Phytochemical Profiling, Structural Characterization, And Cytotoxic Evaluation Of Bioactive Constituents from *Padina Tetrastromatica*

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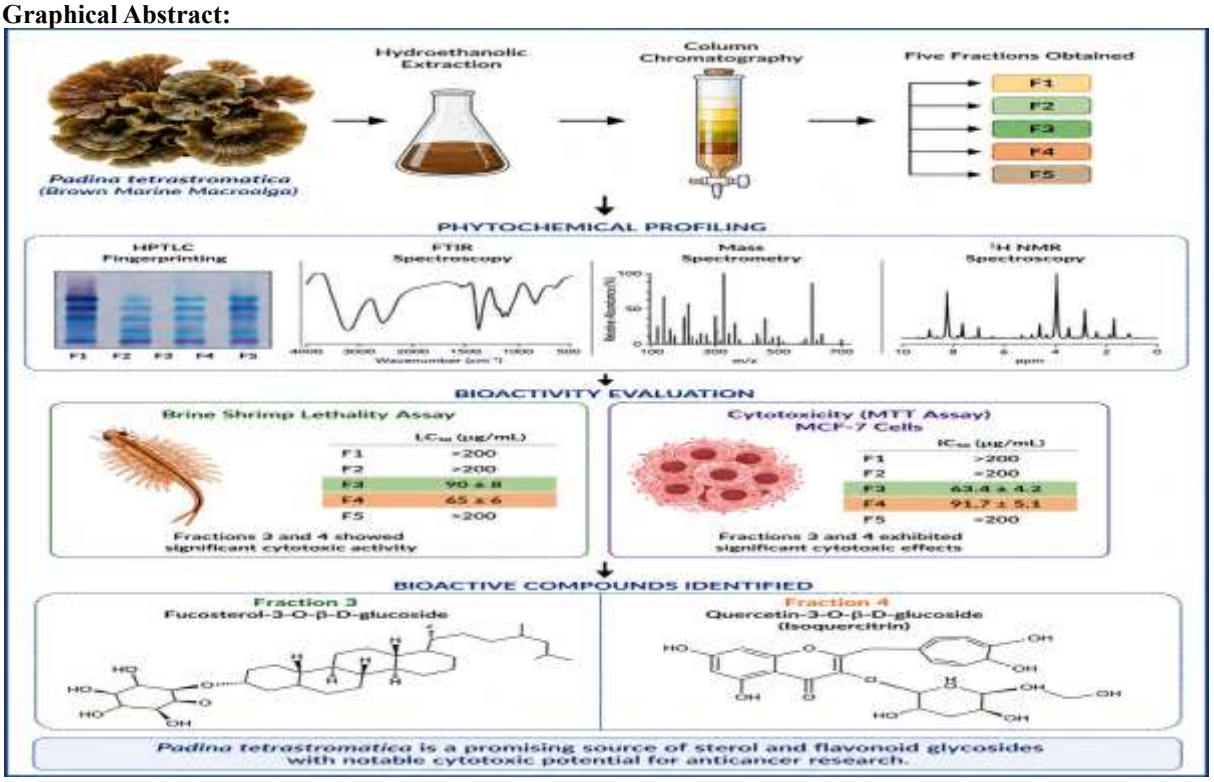
Abstract

Padina tetrastromatica, a brown marine macroalga, is a rich source of bioactive metabolites with potential pharmaceutical applications. The present study aimed to investigate the phytochemical profile, structural characteristics, and *in vitro* cytotoxic potential of the hydroethanolic extract and chromatographic fractions of *P. tetrastromatica*. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, steroids, terpenoids, and carbohydrates. Column chromatography yielded five fractions (F1–F5), which were further characterized using HPTLC, FTIR, Mass Spectrometry, and ^{1}H NMR analyses. Brine shrimp lethality assay demonstrated significant concentration-dependent cytotoxic activity in Fractions 3 and 4, with LC_{50} values of 90 µg/mL and 65 µg/mL, respectively. HPTLC fingerprinting and spectroscopic analyses indicated the presence of sterol glycosides and flavonoid glycosides in the bioactive fractions. Structural elucidation identified Fraction 3 as Fucosterol-3-O-β-D-glucoside and Fraction 4 as Quercetin-3-O-β-D-glucoside (Isoquercitrin). MTT assay against MCF-7 human breast cancer cells revealed significant cytotoxic effects, with IC_{50} values of 63.4 µg/mL and 91.7 µg/mL for Fractions 3 and 4, respectively. These findings demonstrate that *P. tetrastromatica* possesses notable cytotoxic potential and represents a promising source of marine-derived bioactive compounds for anticancer research.

KEYWORDS: *Padina tetrastromatica*, HPTLC Fingerprinting, Fucosterol Glycoside, Quercetin-3-O-glucoside, *In Vitro* Cytotoxicity

Graphical Abstract:



1. Introduction:

Marine macroalgae have been recognized as rich sources of bioactive metabolites and phytoconstituents with significant pharmaceutical potential due to their diverse biological activities, including antioxidant, antimicrobial,

antiviral, antidiabetic, and anticancer properties (1). In addition to their ecological importance, marine algae have been widely utilized in healthcare, nutraceutical, and industrial applications, and are broadly classified into red, brown, and green algae(2). Their potent antioxidant properties have attracted considerable scientific interest because of their potential role in combating oxidative stress-associated disorders.

The emergence of drug-resistant microorganisms and increasing prevalence of chronic and metabolic diseases have intensified the search for novel therapeutic agents from natural sources. Marine macroalgae have gained considerable attention owing to their chemical diversity and broad-spectrum biological activities. Among these, *Padina tetrastromatica*, a brown marine alga, has demonstrated notable pharmacological potential due to the presence of diverse bioactive constituents such as phlorotannin's, flavonoids, sulphated polysaccharides, and other secondary metabolites(3)(4). Previous studies have reported that solvent extracts of *P. tetrastromatica* exhibited significant antioxidant activity, α-amylase inhibitory potential, antimicrobial effects against pathogenic microorganisms, antihyperglycemic activity in streptozotocin-induced diabetic models, cardioprotective effects through PI3K/Akt/Nrf2 signalling pathways, and cytotoxic activity against cancer cell lines including MCF-7 and A549 (4–7).

