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*Original Research Article*

# Biochemical profiling and anticancer properties of brown seaweed *Dictyota dichotoma* (Hudson.) J.V.Lamouroux

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**ABSTRACT**

In the present study, we have extracted the secondary metabolites from *Dictyota dichotoma* and evaluated their anticancer properties against Michigan Cancer Foundation-7 (MCF-7) breast cancer cell lines. Different solvent extracts were used to isolate the bioactive molecules form *D. dichotoma*. Among them, methanol extract showed the highest total phenol content (TPC: 306.31 ± 1.7 GAE mg/g extract), total tannin content (TTC: 291.90 ± 1.2 RE mg/g extract) and total flavonoid content (TFC: 92.89 ± 0.6 GAE mg/g extract). The antioxidant ABTS assay of methanol extract inhibited 74.51 ± 0.10% of free radicals and phosphomolybdenum assay showed 62.36 ± 0.04 mg/g AAE/g extract. Furthermore, bioactive compounds were characterised by gas chromatography-mass spectroscopy (GC-MS), highly performance liquid chromatography (HPLC) and Fourier transmission infrared spectroscopy (FT-IR). The GC-MS results revealed 29 bioactive compounds that are highly potential biological activities. The extracted compound was quantified and confirmed by HPLC and FT-IR. The *D. dichotoma* methanol extract effectively induced the apoptosis in MCF-7 breast cancer cells and recorded $IC_{50}$ value of 44.35 ± 8.62 µg/mL.

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## 1. Introduction

Marine biosphere is one of the richest source of various biodiversity with unrivalled seaweed resources that have potent with enamors bioactive compounds (Ameen et al., 2021). Natural products from marine seaweeds are highly precious for the application of pharmaceutical, cosmeceutical and agricultural (Deyab and Ward, 2016). Seaweeds are used to obtain the industrial products such as phycocolloids (agar-agar), alginate and carrageenan (Ganesan and Shanmugam, 2020). Moreover, it is an excellent source of vitamins, minerals and polysaccharides that could also imply a high level of soluble and insoluble dietary fibers (Kim and Wijesekara, 2010). There are three different types of seaweeds such as green, red and brown which are enormously found in Gulf of Mannar coastal region, Tamil Nadu, India. All the brown seaweeds are with their flexible stems with large size that allow them to withstand in the constant pounding of waves and they will be available in the all seasons. *Dictyota dichotoma* belongs to the family Phaeophyceae the species predominantly grows in rocky intertidal pools and subtidal areas of sea with high amount of polyphenols groups (Bogaert et al., 2020). During the last few decades many novel compounds were identified in the marine seaweeds that possess an interesting biological activity (El Gamal, 2010). *D. dichotoma* is one among brown seaweed distributes in temperate and subtropical region of Gulf of Mannar, it contains various constituents such as polyphenolics, tannins, terpenes, flavonoids, proteins, sulphates, polysaccharides and lipids. Because of its nutritional value, many countries like Europe, India, China, Japan are using it as a food material (Rebours et al., 2014; Rocha et al., 2018; Habeebullah and Alagarsamy, 2023). Bioactive compounds form this seaweed has shown

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high antioxidant, antimicrobial, anti-inflammatory and anticancer properties (Sivakumar and Vignesh, 2014; Rengasamy et al., 2020).

There are only limited data, which exhibit the antiproliferative effect of *D. dichotoma* extract against various cancer cells. Cancer is a non-communicable and life-threatening disease which causes more death worldwide (Raja et al., 2023). Breast cancer is the most commonly diagnosed cancer type and cause 10 million of deaths in the year 2020 (https://www.who.int/health-topics/cancer#tab=tab_1). In 2020, there were 2.3 million women diagnosed with breast cancer and 6,85,000 deaths globally (Ferlay et al., 2021; Ammar et al., 2022). The cancer disease is incurable while treating with chemotherapy that causes more side effects with relatively less success and cells are acquired more resistance (Sakthivel and Devi, 2019). In cancer therapy, methotrexate, cisplatin, doxorubicin, taxanes, etc. are a chemotherapeutic agent can control the proliferative effect of cancer cells (Hosseini et al., 2015; Chaudhary et al., 2019). But, there are some limitations of using synthetic drugs due to its cytotoxicity and affect the non-targeted cells that cause impaired patients quality of life (Pádua et al., 2015). The alternative methods for the treatment of cancer cell are bio-prospecting of natural products from biological sources (Vignesh et al., 2023). The investigation of anticancer drugs originating from natural sources has garnered considerable attention within the realm of cancer research on both a national and global scale. Marine algae as a promising source and emerging trends due to its potential anticancer properties (Xin et al., 2023).

Seaweed derived compounds has shown high efficiency in delivering cancer drugs and also enabling the targeted delivery of chemotherapy or genetic medications to combat cancer. Certain extracts derived from algae have exhibited the capacity to hinder the growth of cancer cells, impede their spread to other parts of the body, and obstruct the formation of blood vessels necessary for tumor development. Simultaneously, these extracts have been found to encourage a process called apoptosis, which is the natural programmed cell death, thereby exerting a beneficial impact in the fight against cancer (Ferdous and Yusof, 2021; Xin et al., 2023). Hence, seaweeds are rich in phytochemical compounds and exhibit a wide range of pharmacological activities, along with diverse therapeutic attributes.

In the context of this study, our objectives were to assess the phytochemical profiles of *D. dichotoma* and analyse its antioxidant activities. Furthermore, we confirmed the presence of bioactive constituents through Gas chromatography-mass spectrometry (GC-MS) analysis, Fourier transform infrared (FT-IR) and High-performance liquid chromatography (HPLC) analysis. Additionally, cytotoxicity studies were carried out against the human breast cancer cell line MCF-7.

## 2. Experimental

### 2.1. Chemicals and reagents

Analytical grade solvents ethyl acetate, chloroform, acetone and methanol used in this study were purchased from S.D Fine chem Ltd. India. The chemicals involving 2,2-azinobis (3-ethyl-benzothiozoline)-6-sulfonic acid disodium salt (ABTS), Folin-ciocalteu phenol reagent, ammonium molybdate and sodium phosphate were purchased from Hi Media Laboratories Pvt. Ltd. (Mumbai, India). Standards of ascorbic acid, gallic acid were provided from Hi Media Laboratories Pvt. Ltd. (Mumbai, India). 6-Hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox) and Rutin were purchased from Sigma Aldrich (Mumbai, India), respectively.

### 2.2. Seaweed collection

Seaweeds were collected by hands picking in period of November-December 2021 from the Islands for the first time from Sayalkudi, Gulf of Mannar Coast [8.47°N 79.02°E] Rameswaram, Tamil Nadu, India.

### 2.3. Ultrasonic assisted extraction

The dried and powdered algal sample (30 g) was successively extracted by absolute chloroform, acetone, ethyl acetate and methanol using Ultrasonic assisted method (Lab man LMUC-2) operated at 40 kHz at 37°C (Kumar et al., 2020). The retrieved extract was filtered using Whatman filter paper no. 1 and concentrated using a rotary vacuum evaporator (Super Fit-Rotavap model: PBU-6D). The concentrated sample was air dried and packed in tight container for further studies.

### 2.4. Phytochemical analysis

#### 2.4.1. Determination of total phenol

The total phenol content in the extract was determined according to the method described by (Makkar and Makkar, 2003). About 100 µL of different solvent extracts (50 mg/mL) were taken in the test tubes and made up to the volume of 1 mL with distilled water. Then, 500 µL of Folin-Ciocalteu reagent (1:1 with distilled water) and 2.5 mL of sodium carbonate solution (20.0%) were added sequentially in each tube. Later the test tubes were incubated in the dark for 40 min and the absorbance was recorded at 725 nm using UV- spectrophotometer. The results were compared with standard gallic acid as positive control and the mixture without solvent extract was taken as blank.

