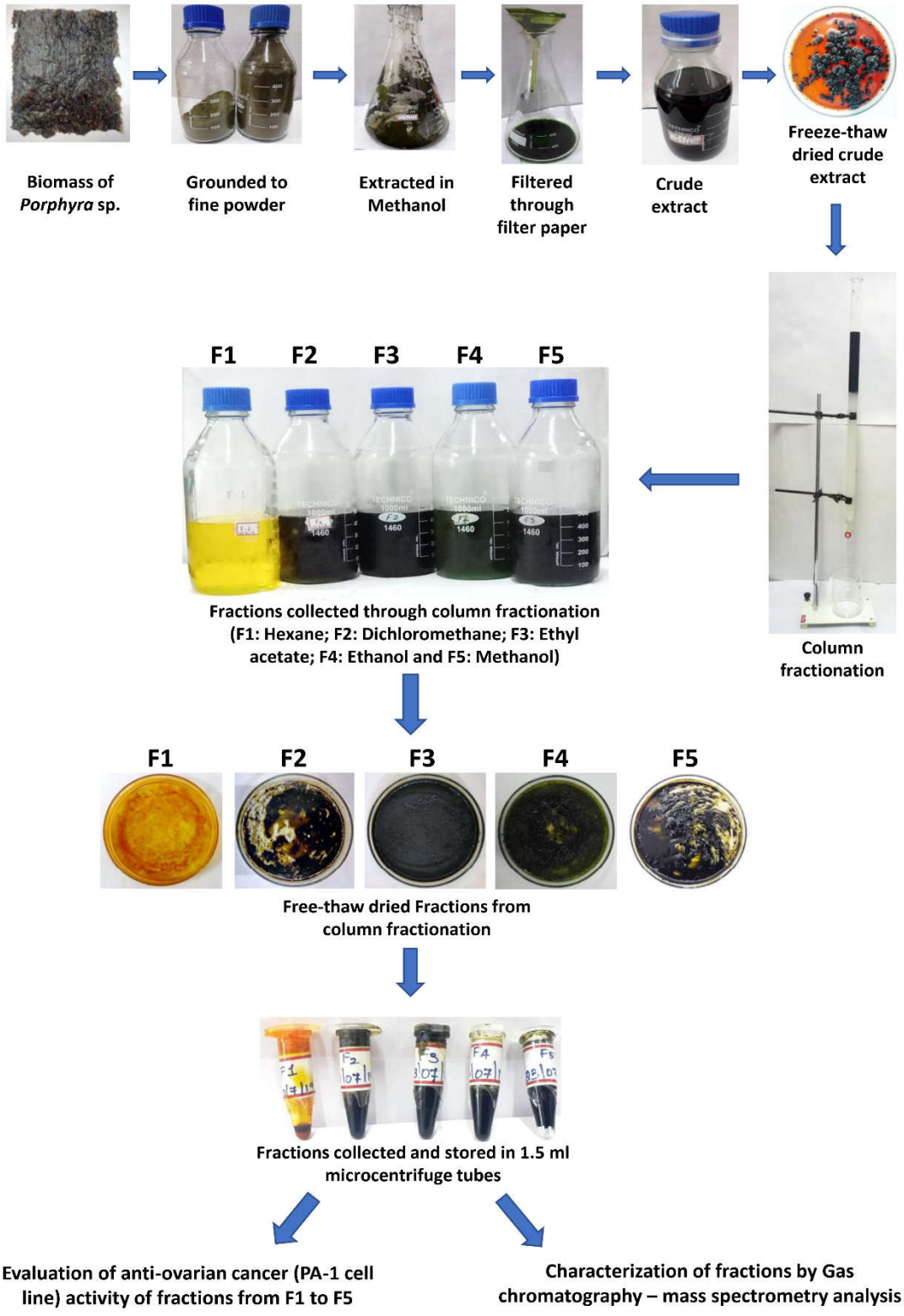

Figure 1. Flow chart illustrates the steps involved in the process of extraction and column fractionation from 'Nori' the red macroalga *Porphyra* sp.

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Table 1. Total yield of all fractions eluted from column fractionation.

S. No.	Solvents used	Fractions	Total weight (g)
1	Hexane	F1	0.031
2	Dichloromethane	F2	0.03
3	Ethyl acetate	F3	**0.436**
4	Ethanol	F4	0.35
5	Methanol	F5	**3.42**
		Total yield of all fractions	**4.267**

3.2. Anticancer activity assay

3.2.1. MTT cytotoxicity assay

All the fractions F1 to F5 derived from 'Nori' inhibited the proliferation of human ovarian tetracarcinoma cells (PA1) in a dose-dependent manner (**Fig. 2 to 4**). The cytotoxicity of the test samples was enhanced while increasing the concentration of the fraction samples. Whereas, the recorded percentage of inhibition for fraction 3 (ethyl acetate) at all three different concentrations (25, 50, and 100 µg/mL) was found greater comparatively with other fractions (**Fig. 5**). However, the calculated IC_{50} values of all the fractions were F1: 67.64 µg/mL; F2: 61.69 µg/mL; F3: 41.1 µg/mL; F4: 52.5 µg/mL; F5: 54 µg/mL; crude extract: 63.45 µg/mL; and positive control (Cisplatin): 1.86 µg/mL. Relatively, the IC_{50} value of fraction 3 (ethyl acetate) was found more effective at lower concentrations.