Figure 1: ***Padina Tetrastromatica***

Marine seaweeds have also been recognized as sustainable functional food resources that support metabolic health through the presence of nutritionally and pharmacologically important compounds. Bioactive polysaccharides present in *P. tetrastromatica* have been associated with glycemic control, further supporting its therapeutic relevance in metabolic disorders. Despite the growing evidence supporting the pharmacological importance of marine macroalgae, systematic investigation correlating their phytochemical composition with functional biological activities remains limited. Moreover, the pharmacological potential of several marine algal species remains insufficiently explored, highlighting the need for focused investigation to identify novel bioactive compounds with therapeutic applications(8),(9),(10). Assessment of the phytochemical composition and antioxidant potential of marine macroalgae is crucial for understanding their biological and therapeutic significance; moreover, it enables the exploration of their applications in pharmaceutical, nutritional, and environmental domains(8). To address these research gaps and further elucidate the phytochemical profile of *P. tetrastromatica*, an integrated analytical approach was employed in the present study. High-performance thin-layer chromatography (HPTLC) was used for rapid phytochemical fingerprinting and comparative analysis of major constituents(11). Sequential column chromatography enabled fractionation of crude extracts and enrichment of bioactive fractions for downstream testing(12). Selected fractions were profiled by HPLC and GC–MS to identify and quantify volatile and non-volatile metabolites(13). Structural characterization of isolated compounds was performed using FTIR and NMR spectroscopy to confirm functional groups and molecular frameworks(14). Chromatographic separations were guided by bioassays (antioxidant, cytotoxicity) to link specific constituents with observed biological activities(15). These integrated analytical approaches permit correlation of phytochemical composition with functional properties, facilitating identification of candidate therapeutic compounds from P. tetrastromatica.

2.Materials and methods:

2.1. Collection & Authentication

The marine macroalga *Padina tetrastromatica* was collected from Colaba, Mumbai, and subsequently authenticated by the Department of Biotechnology, RJSPM College of Pharmacy, Landewadi, Pune.

2.2. Extraction

Dried thalli of *Padina tetrastromatica* were powdered and extracted by maceration with hydroethanolic solvent (ethanol, 70:30 v/v) at room temperature for 24 h. The extract was filtered, re-extracted, and the combined filtrates were concentrated to obtain the crude extract(16–21).

2.3. Preliminary Phytochemical Screening

The obtained hydroethanolic filtrate of *Padina tetrastromatica* was subjected to phytochemical screening for alkaloids, flavonoids, phenols, steroids, terpenoids, and carbohydrates using standard qualitative tests(8).

2.4. Thin Layer Chromatography
Thin layer chromatography (TLC) was performed on the hydroethanolic extract of *Padina tetrastromatica*. The extract was applied onto TLC plates and developed using n-hexane acetate (7.8:1.2:1.0) as the mobile phase. The developed chromatograms were visualized under daylight, and Rf values were recorded for phytochemical characterization(22–24).

2.5. Column Chromatography
Column chromatography was performed using silica gel (60–120 mesh) as the stationary phase. The hydroethanolic extract of *Padina tetrastromatica* was loaded onto the column and eluted with n-hexane acetate (7.8:1.2:1.0) at a flow rate of approximately one drop per second. Five fractions (F1–F5) were collected and subjected to further phytochemical analysis(25).

2.6 The Brine Shrimp Lethality Assay
The brine shrimp lethality assay was performed using *Artemia salina* nauplii. The extract and fractions were tested at concentrations of 5, 10, 25, 50, 100, 250, and 500 µg/mL. Mortality was recorded after incubation and calculated as:

% Mortality = (Dead Nauplii / Total Nauplii) × 100

LC_{50} values were determined from the concentration–mortality curve(26)

2.7.High Performance Thin Layer Chromatography
HPTLC analysis was performed using a CAMAG TLC Scanner 3 on silica gel 60 F_{254} plates. The fractions were applied as bands and developed using n-hexane acetate as the mobile phase. The developed plates were visualized under UV light at 366 nm, and densitometric scanning was performed to obtain Rf values and peak profiles for phytochemical fingerprinting(27,28).

2.8. Fourier Transform Infrared Spectroscopy
FTIR analysis of the fractions obtained from *Padina tetrastromatica* was performed for functional group characterization. Spectra were recorded and analyzed to identify characteristic absorption bands corresponding to hydroxyl (O–H), carbonyl (C=O), aromatic (C=C), and carbon–oxygen (C–O) functional groups present in the fractions(29,30).

2.9.Nuclear Magnetic Resonance
^{1}H NMR spectroscopy was performed on the isolated fractions of *Padina tetrastromatica* for structural characterization. The spectra were analyzed based on chemical shift values, signal multiplicity, and integration patterns to elucidate the proton environment and molecular structure of the bioactive constituents(31).

2.10.Mass Spectroscopy
Mass spectrometric analysis was performed on the isolated fractions of *Padina tetrastromatica* for structural characterization. The mass spectra were analyzed to determine the molecular weights and fragmentation patterns of the bioactive constituents based on their mass-to-charge (m/z) ratios(32).

2.11.MTT Assay
The *in vitro* cytotoxic activity of the hydroethanolic extract of *Padina tetrastromatica* against MCF-7 cells was evaluated using the MTT assay. Cells were treated with different concentrations of the extract, and absorbance was measured at 570 nm. Cell viability was calculated as:

% Cell Viability = (Absorbance of Test / Absorbance of Control) × 100

The IC_{50} value was determined from the concentration–response curve(33).