#### 2.4.2. Determination of total tannin

The total tannin content in solvent extracts was estimated by polyvinyl polypyrrolidone (PVPP) method according to (Makkar and Makkar, 2003). About 75 mg of PVPP was weighed and 900 µL of distilled water and 750 µL of the sample extract were added. The content was vortexed well and the tube was kept in refrigerator at 4 °C for 4 hrs. Further, the sample was centrifuged at

4000 × g for 10 minutes at room temperature and the supernatant was collected. The supernatant has only simple phenolics other than the tannins. The phenolic substance of the supernatant was measured at 725 nm and communicated as the substance of non-tannin phenolic.

#### 2.4.3. Total flavonoid content

Total flavonoid content in the extracts was determined by aluminium chloride colorimetric method described by (Makkar and Makkar, 2003). About 0.5 mL of sample extracts were added to 0.5 mL of aluminium chloride (2.0 *w/w%*) and the mixture was kept in room temperature for 1 hour. The absorbance was measured at 415 nm using UV spectrophotometer and compared to rutin calibration curve. The total flavonoid content was expressed as mg/g rutin equivalents (RE) dried weight.

### 2.5. *In vitro* antioxidant analysis

#### 2.5.1. ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) radical scavenging activity

The ABTS radical cation decolorization assay was determined according to (Blois, 1958). Initially, the ABTS reagent mixture was prepared by adding 7 mM ABTS solution with potassium persulfate (2.4 mM) and kept overnight in the dark condition for 16 hrs. The solution was stabilized to 0.700 ± 0.02 at 734 nm with ethanol (1:89 *v/v*). The reaction mixture was prepared by adding 1 mL of ABTS reagent with sample (*D. dichotoma* extract). It was incubated at 27 °C for 30 min. The results were expressed in trolox equivalents after reading at 734 nm against blank (Ethanol) ethanolic solution of ABTS act as negative control. The percentage of Incubation was determined using this formula.

$$\%Inhibition = [(A_0 - A_1/A_0] \times 100 \quad \text{(Eqn. 1)}$$

Where, $A_0$ and $A_1$ respectively imply the absorbance of control and the absorbance of the sample.

#### 2.5.2. Phosphomolybdenum reduction assay

The total antioxidant properties of *D. dichotoma* were evaluated by the method of (Prieto et al., 1999). An aliquot of 0.4 mL of the sample was added to 4 mL of the phosphomolybdenum reagent solution (0.6 M sulfuric acid, sodium phosphate (28 mM), and ammonium molybdate (4 mM)) and incubated at 95 °C for 90 min. For the blank, 0.4 mL ethanol was mixed with 4 mL of the phosphomolybdenum reagent. The green colour obtained was measured at 695 nm. The results were reported as ascorbic acid equivalence/g sample.

### 2.6. Analytical characterization techniques

#### 2.6.1. Characterization of methanol crude extract of *D. dichotoma* by gas chromatography and mass spectrometry (GC-MS)

The methanol extract of *D. dichotoma* was subjected to GC-MS analysis using Agilent 7890A GC coupled to HP-6890 mass spectrometer operating in EI mode at 70 ev. The GC was equipped with a DB-5 MS capillary non-polar column having the general dimensions of 30 meters, ID: 0.25 mm, Film thickness: 0.25 µm. The oven temperature was programmed from 150 °C for 4 min and increased to 250 °C at 4 °C/min and hold for 10 min at 250 °C for sample analysis. For compounds analyses the oven temperature was set at 265 °C for 40 min. The carrier gas was helium (He) with a flow rate of 1 mL/min and the injector temperature was set at 260 °C in split mode (1:10). The injection volume was 2 µL for the sample. The spectrum showed different compound peak by transferring compound to mass spectrum for detecting their atoms of molecules (Vignesh et al., 2023). The spectrum results were compared with existing MS data library using a NIST Ver. 11.

#### 2.6.2. Fourier transform infrared spectroscopy-attenuated total reflectance (FT-IR-ATR) analysis

FT-IR-ATR analysis of the methanol extract of *D. dichotoma* was performed using Jasco N-4700 FT-IR-ATR spectrophotometer. The IR spectra ranges from 4000 to 600 $cm^{-1}$ were recorded on samples. The IR spectrum detects the chemical structure and characterised their functional groups by the method of (Diem et al., 2004).

#### 2.6.3. Highly performance liquid chromatography (HPLC) analysis

The HPLC analysis of methanol extract of *D. dichotoma* was performed by Shimadzu HPLC system, equipped with a model LC-10AT pump, UV VISIBLE detector SPD-10AT, Rheodyne injector fitted with a 20 µL loop. A Hypersil BDS C-18 column (4.6 x 250 mm, 5 µm size) with a C-18 guard column were used. The gradient solvent systems with a flow rate of 1mL/min at ambient temperature (27 °C). The mobile phase consisted of 1:1 (*v/v%*) methanol and water. The mobile phase was prepared and filtered through a 0.45 µm syringe filter and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µL while the wavelength of the UV-Visible detector was set 254 nm (Raj, 2016). The catechin, colchine and gallic acid standard were used for quantification of the extracted compounds. The concentration of each standard was calculated from the standard graphs.

### 2.7. Cell lines and culture conditions

The breast cancer cell line (MCF-7) stock cells was cultured in DMEM supplemented with 10.0% inactivated Fetal Bovine Serum (FBS (100 IU mL), streptomycin with (100 µg/mL) in a humidified atmosphere of 5.0% $CO_2$ at 37 °C until confluent. The cell was dissociated with cell dissociating solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The viability of the cells was checked and centrifuged. Furthermore, 50,000 cells well were

seeded in a 96 well plate and incubated with 5.0% of $CO_2$ incubation with optimum temperature of 37 °C for 24 hrs.

#### 2.7.2. *In vitro* cytotoxic activity

*In vitro* cytotoxicity effect was analysed by using MTT assay method according to Mosmann (1983) with slight modification. The methanolic extracted sample of *D. dichotoma* was tested on MCF-7 cells by MTT assay. Exponentially growing cells were plated in 96 well plates at a density of $3 \times 10^3$ cells/mL in 100 µL of culture medium and were allowed to adhere for 16 hrs before treatment. MCF-7 cells were subjected to various concentration of extract (10, 20, 40, 80, 160, 320 µg/mL). After culture incubation with MTT solution, proportional number of viable cells was recorded at 570 nm using an ELISA analyser. The percentage of incubation was determined using the following formula (Eqn. 2).

$$\% \text{ Inhibition} = [(1 - A_1)/A_0] \times 100 \quad \text{(Eqn. 2)}$$

Here, $A_1$ and $A_0$ respectively imply the absorbance of the sample and the absorbance of control.

The statistical analysis for cytotoxicity was done by constructing a dose response curve method. The $IC_{50}$ value of a methanol extract can be determined by constructing a dose-response curve with nonlinear regression analysis method.

### 2.8. Statistical analysis

All the experiments were performed three times (triplicates) and results were expressed as mean ± standard deviation. The data were statistically analysed using one-way ANOVA followed by Duncan's test (SPSS Ver. 20). Mean values were considered at $p < 0.05$ statistically significant.