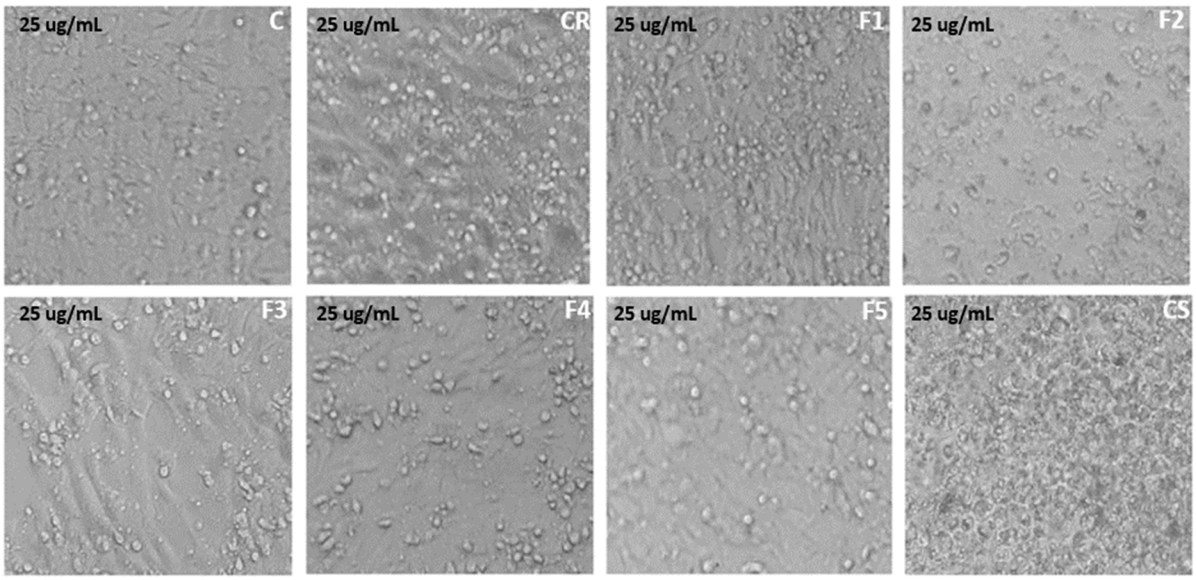

Figure 2. Microphotographs (40X magnification) of human ovarian tetracarcinoma cell treatment with test compounds at a concentration of 25 µg/ml; where, C: Negative Control; CR: Crude extract; F1: Fraction 1 (Hexane); F2: Fraction 2 (Dichloromethane); F3: Fraction 3 (Ethyl acetate); F4: Fraction 4 (Ethanol) and F5: Fraction 5 (Methanol).

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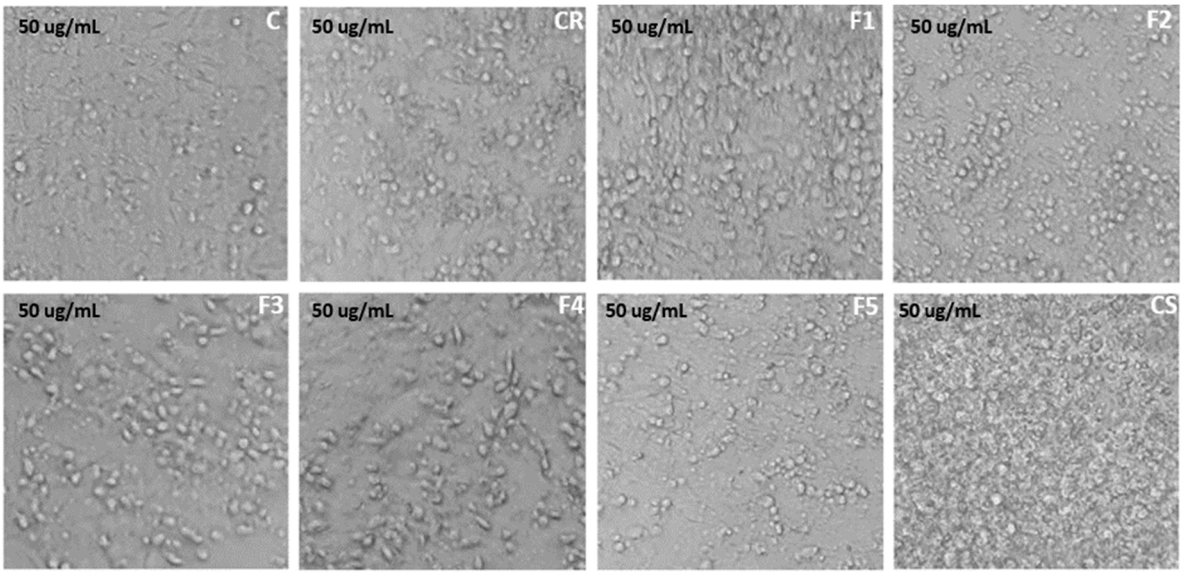

Figure 3. Microphotographs (40X magnification) of human ovarian tetracarcinoma cell treatment with test compounds at a concentration of 50 µg/ml; where, C: Negative Control; CR: Crude extract; F1: Fraction 1 (Hexane); F2: Fraction 2 (Dichloromethane); F3: Fraction 3 (Ethyl acetate); F4: Fraction 4 (Ethanol) and F5: Fraction 5 (Methanol).