3.Result and discussion:
3.1. Extraction
The hydroethanolic solvent system provided efficient extraction of phytoconstituents from *Padina tetrastromatica*(34–36). Table 3.1. shows extraction characteristics of the hydroethanolic extract of *Padina tetrastromatica.* The hydroethanolic extract showed a high extraction recovery of 90%, indicating efficient extraction of phytoconstituents from the algal material. The extract was dark blackish-green in appearance, suggesting the presence of carotenoids, polyphenols, and other bioactive compounds characteristic of marine algae.

Table 3.1. Extraction Characteristics of the Hydroethanolic Extract of *P. tetrastromatica*

Parameter	Observation
Solvent System	Ethanol (70/30)
Initial Volume	100 mL
Extract Recovery	90 mL
Percentage Recovery	90%
Appearance	Dark blackish-green

3.2. Preliminary Phytochemical Screening

Preliminary phytochemical screening of the hydroethanolic extract revealed the presence of several important secondary metabolites, including alkaloids, flavonoids, phenols, steroids, terpenoids, and carbohydrates. The occurrence of these phytoconstituents indicates that the extract possesses a diverse chemical profile and may contribute to various biological and pharmacological activities (Table 3.2)

Table No: 3.2. Phytochemical present in the extract

Phytochemicals	Observations
Alkaloids	Present
Flavonoids	Present
Phenols	Present
Steroids	Present
Terpenoids	Present
Carbohydrates	Present

3.3. Thin Layer Chromatography

As presented in Table 3.3, thin-layer chromatographic profiling of the hydroethanolic extract showed distinct spots corresponding to phenolic compounds (Rf = 0.37) and alkaloids (Rf = 0.70). The observed Rf values suggest the presence of these phytochemical groups in the extract and demonstrate effective chromatographic separation of the detected constituents.

Table 3.3. TLC Analysis of the Hydroethanolic Extract of *Padina tetrastromatica*

Phytochemical Class	Rf Value
Phenolic Compounds	0.37
Alkaloids	0.70

3.4. Column Chromatography

As shown in Table 3.4 Column chromatography resulted in the separation of the hydroethanolic extract of *Padina tetrastromatica* into five distinct fractions (F1–F5) for subsequent phytochemical evaluation.

Table 3.4 Fractions obtained from column chromatography

Fraction	Appearance (Colour)
F1	Dark green
F2	Pale yellow
F3	Green
F4	Light green
F5	Light pale yellowish-green

3.5. The Brine Shrimp Lethality Assay

Table 3.5 summarizes the results of the brine shrimp lethality bioassay performed on the hydroethanolic extract and its chromatographic fractions. A gradual increase in mortality was observed with increasing concentrations of the test samples, indicating dose-responsive biological activity. Among the fractions, F4 showed the greatest lethality with an LC_{50} value of 65 µg/mL, while F3 and the crude extract exhibited LC_{50} values of 90 and 95 µg/mL, respectively. In contrast, F1, F2, and F5 produced relatively lower effects, with LC_{50} values exceeding 500 µg/mL. The standard reference compound displayed the highest potency, yielding an LC_{50} value of 24 µg/mL. These observations indicate that fractions F3 and F4 contain constituents with comparatively stronger cytotoxic properties.

Table 3.5 the Brine Shrimp Lethality Assay

Concentration (µg/mL)	Standard	% Mortality					
		Extract	F1	F2	F3	F4	F5
CTRL	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
5	78.42 ± 0.21***	92.47 ± 0.18*	98.08 ± 0.10	98.67 ± 0.08	94.34 ± 0.22*	92.54 ± 0.18*	97.96 ± 0.09
10	65.31 ± 0.32***	84.71 ± 0.25*	97.13 ± 0.12	97.84 ± 0.11	85.02 ± 0.31*	82.93 ± 0.27*	97.12 ± 0.11
25	49.82 ± 0.44***	73.36 ± 0.41***	95.69 ± 0.18	96.40 ± 0.15	73.37 ± 0.48**	69.21 ± 0.43**	95.68 ± 0.15
50	34.16 ± 0.57***	61.89 ± 0.58**	94.01 ± 0.22	94.84 ± 0.20	61.82 ± 0.65**	55.17 ± 0.61***	94.00 ± 0.19
100	22.08 ± 0.71***	49.10 ± 0.73***	92.58 ± 0.19	93.40 ± 0.18	48.56 ± 0.79***	40.98 ± 0.82***	92.56 ± 0.21
250	10.91 ± 0.83***	32.97 ± 0.92***	90.89 ± 0.25	91.72 ± 0.24	32.76 ± 0.96***	25.12 ± 1.04***	90.76 ± 0.24

500	4.37 ± 0.95***	19.00 ± 1.08***	89.10 ± 0.31	90.04 ± 0.29	18.80 ± 1.08***	12.02 ± 0.95***	88.97 ± 0.30
LC_{50} (µg/mL)	24	95	>500	>500	90	65	>500

3.6. High Performance Thin Layer Chromatography

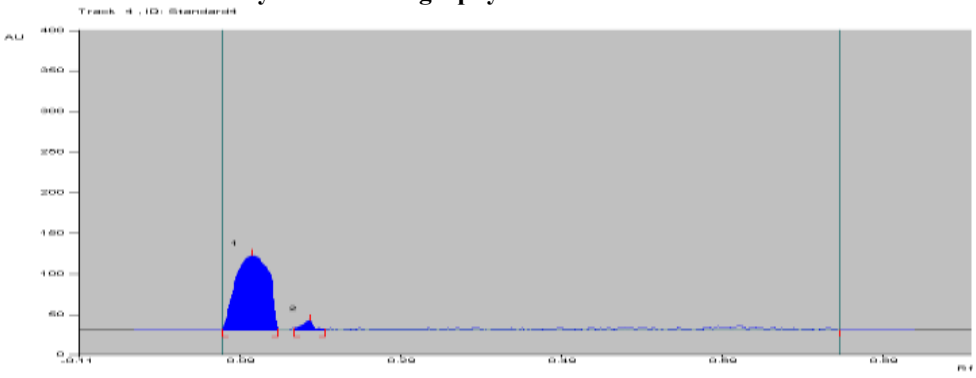


Figure 2: Densitometric Scan of Fraction 3 (F3)