## 3. Results and Discussion

### 3.1. Phytochemical analysis of total phenolics, tannins and flavonoids in *D. dichotoma*

The phytochemical quantification of total phenol, total tannin total flavonoid content was determined in all the extract of *D. dichotoma* (Table 1). The results clearly show the methanol extract of *D. dichotoma* showed highest phenolic contents (306.31 ± 1.7 mg GAE/g extract) tannin content (291.90 ± 1.2 mg RE/g extract) and flavonoid content (92.89 ± 0.6 mg GAE/g extract). Similar result of phenol and tannin content was obtained in acetone extract (282.2 ± 7.1 mg GAE/g extract) and (215.11 ± 2.5 mg RE/g extract). Therefore, flavonoid and tannin also comes under the polyphenol group of compounds. The least amount of polyphenol was found in chloroform extract and due to its in capability eluting the bioactive compounds. However, the methanol extract shows better result of poly phenolic groups in *D. dichotoma*. Generally, polyphenols provide a significant protection against various pathological diseases like cardiovascular disease, diabetes and cancer (Vignesh et al., 2022). The extraction of biomolecules can vary due to the solvent nature and optimal extraction methods (Harborne, 1999; Thouri et al., 2017). Earlier studies were also reported that *Padina tetrastromatica* contains maximum of total phenols (3.6 mg/g) and flavonoids (2.86 mg/g) respectively (Naveen et al., 2021). *Hypnea musciformis* ethanol extracts has showed maximum of (51 mg/g dry wt) tannin (Hasan et al., 2019). Although the quantification of poly phenols and tannin are very important in the phytochemical analysis algae due to their highly commendable antioxidant activities (Paul, 2018).

### 3.2. *In vitro* antioxidant activity

#### 3.2.1. $ABTS^{\cdot+}$ (2,2'-Azino-bis(3-ethylbenzothiozoline-6-sulfonic acid) radical scavenging activity

The ABTS radical scavenging activity of different solvent extracts of *D. dichotoma* were analysed using rutin as standard. The percentage of inhibition was found high when increased the concentration of the extract. The methanolic extract at high concentration showed maximum inhibitory activity (74.51 ± 1.04% at 400 µg/mL). Similarly, acetone extract have showed 72.76 ± 1.10% of inhibition, followed by chloroform and ethyl acetate extracts exhibited inhibition of 67.30 ± 1.09% and 58.55 ± 0.8%, respectively (Fig. 1). Thus, the ABTS activity of *D. dichotoma* methanolic extract exhibited potent radical scavenging activity.

Similar observation of antioxidant activities of the methanolic extract was observed in the previous literature for seaweed (Chakraborty et al., 2015; Rattaya et al., 2015). Because, by these records methanol extracts may have H- donating property which can terminate the oxidation process by converting free radicals to the stable forms. On the other hand, it seems that tannins took part in radical scavenging ability. Similarly, methanolic extract from the red alga *Compsopogon helwanii* has shown an increasing trend in its antioxidant activity (55.8% and 74.3%) in 50 and 100 µg/mL concentration, respectively (Shanab and Shalaby, 2012).

#### 3.1.2. Phosphomolybdenum assay

Phosphomolybdenum is a chemical compound used in antioxidant assays to measure the total antioxidant capacity of extracted substances. Methanolic extract of brown seaweed *D. dichotoma* has strong ability to neutralize the free radicals and act as an antioxidant. The results showed that 62.36 ± 0.04 mg/g ascorbic acid equivalent of methanolic extraction at 100-400 µg/mL. The other solvent extracts such as chloroform, acetone and ethyl acetate exhibited 59.33 ± 0.04, 58.88 ± 0.08 and 53.54 ± 0.13 mg/g ascorbic acid equivalents respectively (Fig. 2). The Phosphomolybdenum method has been routinely used to evaluate the antioxidant potential of extracts. In the presence of extracts Mo (VI) is reduced to Mo (V) and forms a green coloured phosphomolybdenum (V) complex. Previous research has also mentioned that crude extracts of brown and

**Table 1**
Quantitative analysis of various solvent extracts of *D. dichotoma*.

| S. No. | Solvent extraction | Total phenol (GAE mg/g extract) | Total tannin (RE mg/g extract) | Total flavonoid (GAE mg/g extract) |
|---|---|---|---|---|
| 1. | Chloroform | 33.97 ± 8.2$^{d}$ | 35.87 ± 0.8$^{c}$ | 40.79±0.6$^{cd}$ |
| 2. | Acetone | 282.2 ± 7.1$^{b}$ | 215.11 ± 2.5$^{b}$ | 45.07 ± 0.3$^{c}$ |
| 3. | Ethyl acetate | 132.95 ± 3.4$^{c}$ | 33.65 ± 0.69$^{cd}$ | 60.20 ± 0.9$^{b}$ |
| 4. | Methanol | 306.31 ± 1.7$^{a}$ | 291.90 ± 1.2$^{a}$ | 92.89 ± 0.6$^{a}$ |

Values are mean of triplicate analysis (n=3) ± standard deviation the mean values were a>b>c>d statistical different in each column at $p<0.05$ among the extracts. GAE: Gallic Acid Equivalent, RE: Rutin Equivalent.

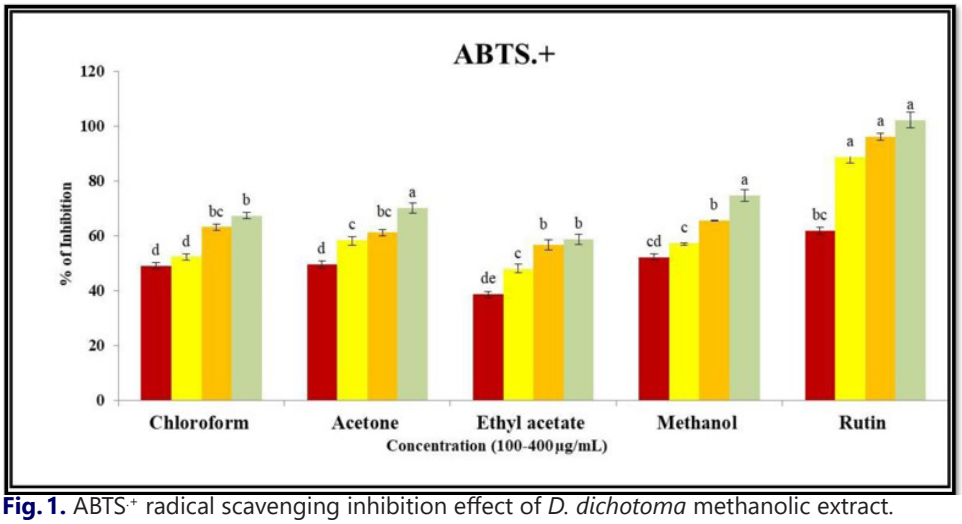


**Fig. 1.** ABTS$^{\cdot+}$ radical scavenging inhibition effect of *D. dichotoma* methanolic extract.

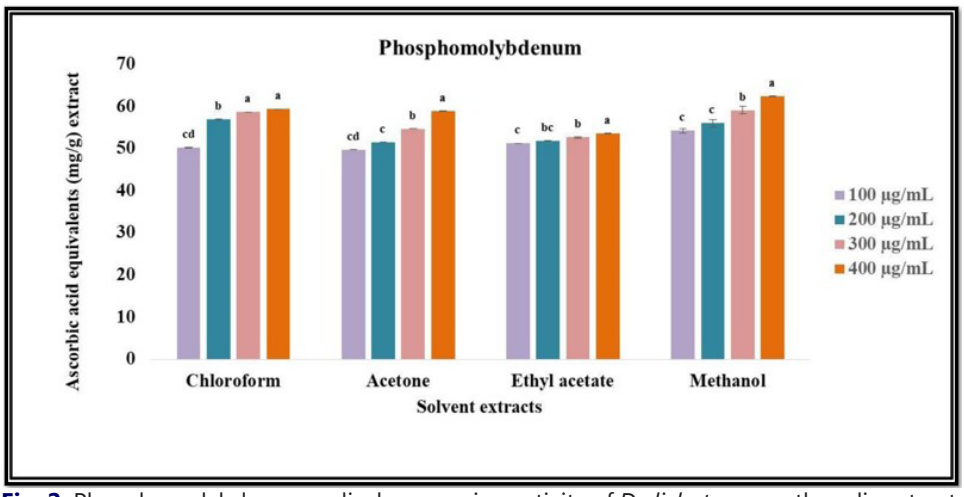


**Fig. 2.** Phosphomolybdenum radical scavenging activity of *D. dichotoma* methanolic extract.

red seaweed can reduce the phosphomolybdenum and the data were (8.2, 32.01 and 39.62 to 9.65 mg/g) in ascorbic acid equivalents respectively, which have been related to our present observations. The various antioxidant assays performed are concerned in different aspects of free radicals scavenging either differing in their mechanisms or in the ionic components taking part in the reaction or the scavenging mechanisms. Likewise, earlier report demonstrated phenolic, polyphenols and terpoenoids in the brown seaweed were one of the most effective and maximum antioxidant activity exhibited (Budhiyanti et al., 2012).