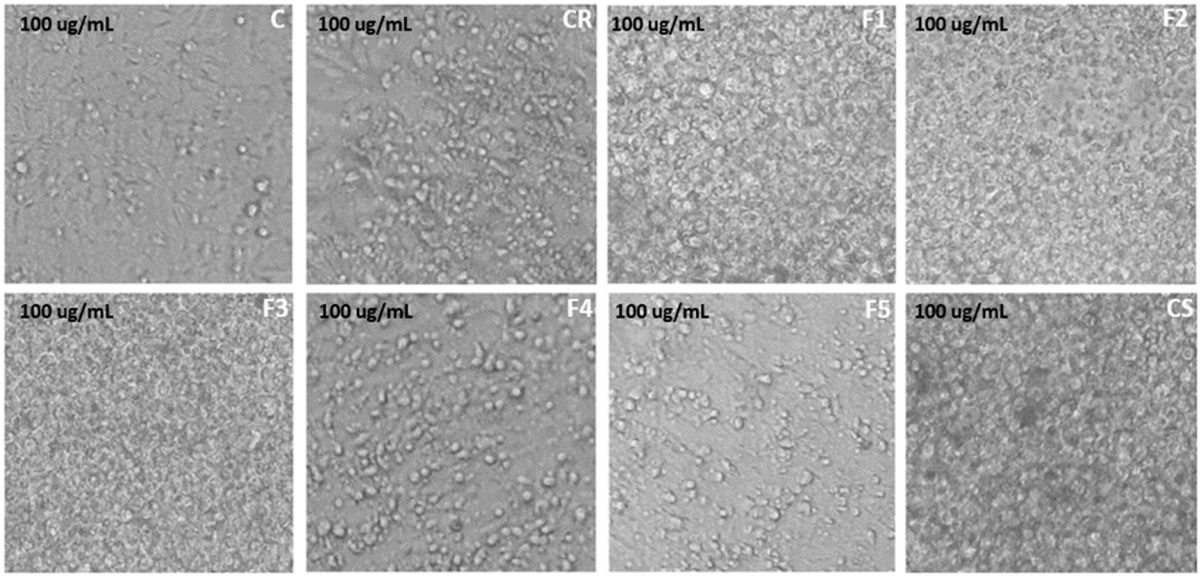

Figure 4. Microphotographs (40X magnification) of human ovarian tetracarcinoma cell treatment with test compounds at a concentration of 100 µg/ml; where, C: Negative Control; CR: Crude extract; F1: Fraction 1 (Hexane); F2: Fraction 2 (Dichloromethane); F3: Fraction 3 (Ethyl acetate); F4: Fraction 4 (Ethanol) and F5: Fraction 5 (Methanol).

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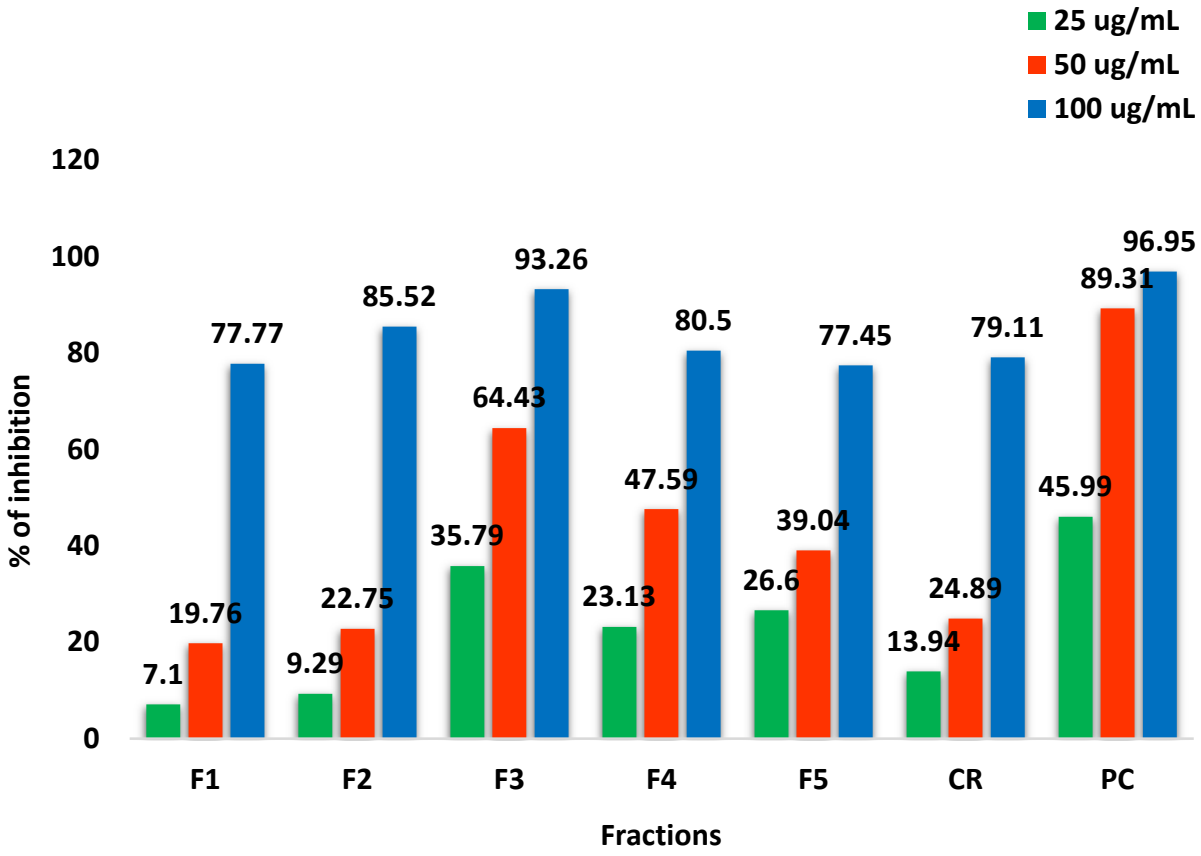

Figure 5. MTT cytotoxicity activity assay of different fractions derived from the edible seaweed 'Nori' (*Porphyra* sp.) on human ovarian tetracarcinoma cells (PA1) (F1: hexane; F2: dichloromethane; F3: ethyl acetate; F4: ethanol; and F5: methanol); where CR: crude extract; PC: positive control (Cis-platin).

3.2.2. DNA (deoxyribonucleic acid) fragmentation assay

DNA fragmentation assay was used to determine the anticancer activity of the fractions derived from 'Nori' (*Porphyra* sp.) by inducing the apoptotic or necrotic activity in the treating cancer cells. In this study, fraction 3 (ethyl acetate) showed promising anticancer activity on human ovarian tetracarcinoma cells (PA1), and thus, it was further taken for DNA fragmentation assay. The agarose gel image for DNA fragmentation assay clearly visualizes that the ovarian cancer cells underwent apoptosis (**Fig. 6**) upon treatment with fraction 3 (ethyl acetate) of the macroalga *Porphyra* sp. (Nori).