Table 3.6.1. HPTLC Profile of Fraction 3 (F3)

HPTLC Rf	% Area
0.10	95.52%
0.18	4.48%

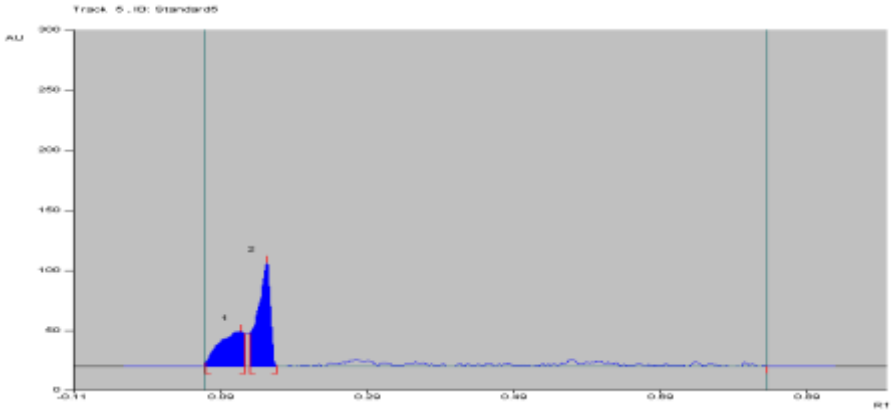


Figure No 3: Densitometric Scan of Fraction 4 (F4)

Table 3.6.2. HPTLC Profile of Fraction 4 (F3)

HPTLC Rf	% Area
0.12	40.91%
0.15	59.09%

HPTLC analysis of Fraction 3 revealed two major phytochemical groups, with phenolic compounds predominating (95.52% area) followed by terpenoids (4.48% area) (39,40) HPTLC analysis of Fraction 4 showed the presence of phenolic compounds (40.91% area) and terpenoids (59.09% area), with terpenoids representing the dominant constituent class(14,40).

3.7. Fourier Transform Infrared Spectroscopy

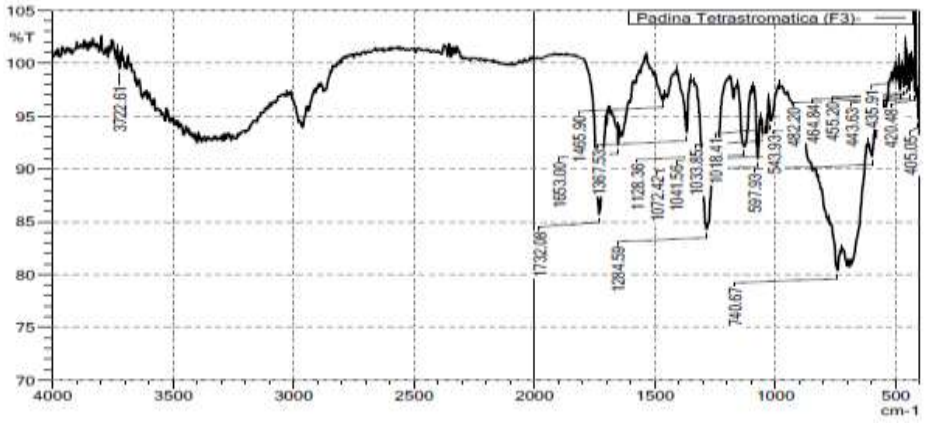


Figure No. 4. FTIR Spectra of Fraction 3

FTIR spectrum of fraction 3 of *Padina tetrastromatica* showing characteristic bands for hydroxyl, carbonyl, aromatic, carbohydrate, and sulphate/halogen-associated groups. The broad band at 3722.61 cm^{-1} was attributed to O–H stretching, while the peaks at 1732.08 cm^{-1} and 1653.00 cm^{-1} indicated C=O and C=C stretching, respectively. Bands at 1455.90 cm^{-1}, 1373.53 cm^{-1}, and 1282.89–1014.21 cm^{-1} were assigned to C–H and C–O stretching vibrations related to lipids, phenolics, sugars, and polysaccharides. Low-wavenumber peaks at 740.67 cm^{-1}, 597.93 cm^{-1}, and 543.93 cm^{-1} suggested aromatic, sulphate-associated, or halogenated constituents, while the fingerprint-region peaks reflected complex marine bioactive compounds(41,42). The FTIR spectrum of fraction 3 of *Padina tetrastromatica* indicates that the fraction is predominantly enriched in sulphated polysaccharides, most likely fucoidan, with associated phenolic marine metabolites. This interpretation is supported by the strong C-O stretching bands in the 1128.36–1014.21 cm^{-1} region, the sulphate-associated vibration at 597.93 cm^{-1}, and the O-H/aromatic bands characteristic of brown algal bioactive compounds(43,44).