## 3.2. Analytical characterization studies

### 3.2.1. Bioactive compound analysis in methanolic extract of *D. dichotoma* using GC-MS

In this study, GC-MS were used to identify the bioactive compound in methanolic extract, maximum of 29 bioactive compounds were identified based

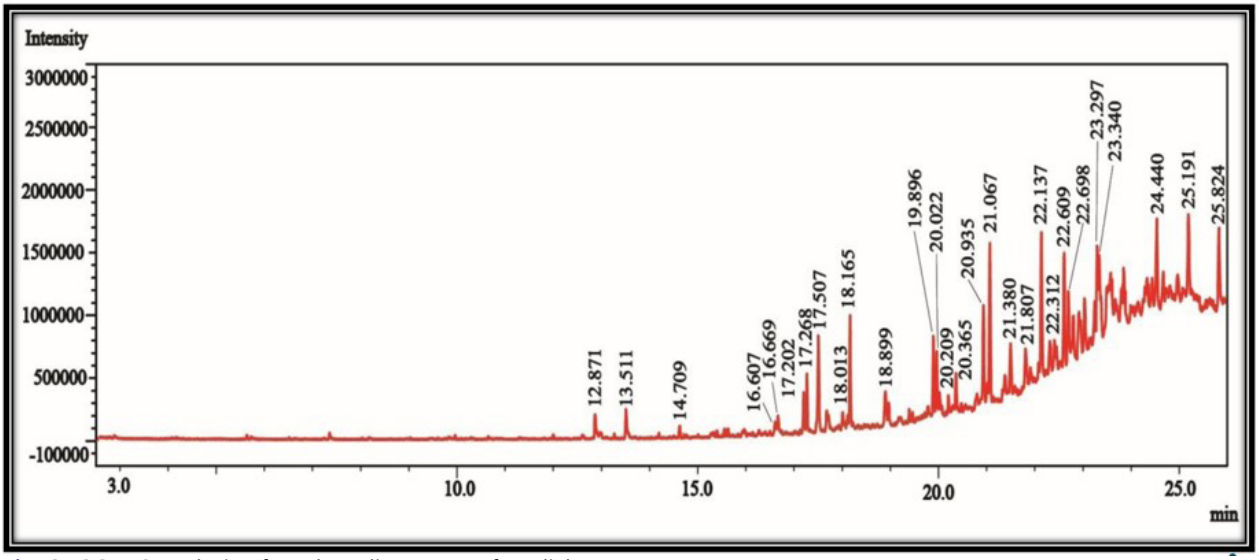


**Fig. 3.** GC-MS analysis of methanolic extract of *D. dichotoma*.

on their retention time (RT) and chemical profiling of extracted compounds (Fig. 3). All the major marine compounds were recorded, caryophyllene oxide (RT-21.067), thunbergol (RT-22.137), spiro[2.5] octane, 5,5 dimethyl-4- (RT-17.507), hexadecenoic acid, methyl ester (RT-18.165), 9,12-Octadecadienoic acid, methyl ester (RT-19.896), octane, 3,7-dimethyl-1-(2,5-xylyl- (RT-22.609), 1,3-Dioxane-5-carboxylic acid, 2-(4-methoxyphenyl)-5-methyl- (RT-25.191) and 13-docosenamide (*Z*)- (RT-25.824). The chemical structures of major bioactive compounds were detected in GC-MS analysis with chemical profiling (Fig. 4). Furthermore, extracted compounds were compared with previous studies and observed their biological activities. Moreover, the result indicates the *D. dichotoma* methanolic extract has shown major compounds of some phenols and polyphenolics. The extracted compounds showed potential antioxidant, antimicrobial and anticancer properties (Table 2). Similarly, *Sargassum whitii* possesses rich fatty acids and polyphenols with antioxidants, anti-inflammation activities (Giriwono et al., 2019).

The major bioactive compounds such as Thunbergol, Caryophyllene oxide, Octane, 3,7-dimethyl-1-(2,5-xylyl), spiro [2.5] octane, 5,5-dimethyl-4-(3-oxobutyl) were identified by using GC-MS analysis. Aldarhami et al. (2023) reported that major bioactive compounds 9,12-hexadecanoic acid, methyl ester with retention time 23.515 has been reported in Indian brown seaweed species. This compound has well known for its antiviral, antibacterial and antibiofilm activities. In addition, terpenoids like caryophyllene oxide had high antioxidant activity. Terpenoids are sort of terpenes that have oxygen molecules in their structure (Akbari et al., 2022; Jayapala et al., 2022). *D. gracilis* Blume oleoresin soluble ethanol 95% identified 17.84% of caryophyllene oxide compounds. Caryophyllene oxide compounds have the function of gastroprotective activity (Sánchez-Mendoza et al., 2014). Caryophyllene oxide exerts strong anticancer effects against MG-63 human osteosarcoma cells by inhibiting cancer cell migration tendency and including apoptosis characterized by cellular shrinkage, membrane blebbing, chromatin condensation and apoptotic body formation (Pan et al., 2016). Thus, large number of bioactive compounds with biological properties depends upon the extraction of solvent and its polarity. The solvent polarity may significantly play a crucial role in extraction of various bioactive compounds (Azmir et al., 2013; Zhang et al., 2020).

### 3.2.2. FT-IR-ATR analysis

The Fourier transform infrared spectroscopy with attenuated total reflectance (FT-IR-ATR) was employed to detect the functional group of the bioactive components based on peak value in the area of infrared radiation. The main functional groups of the bioactive compounds were detected and their respective peaks were obtained in the range of 4000 $cm^{-1}$ to 600 $cm^{-1}$ (Fig. 5). The characteristic peaks of methanolic extracts revealed C-H stretching vibration at strong intense peak at 2833.34 $cm^{-1}$, indicating the aldehydes groups. The C=O stretching vibration at 1736.58 $cm^{-1}$ indicated strong fatty acid groups. The peaks 1447.31, 1372.10, 1234.22 and 1097.3 $cm^{-1}$ correspond to the aromatic compound, aliphatic group, carbohydrate and glycosidic bond respectively. The intense characteristic peak at 846.597 $cm^{-1}$ as a medium signal with C=O bond stretching, indicates the presence of carboxylic acids. The C=C stretching vibration at 785.85 and 607.16 $cm^{-1}$ indicated strong aromatic amine and alkane groups (Table 3).

The structural and functional groups of marine compounds were analysed by FT-IR-ATR spectroscopy.