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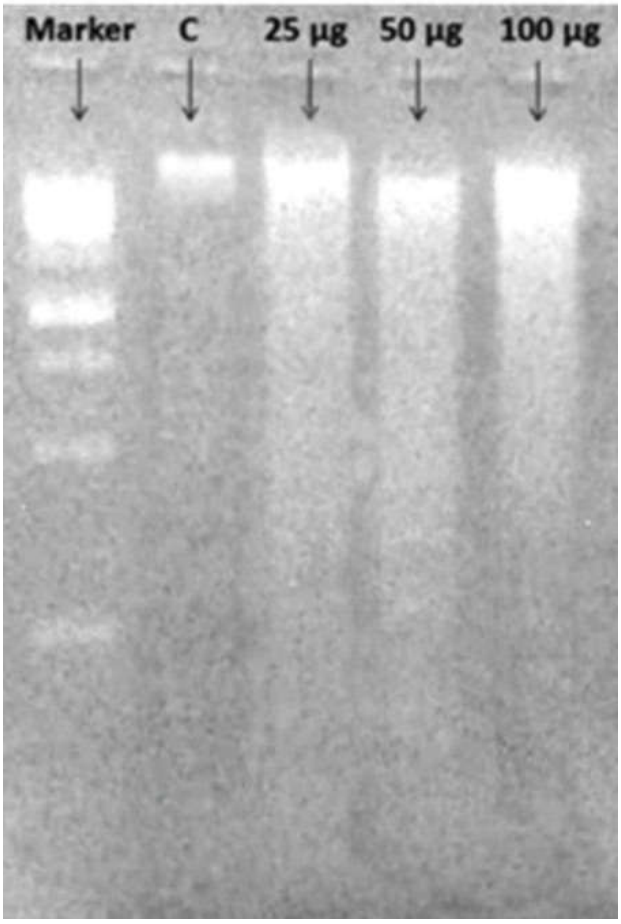

Figure 6. DNA fragmentation assay on Fraction 3 (ethyl acetate) on human ovarian tetracarcinoma cancer cells showing fragmented DNA smear in lanes 3 (25 µg/ml), 4 (50 µg/ml) and 5 (100 µg/ml), where lane 1 and lane 2 were DNA marker and Control respectively.

3.3. Gas chromatography-mass spectrometry (GC-MS) analysis

Based on the GC-MS analysis of fraction 3 (ethyl acetate) derived from the edible seaweed 'Nori' (*Porphyra* sp.), three different compounds were identified, which are Trans-traumatic acid at RT: 27.94 with a molecular weight of 228.28 Da and chemical formula $C_{12}H_{20}O_4$, 1-naphthalene propanal, alpha-ethyldecahydro-5-(hydroxymethyl)- at RT: 28.48 with a chemical formula of $C_{20}H_{36}O_2$ and molecular mass of 308 Da and Tatridin B ($C_{15}H_{20}O_4$) (RT: 29.47) with a molecular mass of 264.32 Da (**Fig. 7 to 10**). Tatridin B is found in resemblance with 3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene- with similar structure (**Fig. 11**).

Trans-traumatic acid is a monounsaturated straight chain with a dicarboxylic acid, it is a common plant-wound healing hormone found in land plants (NCBI, 2022). However, 1-naphthalene propanal, alpha-ethyldecahydro-5-(hydroxymethyl)- reported possessing antimicrobial, anti-inflammatory, and antioxidant properties (Sulthanabegum et al., 2019). Tatridin B (3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-) is an oxygenated germacrane sesquiterpenoid found in land plants reported for its inhibition of nuclear receptor binding SET domain protein 2 (NSD2) (human), and nuclear factor kappa B (NFkB1 and NFkB2) (human), and RELA – proto-oncogene (human) (National Center for Biotechnology Information, 2022b, 2022a) (**Table 2**).

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Table 2. Biological assay results of Tatridin B or 3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene- obtained from PubChem, NCBI.

S. No.	Chemical Name	Chemical formula	Molecular weight (Da)	Substance ID	BioAssay ID	Bioassay Name	Target Name	Gene ID	Activity	Activity value (uM)
1	3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-	$C_{15}H_{20}O_4$	264.32	363679980	1645877	Primary qHTS for inhibitors of nuclear receptor binding SET domain protein 2 (NSD2) in an RCH-ACV wild type WT cells (2C)	NSD2 - nuclear receptor binding SET domain protein 2 (human)	7468	Active	16.29
2	3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-	$C_{15}H_{20}O_4$	264.32	363679980	1645876	Primary qHTS for inhibitors of nuclear receptor binding SET domain protein 2 (NSD2) in an isogenic RCH-ACV NSD2 p.E1099K mutant (9B)	NSD2 - nuclear receptor binding SET domain protein 2 (human)	7468	Active	18.28
3	3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-	$C_{15}H_{20}O_4$	264.32	363679980	1345084	Primary qHTS to identify gynecologic anti-cancer compounds using libraries of approved drugs and bioactive compounds			Inactive	
4	3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-	$C_{15}H_{20}O_4$	264.32	363679980	1296009	qHTS assay to identify novel small-molecules to repurpose for Merkel cell			Unspecified	

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						carcinoma treatment			
5	Tatridin B	$C_{15}H_{20}O_4$	264.32	103458631	242927	Concentration required for complete inhibition of Nuclear factor kappa B DNA binding	NFKB1 - nuclear factor kappa B subunit 1 (human)	4790	Unspecified
6	Tatridin B	$C_{15}H_{20}O_4$	264.32	103458631	242927	Concentration required for complete inhibition of Nuclear factor kappa B DNA binding	NFKB2 - nuclear factor kappa B subunit 2 (human)	4791	Unspecified
7	Tatridin B	$C_{15}H_{20}O_4$	264.32	103458631	262798	Inhibition of NF-kappaB DNA binding by EMSA	RELA - RELA proto-oncogene, NF-kB subunit (human)	5790	Unspecified
8	Tatridin B	$C_{15}H_{20}O_4$	264.32	103458631	243231	Concentration required for complete inhibition of Nuclear factor kappa B DNA binding	NFKB2 - nuclear factor kappa B subunit 2 (human)	4791	Unspecified