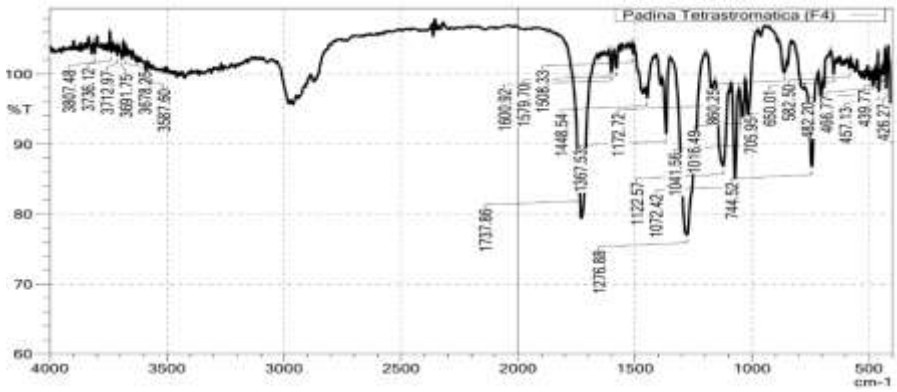


Figure No. 5 FTIR Spectra of Fraction 4

The FTIR spectrum of fraction 4 of *Padina tetrastromatica* showed characteristic absorption bands at 3807.48–3587.60 cm^{-1} for O-H stretching, 1737.86 cm^{-1} for C=O stretching, 1600.92–1508.33 cm^{-1} for aromatic/C=C and amide-related vibrations, 1448.54–1276.88 cm^{-1} for C-H and C-O stretching, and 1172.72–1016.49 cm^{-1} for C-O-C, S=O, and C-O vibrations. Low-wavenumber peaks at 860.25–426.27 cm^{-1} were assigned to aromatic bending, halogenated or sulphate-associated groups, and fingerprint-region vibrations. These features indicate the presence of phenolics, polysaccharides, proteins, lipids, sulphated constituents, and other complex marine bioactive metabolites(42,45,46).The FTIR spectrum of fraction 4 of *Padina tetrastromatica* suggests the predominant presence of sulphated polysaccharides, most likely fucoidan, along with associated phenolic compounds and minor lipid-related constituents. This interpretation is supported by the broad O-H stretching bands, strong C-O/C-O-C vibrations of polysaccharides, and the characteristic S=O band at 1172.72 cm^{-1}, which is a key marker of sulphated brown algal polysaccharides(43,47,48).

3.8 Nuclear Magnetic Resonance

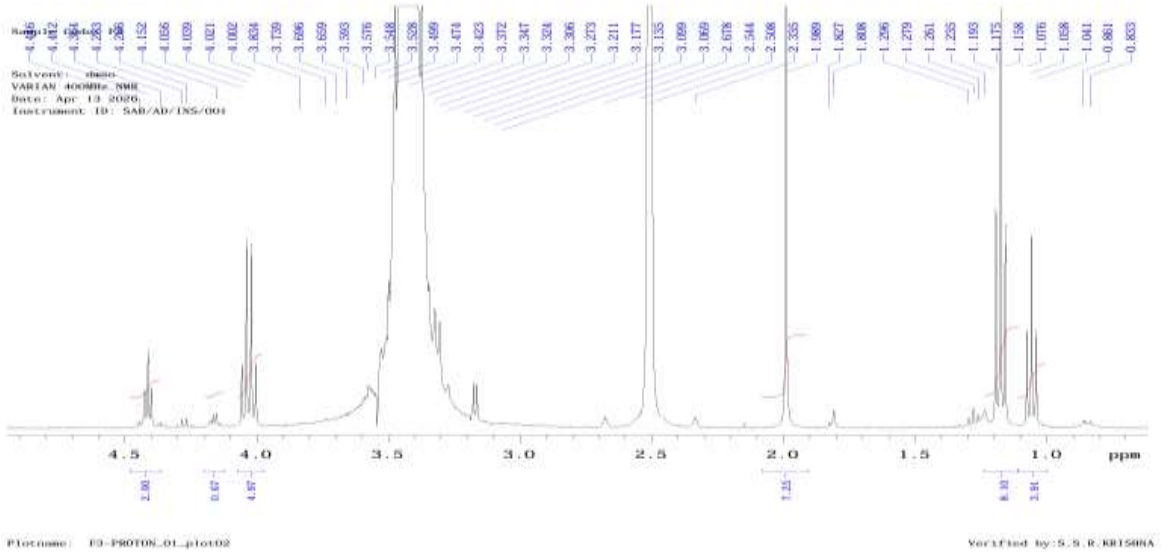


Figure No. 6. NMR Spectra of Fraction 3

(Figure No. 6 and 7) ^{1}H NMR spectrum of fraction F3 revealed prominent oxygenated proton signals in the δ 4.5–3.1 ppm region, corresponding to glycosidic or polyhydroxylated constituents, along with aliphatic methyl and methylene signals in the δ 2.0–0.8 ppm region, indicating the presence of glycosidic, oxygenated, and aliphatic phytoconstituents, possibly including saponin-like or lipid-type compounds(49).

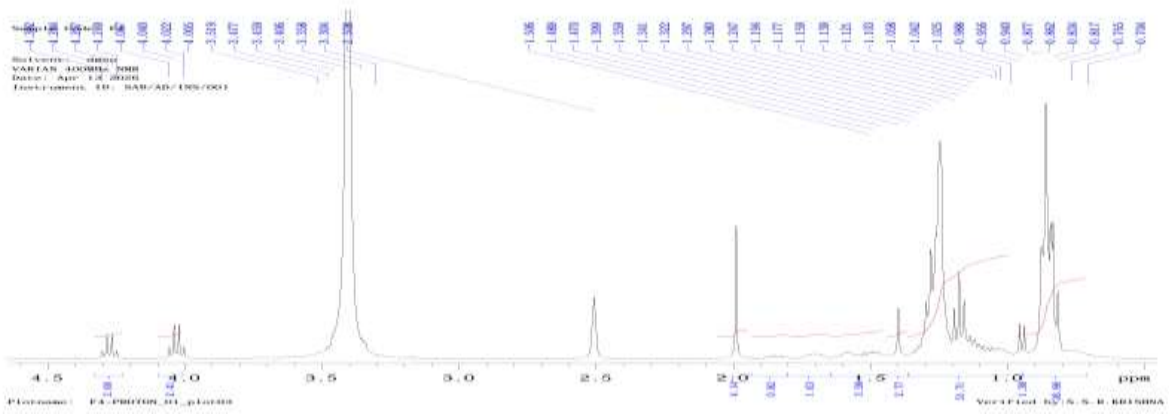


Figure No. 7. NMR Spectra of Fraction 4

The ^{1}H NMR spectrum of fraction F4 showed dominant aliphatic proton resonances in the δ 1.9–0.7 ppm region, corresponding to methylene and methyl protons of long hydrocarbon chains, along with minor oxygenated proton signals in the δ 4.4–3.3 ppm region. This pattern suggests that F4 is enriched in aliphatic phytoconstituents, likely including lipid/fatty acid- or terpenoid-type compounds, with only minor oxygenated constituents(50).