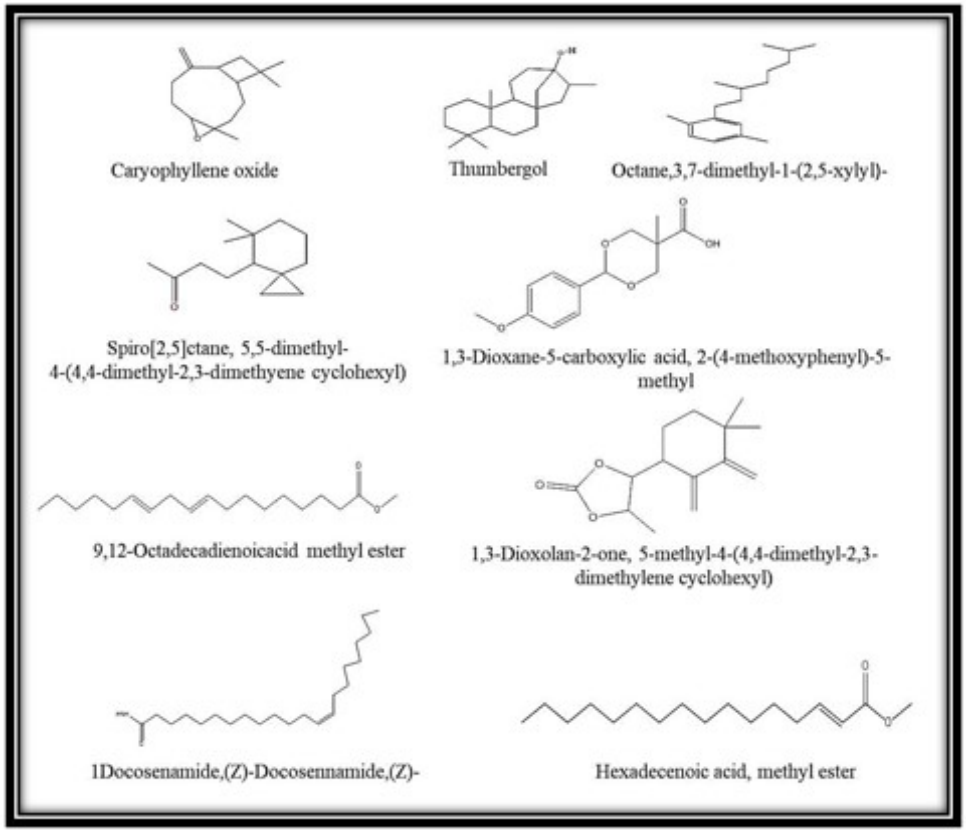


**Fig. 4.** Chemical structure of major compounds detected in methanolic extract of *D. dichotoma* in GC-MS analysis.

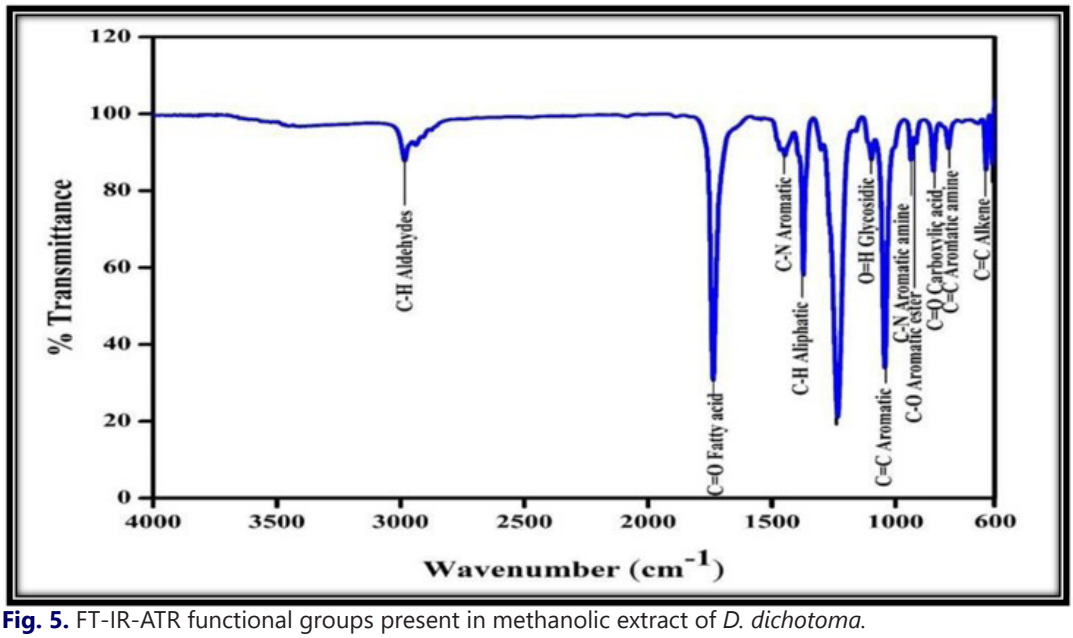


**Fig. 5.** FT-IR-ATR functional groups present in methanolic extract of *D. dichotoma*.

In the present study, methanolic extract of *D. dichotoma* showed the presence of major functional groups especially aldehydes, aromatic compound, aliphatic groups, aromatic amine, fatty acids, carboxylic acids and alkenes. Earlier report showed that the functional groups amine salts, alcohol, phenol, aliphatic, aromatics and aliphatic amines were found in *Sargassum tenerrimum* (Hakim and Patel, 2023). Likewise, based

**Table 2**
Identification of major bioactive compounds of *D. dichotoma* methanolic extract using GC-MS analysis along with biological activities.

| S. No. | Retention Time | Compound | Area | Area | Molecular formula | Molecular weight | Biological activity |
|---|---|---|---|---|---|---|---|
| 1. | 12.871 | 1-(4-Ethoxyphenyl)propan-1-ol | 393771 | 1.46 | $C_{11}H_{16}O_2$ | 180 | No activity reported |
| 2. | 13.511 | Benzoic acid, 4-ethoxy-, ethyl ester | 510501 | 1.9 | $C_{11}H_{14}O_3$ | 194 | Anti-oxidant and anti-inflammtory compound (Ahmed et al., 2017) |
| 3. | 14.709 | *N*-(Trifluoroacetyl)-*N*,*O*,*O*′,*O*′′-tetrakis(trimethylsilyl)norepinephrine | 44301 | 0.16 | $C_{22}H_{42}F_3NO_4Si_4$ | 553 | Antimicrobial potential (Soliman et al., 2016) |
| 4. | 16.607 | 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one | 121801 | 0.45 | $C_{11}H_{16}O_3$ | 196 | Anti-inflammatory potential (Jayawardena et al., 2019) |
| 5. | 16.669 | (2,6,6-Trimethylcyclohex-1-phenylmethanesulfonyl) benzene | 152494 | 0.57 | $C_{16}H_{22}O_2S$ | 278 | No activity reported |
| 6. | 17.202 | Neophytadiene | 506403 | 1.88 | $C_{20}H_{38}$ | 278 | Analgesic, anti-inflammatory and antioxidant compound (Raman et al., 2012) |
| 7. | 17.268 | 2-Pentadecanone, 6,10,14-trimethyl- | 675233 | 2.51 | $C_{18}H_{36}O$ | 268 | Antimicrobial activity (Amos-Tautua et al., 2020) |
| 8. | 17.507 | Spiro[2.5]octane, 5,5-dimethyl-4-(3-oxobutyl)- | 1583529 | 5.89 | $C_{14}H_{24}O$ | 208 | No activity reported |
| 9. | 18.013 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 177862 | 0.66 | $C_{17}H_{24}O_3$ | 276 | Antioxidant properties (Adeosun et al., 2013) |
| 10. | 18.165 | Hexadecanoic acid, methyl ester | 1228651 | 4.57 | $C_{17}H_{34}O_2$ | 270 | Antimicrobial compound (Shaaban et al., 2021) |
| 11. | 18.899 | 3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)- | 530027 | 1.97 | $C_{13}H_{20}O_2$ | 208 | No activity reported |
| 12. | 19.896 | 9,12-Octadecadienoic acid, methyl ester | 971998 | 3.61 | $C_{19}H_{34}O_2$ | 294 | Compound with Analgesic, anti-inflammatory and ulcerogenic properties (Hadi et al., 2016) |
| 13. | 20.022 | *Z*-(13,14-*Epoxy*)tetradec-11-en-1-ol acetate | 172679 | 0.64 | $C_{16}H_{28}O_3$ | 268 | Antioxidant compound (Khan et al., 2023) |
| 14. | 20.209 | Methyl stearate | 200883 | 0.75 | $C_{19}H_{38}O_2$ | 298 | Antimicrobial |
| 15. | 20.365 | 4-Methyl-4-nonadecene | 419419 | 1.56 | $C_{20}H_{40}$ | 280 | No activity reported |
| 16. | 20.935 | Caryophyllene oxide | 1262393 | 4.69 | $C_{15}H_{24}O$ | 220 | Compound with Anticancer properties (Fidyt et al., 2016) |
| 17. | 21.067 | Caryophyllene oxide | 2041508 | 7.59 | $C_{15}H_{24}O$ | 220 | Compound with Anticancer properties (Fidyt et al., 2016) |
| 18. | 21.38 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 242901 | 0.9 | $C_{19}H_{38}O_4$ | 330 | Antimicrobial compound (Tyagi and Agarwal, 2017) |