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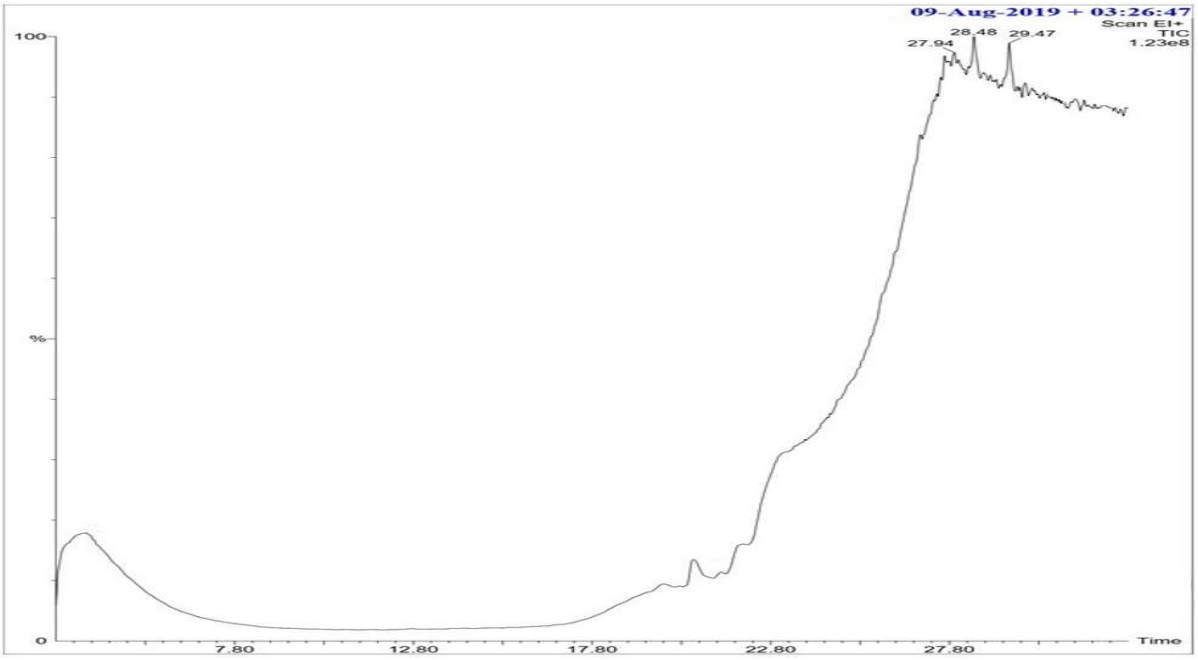

Figure 7. Gas chromatogram of the Fraction 3 (F3) of *Porphyra* sp. in Ethyl acetate.

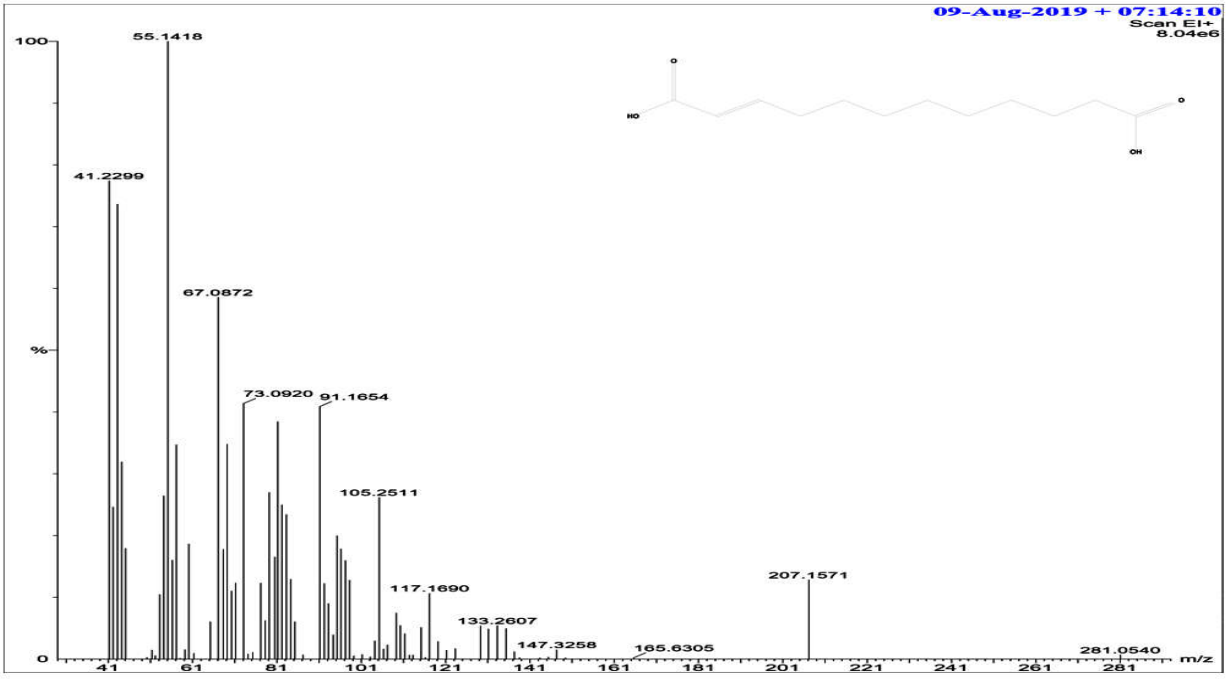

Figure 8. Gas chromatography – mass spectrum of Retention time RT: 27.94 (Trans-traumatic acid) of Fraction 3 (F3) of *Porphyra* sp. in Ethyl acetate.