3.9.Mass Spectroscopy

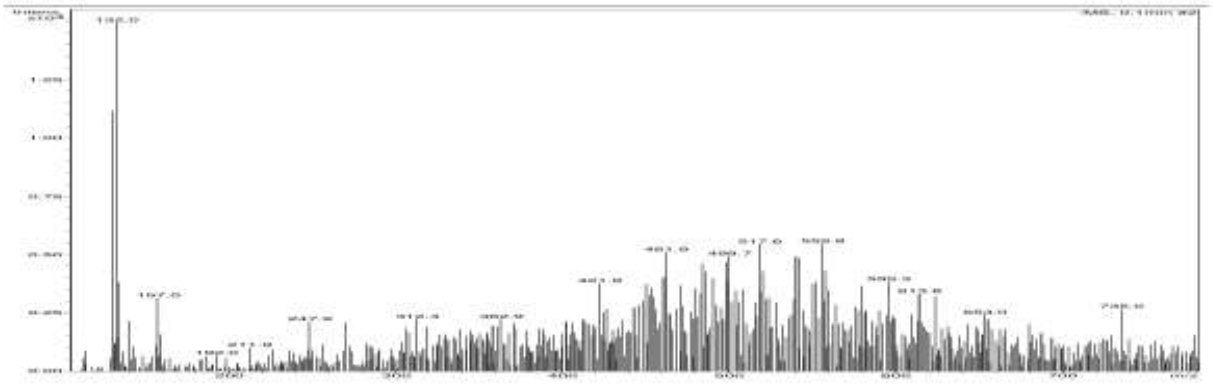


Figure No. 8. Mass Spectra Of Hydroethanolic Extract Of Padina Tetrastromatica

(Figure No. 8) The negative-ion mass spectrum of *Padina tetrastromatica* (m/z 132–735) indicates a chemically diverse extract dominated by acidic and anionic metabolites: low-mass ions (m/z 132–248) are consistent with phenolics and small organic acids or degradation fragments, mid-range ions (m/z ~312–422) are consistent with oxygenated terpenoid/glycosidic species, and high-mass ions (m/z 462–735) most likely represent complex polar lipids (glycolipids, sulfoxides) and long-chain acyl glycerides; this interpretation is supported by the prevalence of deprotonated and anionic species in negative-mode MS and by literature reports of sulphated glycolipids and diverse lipid/glycolipid profiles in marine brown algae(51–53).

3.10. MTT Assay (Figure No. 9, 10, 11 and 12)

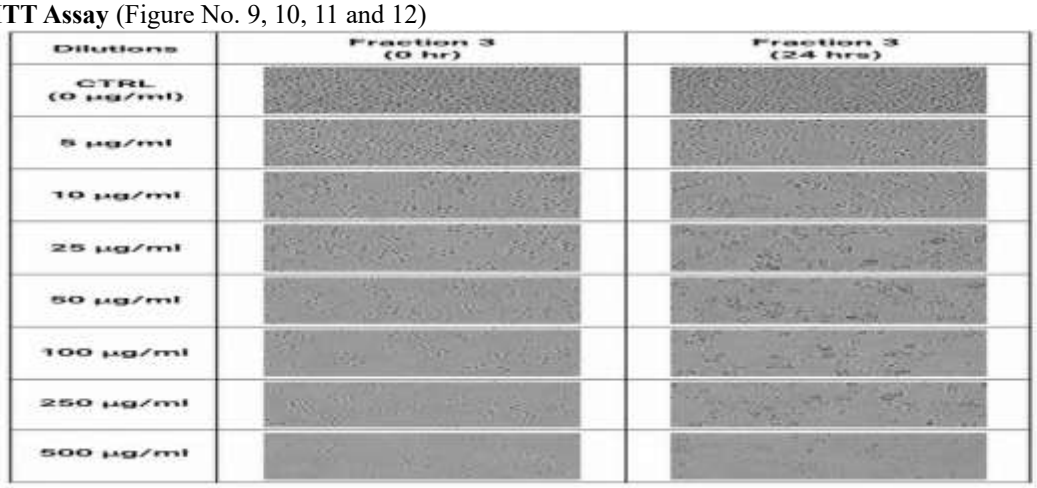


Fig. 9. Morphological Observations of Fraction 3 (MTT assay)