**Table 2**
**Continued**

| S. No. | Retention Time | Compound | Area | Area | Molecular formula | Molecular weight | Biological activity |
|---|---|---|---|---|---|---|---|
| 19. | 21.807 | Glycidyl palmitate | 687585 | 2.56 | $C_{19}H_{36}O_3$ | 312 | No activity reported |
| 20. | 22.137 | Thunbergol | 2101406 | 7.81 | $C_{20}H_{34}O$ | 290 | Antimicrobial compound (Mitić et al., 2019) |
| 21. | 22.312 | 2-Dodecen-1-yl(-)succinic anhydride | 600319 | 2.23 | $C_{16}H_{26}O_3$ | 266 | Used in microbial hydroxylation (Olejniczak, 2010) |
| 22. | 22.609 | Octane, 3,7-dimethyl-1-(2,5-xylyl)- | 1655830 | 6.16 | $C_{18}H_{30}$ | 246 | No activity reported |
| 23. | 22.698 | 1,3-Dioxolan-2-one, 5-methyl-4-(4,4-dimethyl-2,3-dimethylenecyclohexyl) | 1052313 | 3.91 | $C_{14}H_{20}O_3$ | 236 | No activity reported |
| 24. | 22.803 | Androst-5-en-3.beta.-ol, 4,4-dimethyl-, acetate | 553916 | 2.06 | $C_{23}H_{36}O_2$ | 344 | No activity reported |
| 25. | 23.297 | Irone .alpha. b | 612690 | 2.28 | $C_{14}H_{22}O$ | 206 | No activity reported |
| 26. | 23.34 | 1-Heptatriacotanol | 380487 | 1.41 | $C_{37}H_{76}O$ | 536 | Antioxidant, anti-inflammatory, hypocholesterolemic, antimicrobial, and anticancer properties (Madhavan, 2015; Al-Rubaye et al., 2017) |
| 27. | 24.44 | 2-Heptanone, 6-(3-acetyl-1-cyclopropen-1-yl)-3-hydroxy-6-methyl-, (R*,R*)- | 302657 | 1.13 | $C_{13}H_{20}O_3$ | 224 | No activity reported |
| 28. | 25.191 | 1,3-Dioxane-5-carboxylic acid, 2-(4-methoxyphenyl)-5-methyl- | 1333355 | 4.96 | $C_{13}H_{16}O_5$ | 252 | No activity reported |
| 29. | 25.824 | 13-Docosenamide, (*Z*)- | 1248167 | 4.64 | $C_{22}H_{43}NO$ | 337 | Antifungal activity (Prasher and Manju, 2019) |

**Table 3**
FTIR-ATR analysis of different functional groups present in methanolic extract of *D. dichotoma*.

| Absorption ($cm^{-1}$) | Functional group | Phytocompounds identified |
|---|---|---|
| 2833.34 | C-H Stretching | Aldehydes |
| 1736.58 | C=O | Fatty acid group |
| 1447.31 | C-N | Aromatic compound |
| 1372.1 | C-H Stretching | Aliphatic group |
| 1234.22 | C-O | Carbohydrate |
| 1097.3 | O=H | Glycosidic bond |
| 1043.3 | C=C | Aromatic compound |
| 938.109 | C-N | Aromatic amine |
| 917.95 | C-O | Aromatic ester |
| 846.597 | C=O | Carboxylic acid |
| 785.85 | C=C | Aromatic amine |
| 634.466 | C=C | Alkene |
| 607.16 | C-H | Alkane |

on the previous studies and report confirmed the presence of functional groups of alkynes, aromatic compounds, carboxylic acids, aliphatic amines, alkanes and alcohols in the methanolic extracts were reported in the methanolic extracts of brown seaweed *Sargassam wightii* (Venkatesan et al., 2023).

### 3.2.3. HPLC analysis

The HPLC fingerprint profile for methanolic extract of *D. dichotoma* was carried out to quantify the secondary metabolites by using standard bioactive compounds. The HPLC results exhibited the presence of polyphenols and tannin by comparing the identified compounds with standard. The identified major bioactive compounds and its retention times were 2.499, 4.126, 4.344 and 14.764 min (Fig. 6). It was matched with standard compounds viz. catechin, gallic acid, colchine and quercetin and confirms the identity of unknown compounds (Fig. 7 (a-d)). The extracted compounds were quantified using known standards, resulting in concentrations of 0.0140 µg/mg for catechin, 0.0025 µg/mg for gallic acid, 0.0028 µg/mg for rutin, and 0.0034 µg/mg for quercetin, respectively (Table 4).

HPLC analysis will be performed to find out the existence of active ingredients and any additives in the extracts (Yamuna Devi et al., 2012). In the present study, the HPLC profile of *D. dichotoma* showed the presence of important bioactive compounds tannin and phenolic compounds. Dang et al. (2018) reported that the highest amount of tannin was recorded in *Padina* sp. (17.83 mg catechin $mg^{-1}$) and *Sargassum vestitum* (24.39 mg catechin $mg^{-1}$). The methanolic extracts of *D. dichotoma* consists of gallic acid, rutin and quercetin in the extracts. There is an inadequate number of research were conducted on the identification of phenolic compounds from the ethanolic extract of *Padina pavonica* and *Zanardinia typus* (Keskinkaya et al., 2023). The previous studies revealed that marine macroalgae species contain many phenolic compounds such as gallic, ferulic and catechin, in addition to phlorotannin that can only be synthesized by macroalgae species in nature (Jimenez-Lopez et al., 2021). The phenolic acid of the hydroxycinnamic class, has been recorded to have antioxidant, antimicrobial, anticancer, antidiabetic and anxiolytic activities There are some variations and similarities between our results the literature. These differences may be caused by the collection localities of samples, extraction, purification, quantification and characterization methods of the phenolic compounds (Freile-Pelegrín and Robledo, 2013).

### 3.3. *In vitro* cytotoxicity by MTT assay

The cytotoxic effect of methanolic extracted compounds of *D. dichotoma* on MCF-7 cell lines was evaluated through MTT assay and exhibited significant anti-proliferation activity in a dose dependent manner. The concentration at 320 µg/mL has shown 79.09% of inhibition and standard doxorubicin at a same concentration showed an inhibitory activity of 82.06 ± 0.38%. The $IC_{50}$ value of methanolic extract showed 44.35 ± 8.62 µg/mL (Table 5). These values represent the concentration of the extracts required to deserve 50% inhibition of the cell proliferation. The morphological changes in MCF-7 cell culture were observed in both treated and untreated samples. The cell shrinkage and apoptotic cell death were observed after 24 hrs of treatment. The cancer cell shape and size was reduced at 160 µg/mL and obtained apoptotic cell death at 320 µg/mL concentration (Fig. 8 and Fig. 9).