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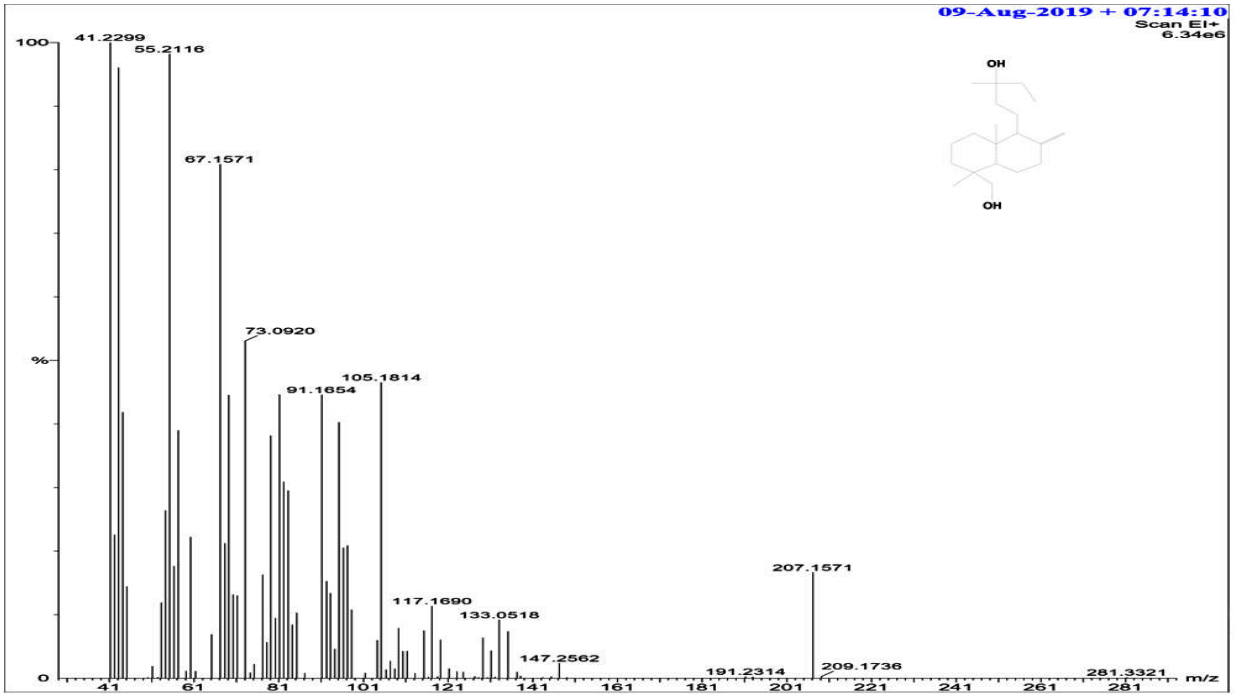

Figure 9. Gas chromatography – mass spectrum of Retention time RT: 28.48 (1-naphthalene propanal, alpha.-ethyldecahydro-5-(hydroxymethyl)-) of Fraction 3 (F3) of *Porphyra* sp. in Ethyl acetate.

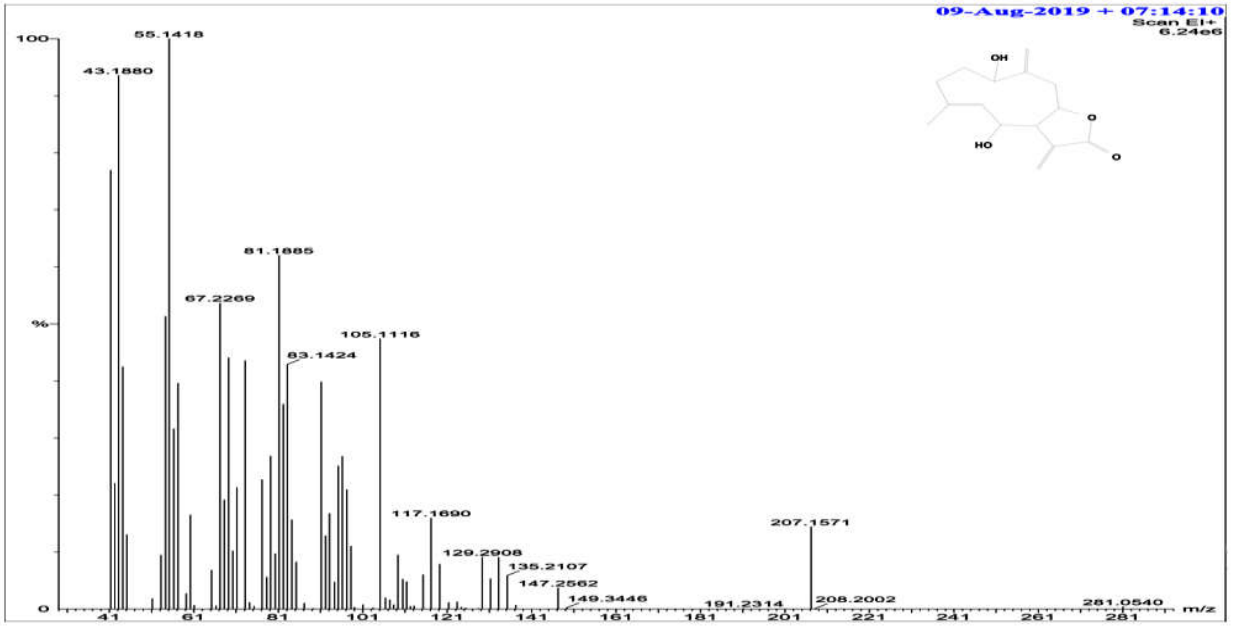

Figure 10. Gas chromatography – mass spectrum of Retention time RT: 29.47 Tatridin B or (3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-) of Fraction 3 (F3) of *Porphyra* sp. in Ethyl acetate.