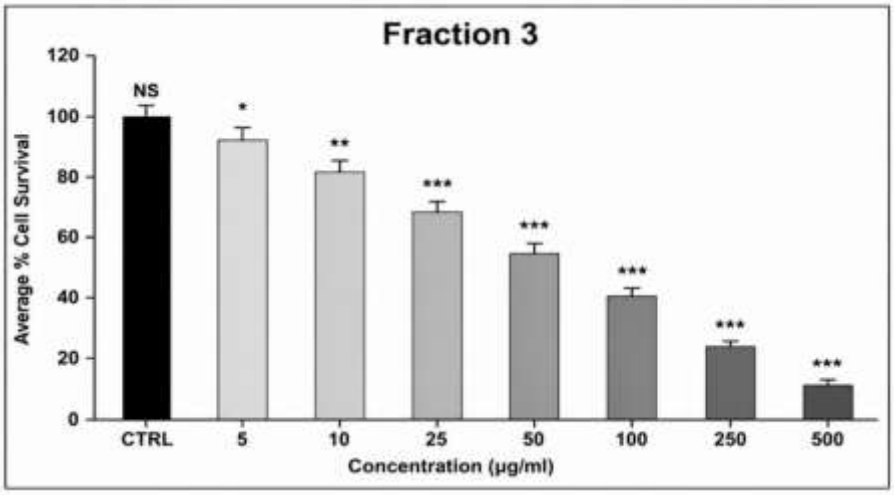


Figure 10. Graph of concentration vs % cell Viability

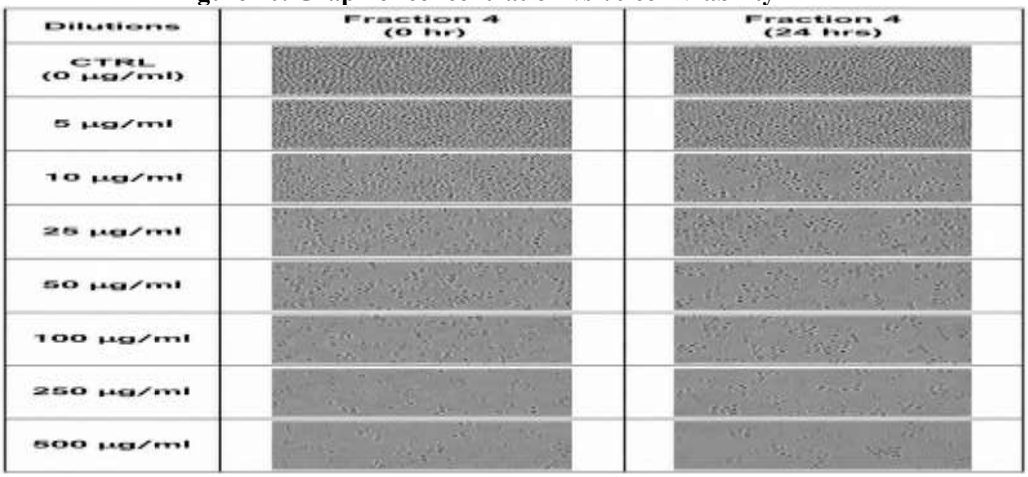


Figure 11. Morphological Observations of Fraction 4

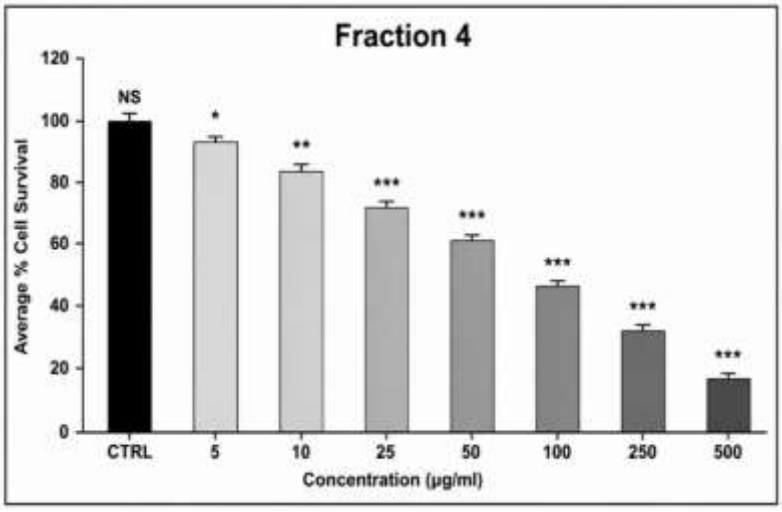


Figure 12. Graph of concentration vs % cell Viability

Fraction 3 exhibited significant cytotoxic activity against MCF-7 cells, producing a marked concentration-dependent decrease in cell viability. The IC_{50} value of 63.4 µg/mL indicates potent anticancer potential. Fraction 4 demonstrated pronounced cytotoxic activity against MCF-7 cells, with cell viability decreasing to 18.66% at 500 µg/ML. The IC_{50} value of 91.7 µg/mL confirms substantial growth inhibitory activity.

Table 3.7 Cytotoxicity Assessment of fractions against MCF-7 Cells

Conc (µg/ml)	Avg % Survival (F3)	Avg % Survival (F4)
CTRL	100.0 ± 0.00	100.0 ± 0.00
Standard	10.68 ± 0.13*	10.42 ± 0.21*
5	92.08 ± 0.08*	94.48 ± 0.12*
10	82.20 ± 0.19**	85.03 ± 0.26**
25	68.65 ± 0.41***	73.31 ± 0.55***

50	54.85 ± 0.65***	61.81 ± 0.59***
100	40.62 ± 0.79***	48.44 ± 0.71***
250	24.74 ± 1.02***	32.63 ± 0.91***
500	11.83 ± 0.92***	18.66 ± 0.87***
IC_{50} (Extract) = **63.4 µg/mL**		**IC_{50} = 91.7 µg/mL**

3.11.Chemical And Structural Elucidation (Figure No. 13)

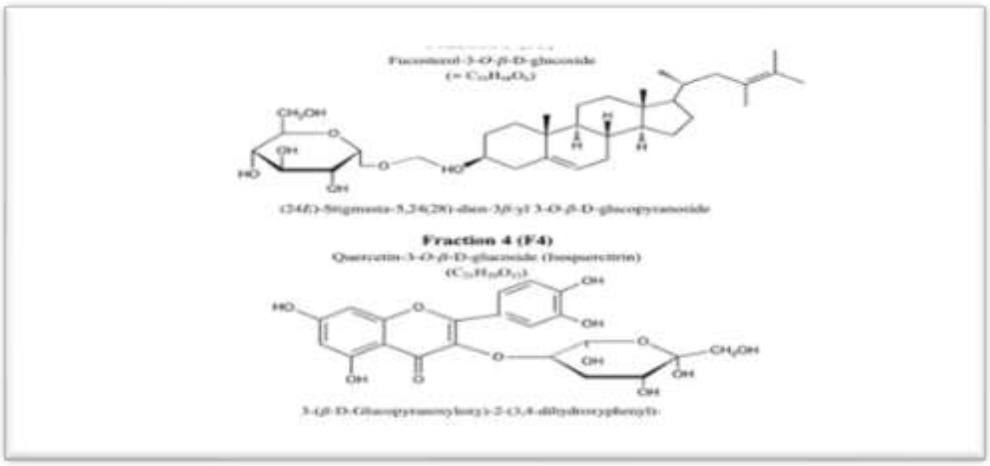


Figure 13: Chemical Structures of Bioactive Compounds Identified in Fraction 3 (F3) and Fraction 4 (F4) of *Padina tetrastromatica*

The FTIR and ^{1}H NMR spectra of Fraction 3 indicated the presence of oxygenated glycosidic metabolites containing aliphatic and sterol-associated moieties. The occurrence of anomeric proton signals together with ester-associated bands suggested a sterol glycoside structure. Based on the integrated spectral profile and known phytochemistry of Padina species, Fraction 3 was tentatively assigned as a fucosterol glycoside-type constituent possessing a sterol nucleus linked to a sugar residue. The proposed molecular framework consisted of a steroidal aglycone attached to a glycosidic moiety through an O-glycosidic linkage. Based on the structural identification of Fraction 3 as a fucosterol glycoside-type constituent, this compound is expected to exhibit cytotoxic activity (54).