The MTT assay was useful for the measurement of

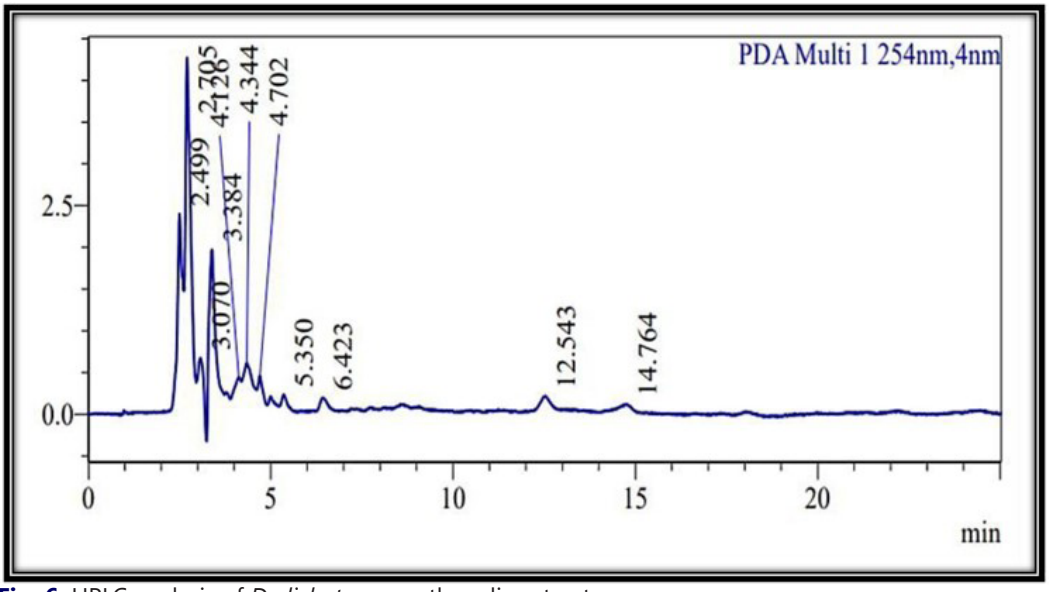


**Fig. 6.** HPLC analysis of *D. dichotoma* methanolic extract.

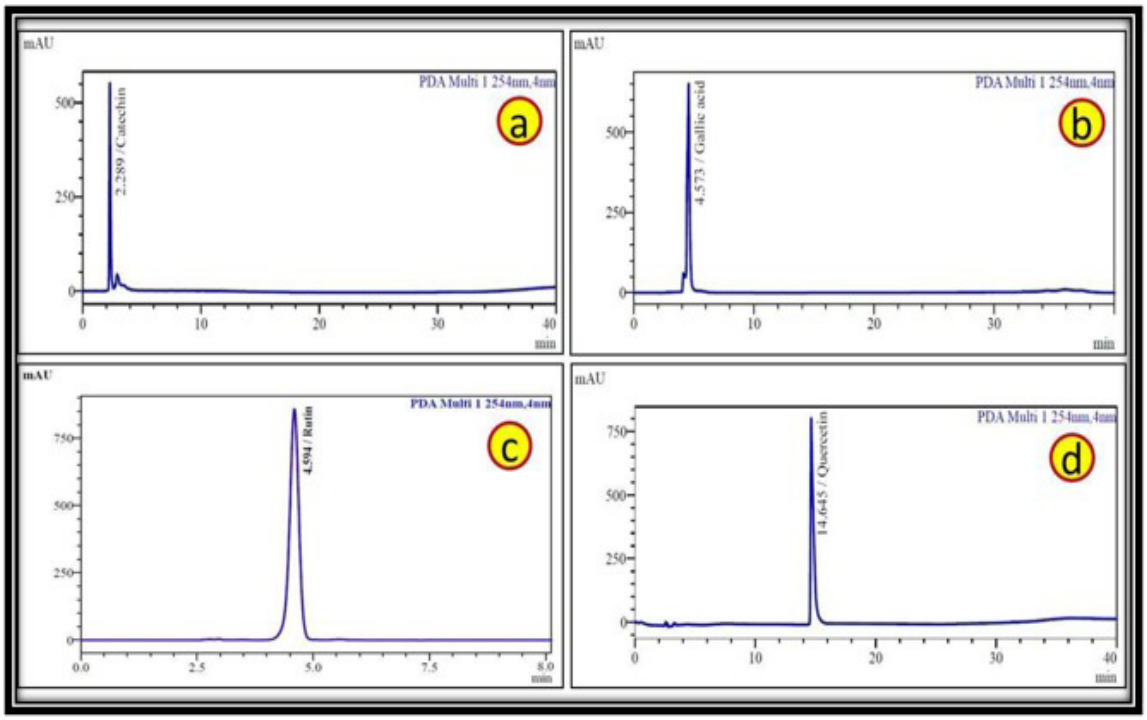


**Fig. 7.** Chromatogram of standards by HPLC analysis **a**: Catechin, **b**: Gallic acid, **c**: Rutin and **d**: Quercetin.

**Table 4**
Quantification of compounds present in methanolic extract of *D. dichotoma* by HPLC analysis.

| Compound name | Retention Time | Area | Height | Concentration (µg/mg) |
|---|---|---|---|---|
| Catechin | 2.499 | 8811 | 1402 | 0.014 |
| Gallic acid | 4.126 | 581 | 84 | 0.003 |
| Rutin | 4.344 | 68239 | 10297 | 0.003 |
| Quercetin | 14.764 | 2975 | 117 | 0.003 |

**Table 5**
Cytotoxic effect of MCF-7 cell line after treatment with methanolic extract of *D. dichotoma vs* positive control doxorubicin showing percentage of inhibition.

| S. No | Concentration (µg/mL) | % of Inhibition | |
|---|---|---|---|
| | | Standard doxorubicin | Methanol extract |
| 1 | 10 | 14.43 ± 0.13$^{f}$ | 6.25 ± 0.13$^{f}$ |
| 2 | 20 | 28.34 ± 0.13$^{e}$ | 13.95 ± 0.27$^{e}$ |
| 3 | 40 | 38.53 ± 0.07$^{d}$ | 30.97 ± 0.39$^{d}$ |
| 4 | 80 | 57.52 ± 0.33$^{c}$ | 42.51 ± 0.13$^{c}$ |
| 5 | 160 | 63.77 ± 0.26$^{b}$ | 64.87 ± 0.07$^{b}$ |
| 6 | 320 | 82.06 ± 0.38$^{a}$ | 79.09 ± 0.20$^{a}$ |
| $IC_{50}$ | | 21.40 ± 4.84 µg/mL | 44.35 ± 8.62 µg/mL |