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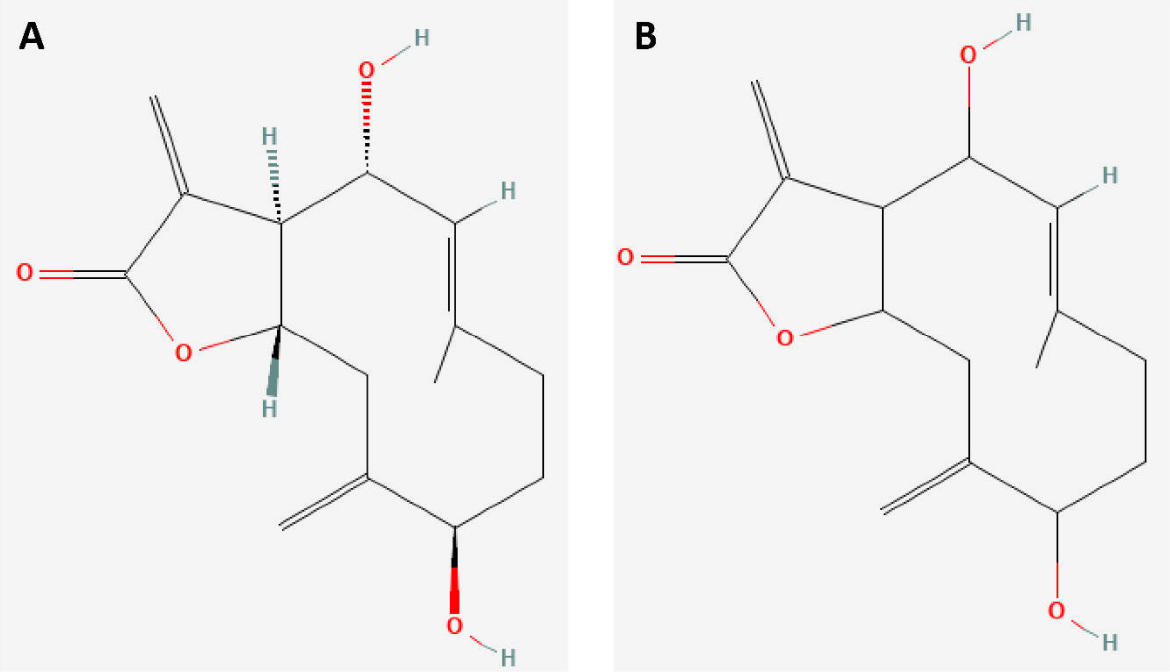

Figure 11. Structural similarity between A) Tatridin B and B) 3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-.

4. Discussion

The 'Nori' or 'Wakame' is an edible form of seaweed obtained commercially either press-dried in sheets or flakes most commonly used to wrap a favourite cuisine 'Sushi' in Japan (Baweja et al., 2016). 'Nori' is a thallophytic red seaweed named *Porphyra yezoensis* purple in colour rich in vitamins A, B12, and C (Hamid et al., 2015), eicosapentaenoic acid, choline, inositol, and minerals include Zn, Mn, Se, and Cu (Noda, 1993). In our present investigation, to valorize the anticancer potential of 'Nori', it was obtained commercially from Taipei, Taiwan, and evaluated for the anticancer bioactive compounds.

Solvent extraction is an important chemical process of extracting bioactive metabolites from biomass, which involves non-polar and polar organic solvents. Hexane, petroleum ether, diethyl ether, ethanol, isopropanol, n-heptane, acetone, chloroform, methanol, and 1-butanol are some of the common organic solvents used for the extraction of volatile compounds (Miazek et al., 2017). Whereas, the polarity of the solvent plays a significant role in extracting a wide range of compounds, in which, non-polar to low-polar solvents extract a greater number of compounds than polar or high-polar solvents (Zarrinmehr et al., 2022). However, in this study, fractionation was performed by using different polar solvents ranging from low to high polarities such as hexane, dichloromethane, ethyl acetate, ethanol, and methanol, and marked as fractions F1 to F5 respectively. The total crude extract obtained from 'Nori' was 9.08 % of its dry biomass and among different solvent fractionations, F3 yielded about 10.21 % of the obtained crude extract, ranked next to F5 (80.14 %).

Marine seaweeds are rich sources of polysaccharides, such as laminarin, alginate, and fucoidan in brown seaweeds, ulvan, and rhamnan in green seaweeds, agar, galactan, and carrageenan in red seaweeds (Alba & Kontogiorgos, 2019). Porphyran is a sulphated polysaccharide found in *Porphyra sp*. (Nori) reported with antitumor, anti-inflammatory, immunomodulatory, antihyperlipidemic, and hyper-cholesterolemia activities (Ueno & Oda, 2020). Though polysaccharides from marine seaweeds are reported with a wide range of bioactivities, other constituents like polyphenols (phlorotannin, eckol, dieckol, phloroglucinol), polyunsaturated fatty acids (PUFA), flavonoids, and alkaloids have attracted much attention in the recent decades due to their antioxidant, antidiabetic (Park et al., 2012), and anticancer (Wang et al., 2019) bioactivities. Bisindole alkaloids, terpenes,

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sesquiterpenoid lactone, and diterpenoids from green seaweeds have promising anti-tumor activity by suppressing tumor-inducing molecular markers (Pradhan et al., 2020; Tanna et al., 2020). Fucoidan arrests the cell growth cycle and enhances apoptosis by inhibiting VEGF (vascular endothelial growth factor) and natural killer cell activation (Ganesan et al., 2019; Jin et al., 2022). Similarly, laminarin, dieckol, and phloroglucinol enhance the apoptosis signal pathways in colon cancer (Weinberg, 2013), pancreatic cancer (Xu et al., 2021), and breast cancer (Hoadley et al., 2018) respectively. Phlorotannin inhibits the proliferation of liver and leukemia cancer (Lee et al., 2021). In an interesting study, diterpenoids isolated from *Dictyota ligulata* inhibit doxorubicin-resistant leukemic cancer (IC_{50}: 5.95 to 12.9 μM) (Bouaïcha et al., 1993).