Fraction 4 displayed characteristic aromatic ring vibrations at 1600, 1579, and 1508 cm^{-1} in the FTIR spectrum together with aromatic proton signals in the ^{1}H NMR spectrum. Correlation with mass spectral data suggested the presence of quercetin-3-O-glucoside (C21H20O12; molecular weight 464.38 g/mol). The aromatic proton pattern, glycosidic signals, and multiple hydroxyl functionalities supported the flavonoid glycoside structure. Therefore, Fraction 4 was identified as quercetin-3-O-glucoside, a bioactive flavonoid known for antioxidant and cytotoxic properties.

Findings confirm that Fraction 4, as quercetin-3-O-glucoside, possesses cytotoxic properties suitable for anticancer applications(55).

4.Conclusion:

The present study comprehensively evaluated the phytochemical composition, structural characteristics, and in vitro cytotoxic activity of the hydroethanolic extract of *Padina tetrastromatica*. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, steroids, terpenoids, and carbohydrates, indicating the richness of biologically active constituents within the seaweed. Chromatographic fractionation yielded five fractions (F1–F5), which were subsequently characterized using HPTLC, FTIR, Mass Spectrometry, and ^{1}H NMR analyses.

Bioactivity-guided investigations demonstrated that Fractions 3 (F3) and 4 (F4) exhibited significant concentration-dependent cytotoxic activity in both brine shrimp lethality and MTT assays, while the remaining fractions showed comparatively lower activity. Structural elucidation identified Fraction 3 as Fucosterol-3-O-β-D-glucoside and Fraction 4 as Quercetin-3-O-β-D-glucoside (Isoquercitrin)**.** These compounds displayed notable cytotoxic effects against MCF-7 human breast cancer cells, with IC_{50} values of 63.4 µg/mL and 91.7 µg/mL, respectively, indicating that they are the principal contributors to the observed biological activity.

Overall, the findings confirm that *Padina tetrastromatica* possesses significant **in vitro cytotoxic potential**, and that **Fucosterol-3-O-β-D-glucoside** and **Quercetin-3-O-β-D-glucoside (Isoquercitrin)** are major bioactive constituents responsible for this activity. The study provides scientific evidence supporting the potential application of *Padina tetrastromatica* as a source of marine-derived cytotoxic compounds for anticancer research.

5.Future scope:

The findings of the present study provide a foundation for further exploration of the cytotoxic constituents of *Padina tetrastromatica*. Future research should focus on the large-scale isolation and purification of Fucosterol-3-O-β-D-glucoside and Quercetin-3-O-β-D-glucoside (Isoquercitrin) to facilitate detailed pharmacological

investigations. The cytotoxic activity of these compounds should be evaluated against a wider range of cancer cell lines, along with studies aimed at elucidating their mechanisms of action through apoptosis, cell cycle regulation, and molecular signaling pathways. Additionally, in vivo studies are necessary to validate their efficacy and safety profiles. Advanced analytical techniques may further strengthen structural characterization and aid in identifying additional bioactive metabolites. Such investigations will contribute to a better understanding of the therapeutic value of *Padina tetrastromatica* and its bioactive constituents in anticancer research.

6.AUTHORS CONTRIBUTIONS:

Shruti Garde performed the experimental work, data analysis, and manuscript preparation. Pranali Dhole and Jyoti Kadam assisted in data collection and interpretation. Jeevan Dhumal supervised the study and critically reviewed the manuscript. Amol Kumbhar contributed to analytical studies and data interpretation. Nilesh Jadhav and Archana Thikekar provided guidance, reviewed the manuscript, and approved the final version. All authors read and approved the final manuscript.

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The authors declare no conflict of interest

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10. ABBREVIATIONS

Abbreviation	Full Form
TLC	Thin Layer Chromatography
HPTLC	High-Performance Thin-Layer Chromatography
FTIR	Fourier Transform Infrared Spectroscopy
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR	Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
HPLC	High-Performance Liquid Chromatography
GC–MS	Gas Chromatography–Mass Spectrometry
MEM	Minimum Essential Medium
FBS	Fetal Bovine Serum

Abbreviation	Full Form
CO_2	Carbon Dioxide
MCF-7	Michigan Cancer Foundation-7 Human Breast Cancer Cell Line
ATR	Attenuated Total Reflectance
KBr	Potassium Bromide
Rf	Retardation Factor
IC_{50}	Half-Maximal Inhibitory Concentration
DPPH	2,2-Diphenyl-1-picrylhydrazyl
PI3K	Phosphoinositide 3-Kinase
Akt	Protein Kinase B
Nrf2	Nuclear Factor Erythroid 2-Related Factor 2
v/v	Volume per Volume
cm^{-1}	Reciprocal Centimeter (Wavenumber)
O–H	Hydroxyl Group
C=O	Carbonyl Group
C=C	Carbon–Carbon Double Bond
C–O	Carbon–Oxygen Bond
C–H	Carbon–Hydrogen Bond
S=O	Sulphur–Oxygen Bond
LC_{50}	Lethal Concentration Causing 50% Mortality
m/z	Mass-to-Charge Ratio
UV	Ultraviolet
ppm	Parts Per Million
ppm (NMR)	Parts Per Million (Chemical Shift)