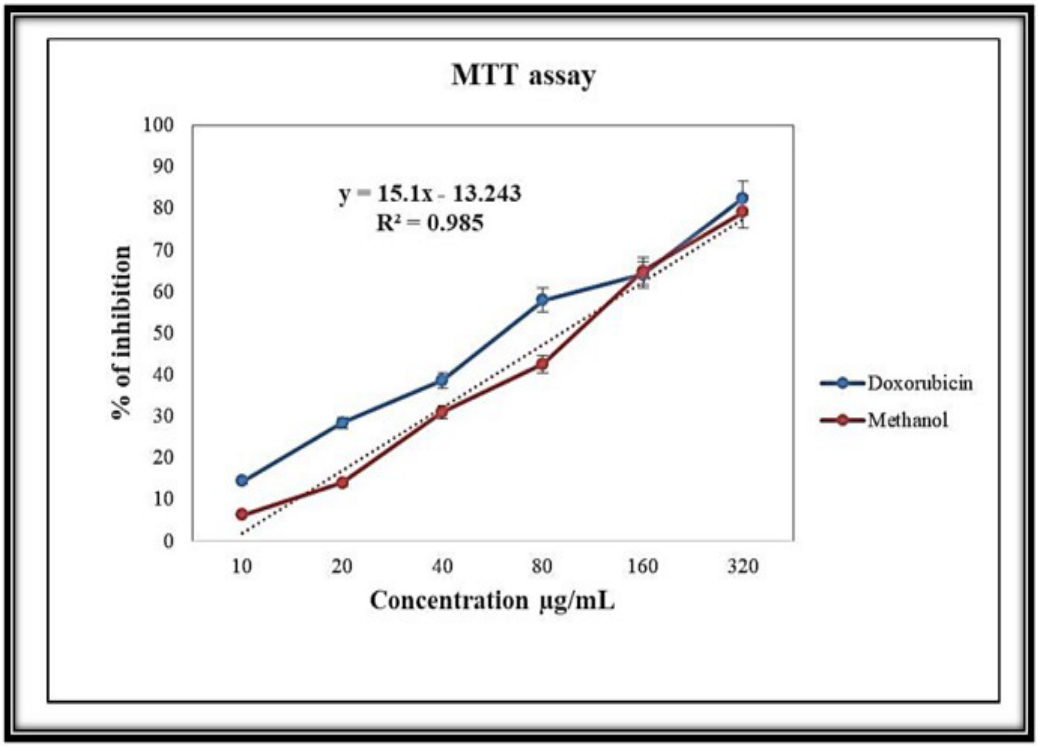


**Fig. 8.** Cytotoxicity effect of methanolic extract of *D. dichotoma* on MCF-7 breast cancer cell line.

cell growth, response to mitogens, growth factors, membrane stability, cytotoxicity and to derive growth curves (Akhir et al., 2022; Mahendran et al., 2022). The cytotoxicity studies provide a preliminary knowledge about the nature of the activity of the herbal products on the cancer cells. In recent years, many researchers also performed the cytotoxicity studies using MTT assay to test the ability of the phytocompounds against different cancer cell lines (Popwo Tameye et al., 2020; Mahdavi and Mohammadhosseini, 2022). Previously, ethanol extract of brown seaweed *Sargassum muticum* exhibited cytotoxicity activity against MCF-7 cells and increased the apoptotic cells death from 0.8% to 49% after 24 hours (Namvar et al., 2014). Numerous studies reported that, seaweed derived compounds have provided effective protection with intervention to any stage of cancer especially induce apoptotic genes.

## 4. Concluding remarks

Seaweed-derived compounds have demonstrated promising medicinal potential with diverse applications

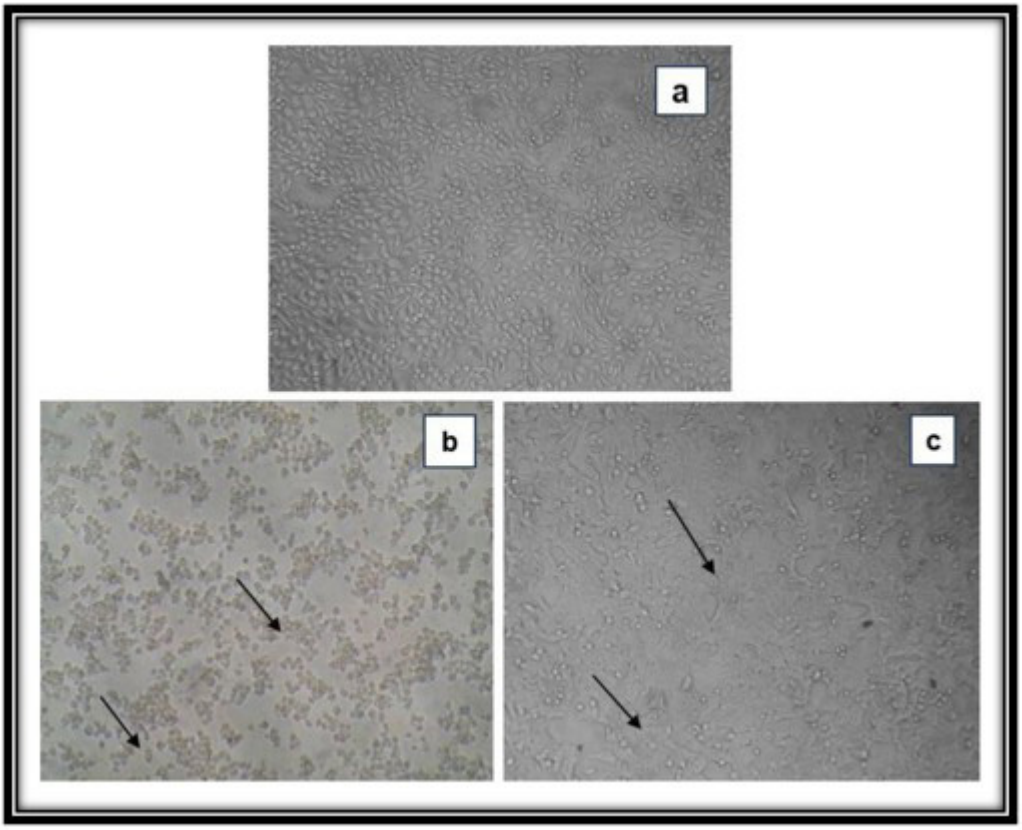


**Fig. 9.** Microscopic images of *D. dichotoma* methanolic extract showing *in vitro* cytotoxic effect on MCF-7 cell line. The microscopic images shows the morphological changes observed in Breast cancer (MCF-7) cell in different concentrations of methanol extract of *D. dichotoma* **a**: Control, **b**: 160 µg/mL and **c**: 320 µg/mL (Arrow indicates **b**: cell shrinkage and **c**: Apoptotic bodies).

in medicine. Our investigation provides a comprehensive evaluation of the biochemical profile, antioxidant activity, and cytotoxic properties of phenolics and polyphenols extracted from the brown seaweed *D. dichotoma* using an ultrasonic-assisted extraction method. The methanolic extracts of *D. dichotoma* exhibited potential antioxidants and antiproliferative activities. The extracted compounds from seaweeds are a remarkable source for the natural antioxidants. Further investigation exhibited that methanolic extract of *D. dichotoma* possess antiproliferative effect against MCF 7 cancer cells. Thus, this study could be a promising way for discovering new drugs from marine resources and extraction of various metabolites from seaweeds are highly potent with pharmaceutical values. Furthermore, there is an urgent need to elucidate the pathways for an efficient targeted delivery system to combat cancer.

## Abbreviations

**ABTS**: 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulphonic Acid; **ATTC**: American Type Culture Collection; **DMEM**: Dulbecco's Modified Eagle Medium; **DMSO**: Dimethyl Sulfoxide; **EDTA**: Ethylene Diamine Tetra acetic Acid; **FBS**: Fetal Bovine Serum; **FT-IR-ATR**: Fourier Transmission Infrared Spectroscopy-Attenuated Total Reflectance; **GAE**: Gallic Acid equivalents; **GC-MS**: Gas Chromatography-Mass Spectroscopy; **He**: Helium; **HPLC**: Highly Performance Liquid Chromatography; **MCF-7**: Michigan Cancer Foundation-7; **MTT**: 3-(4,5-Dimethylthi-azol-2-yl)-2,5-Diphenyltetrazolium Bromide; **RE**: Rutin Equivalents; **TE**: Trolox Equivalents; **TFC**: Total Flavonoid Content; **TPC**: Total Phenol Content; **TTC**: Total Tannin Content.

## Author contribution statement

The first draft of the manuscript was prepared by Arputhaswamy Velankanni. Review and editing of the manuscript were performed by Rajkumar Vasanthkumar and Thomas Nancy Mary. Validation and Data curation were performed by Sathasivam Vinoth and Muthukrishnan Arun. Supervision, Conceptualization, writing review and editing were performed by Packiaraj Gurusaravanan. All authors read and approved the final manuscript.

## Conflict of interest

The authors declare that there is no conflict of interest.

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