In this present investigation, a dose-dependent cytotoxic activity was obtained in all the fractions obtained from 'Nori' F1 to F5 on human ovarian tetracarcinoma cells (PA1). Moreover, the percentage of inhibition of ovarian cancer cell proliferation was obtained higher in F3 (ethyl acetate) than in other fractions. Simultaneously, the IC_{50} value for the respective fraction F3 was 41.1 μg/mL, showing greater activity at lower concentrations than that of other fractions. DNA fragmentation study also confirms the apoptotic inhibition of human ovarian tetracarcinoma cells by the ethyl acetate fraction. The polysaccharide extract of *Porphyra haitanensis* showed in vitro anticancer activity on HT-29, LoVo, and SW-480 cancer cell lines with IC_{50} values of 664.4, 825.3, and 862.2 μg/mL respectively (Yao et al., 2020). Galactan isolated from *Pyropia yezoensis* (Porphyra) had induced apoptotic activity in DU145 prostate cancer cell line by modulating the PI3K/AKT/mTOR signaling pathway (Pham et al., 2021). Moreover, epicatechin reported in edible 'Nori' (*Porphyra tenera*) (Machu et al., 2015) inhibits the proliferation of breast cancer (MDA-MB-231, and MCF-7) and induces apoptosis (Bad and Bax) with an IC_{50} value of 350 μM (Pereyra-Vergara et al., 2020).

However, Tatridin B (3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-) is a key compound responsible for the anticancer effect on human ovarian tetracarcinoma cells (PA1) in this study. Tatridin B ($C_{15}H_{20}O_4$) is a germacrane sesquiterpene lactone reported for the first time in the edible seaweed 'Nori' (*Porphyra* sp.) with potent cytotoxic efficacy against human ovarian tetracarcinoma cells (PA1). Sesquiterpene lactones are natural metabolites with 5000 different structures and their derivatives are renowned for their potential inhibition of the NF-kB (nuclear factor-kB) pathway (babaei et al., 2018; Castro et al., 2000; Rüngeler et al., 1999). Sesquiterpene lactones have a wide range of bioactivities including anti-inflammatory, antimigraine, antifeedants, antitumor, antiulcer, and cardiotonic activities (Shoaib et al., 2017). Intriguingly, the Tatridin B (3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-) was found as an active inhibitor of nuclear receptor binding SET domain protein 2 (N2SD2) (National Center for Biotechnology Information, 2022b, 2022a).

NSD2 gene codes for Histone methyltransferase which di-methylates nucleosomal histone H3 at 'Lys-36' (H3K36me2). Despite aberrant upregulation of H3K36me2 leads to overexpression of NSD2 or point mutation in several types of cancers (Sato et al., 2021). NSD2 is recently determined as an important molecular marker for the progression of tumors (oncogene activation) in several types of cancer (Lhoumaud et al., 2019). Based on several investigations, NSD2 is uncontrollably expressed in aggressive solid tumors, including renal cancer, breast cancer, cervical cancer, prostate cancer, and osteosarcoma, and mainly involves in cancer cell proliferation, metastasis, and invasion (Chen et al., 2020). NSD2 progressively accumulated and mediates metastasis in lethal prostate cancer *in vivo* (Aytes et al., 2018). NSD2 dimethylation at H3K36me2 promotes robust malignant tumor progression of lung adenocarcinoma *in vivo* (Sengupta et al., 2021); oral squamous cell carcinoma (L. Zhang & Hu, 2022); breast cancer (Gao et al., 2021). Zhao et al. proposed histone methyltransferase NSD2 as an important gene marker for the progression of colorectal cancer (Zhao et al., 2021). NSD2 inhibitor plays a vital role in the treatment of multiple myeloma (Liu et al., 2021).

Most recent oncology reports clearly highlight the overexpression of NSD2 in the tumor progression and metastasis of cancer, and thus, NSD2 inhibitors are being proposed

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for the treatment of tumor progression in chemotherapy of cancer. As a result of our present study, a natural sesquiterpene lactone Tatridin B (3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-) from 'Nori' (*Porphyra* sp.) is an active inhibitor of NSD2, showed a potent activity on human ovarian tetracarcinoma cells (PA1) and hence, may be considered as a future chemotherapeutic drug for cancer therapy.

5. Conclusion

In our present study, a renowned edible seaweed 'Nori' (Porphyra sp.) was extracted and fractionated with different solvents, and subjected to evaluate the MTT cytotoxic activity on human ovarian tetracarcinoma cells (PA1). All the fractions showed anticancer activity in a dose-dependent manner, however, among different fractions, the F3 (ethyl acetate) fraction was found effective even at low concentrations with an IC_{50} value of 41.1 µg/mL. Based on the DNA fragmentation assay, the anticancer activity was due to apoptosis. The key compound responsible for the anticancer activity was determined as Tatridin B (3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-), as sesquiterpene lactone. Most commonly, sesquiterpene lactones are inhibitors of NF-kB, moreover, this specific compound was found active in inhibiting NSD2 (nuclear receptor binding SET domain protein 2) an important marker in cancer therapy. Therefore, this is a *hitherto* report on the presence of Tatridin B (3h-cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-) in 'Nori' (*Porphyra* sp.) showing potent anticancer activity on human ovarian tetracarcinoma (PA1).

Conflicts of Interest: The authors declare that there is no conflict of interest.

Contribution by authors: This is an M.Phil. dissertation work of Mrs. Saraswathi, guided by Rajesh Kanna Gopal, and Preethy P. Raj, under the supervision of Prof. S. Elumalai, and Dr. A. Umamaheswari. The manuscript was drafted by RKG, and PPR was checked and corrected by SE and AU.

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