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BIOCHEMISTRY

Cytotoxicity, Apoptotic, Antioxidant and Antimicrobial Activity of *Dictyota dichotoma* (Hudson) J.V. Lamouroux 1809 Collected from Antalya Coast, Turkiye

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Abstract—Current study was purposed to reveal the cytotoxic, apoptotic, antimicrobial and antioxidant potentials of oil extracted from *Dictyota dichotoma* (Hudson) J.V. Lamouroux 1809 collected from Antalya coast of Turkiye. *Dictyota dichotoma* oil showed better cytotoxic activity towards two different adenocarcinoma cell lines (Caco-2 (8.83 µg/mL) and LnCap (10.86 µg/mL)) compared to HEK293 cells (non-cancerous). Annexin V/PI staining and *q*PCR studies revealed that oil caused induction of apoptosis. *Dictyota dichotoma* oil inhibited the growth of *Aspergillus niger* ATCC 16404 and *Enterococcus faecalis* ATCC 29212. Nevertheless, the lower antioxidant activity of *Dictyota dichotoma* oil was determined compared with trolox standard for both DPPH and ABTS assays (4.8 fold less). In conclusion, *Dictyota dichotoma* oil has a promising potential in medical usage.

Keywords: *Dictyota dichotoma*, cytotoxic activity, apoptosis antimicrobial potential, antioxidant activity, gene expression

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INTRODUCTION

Nowadays, sustainable, non-toxic and biodegradable natural products attract the attention of researchers (Sirajunnisa et al., 2021). Researchers pointed out that an important source of these natural resources is the sea (Hentati et al., 2020). It contains diverse aquatic plants and animals that are the source of novel secondary metabolites, with structural and chemical features generally not found in terrestrial natural products. Therefore, aquatic organisms are screened to find a new bioactive secondary metabolites that have different biological activities including antiviral, antibacterial, antioxidant, anticancer, neuroprotective activity, anti-inflammatory properties, anticoagulant activity, apoptotic activity, prebiotic activity, antiaging, antiallergic, antiproliferation, hypolipidemia properties, antidiabetic, antifungal, antidiarrheal, analgesic, neuroprotective and hepatoprotective activities (Lima-Filho et al., 2002; Kang et al., 2003, 2004; Aisa et al., 2005; Wang et al., 2008; Pangestuti and Kim, 2011; Chan et al., 2015; Ibraheem et al., 2017a; Agregán et al., 2017; El-Din and El-Ahwany; Rashad et al., 2019; Aumeerun et al., 2019; Kumar et al., 2020; Pradhan et al., 2020a, 2020b; Sharafi and Adhami, 2020). Wang et al. (2017) reported that interest in macroalgae especially for the Phaeophyta, Chlorophyta and Rhodophtya groups has increased due to the above-mentioned biological activities. Alsenani et al. (2020) revealed that macroalgae exhibited excellent performance in producing bioactive metabolites.

Dictyota dichotoma (Hudson) J.V. Lamouroux is planar, dichotomous, parallel-sided brown algae that

can grow up to approximately 30 cm. It lives in litoral rocky pools and sub-litoral zones. Although it is found throughout Turkey, it has been reported to be found on the coasts of Adana (Aysel et al., 2006a), Hatay (Aysel et al., 2006b), Bozcaada (Aysel et al., 2005) and Çeşme (Parlakay et al., 2005). *Dictyota dichotoma* is capable of producing various types of bioactive molecules, including polyphenols, diterpenes, sulfated polysaccharides and lipids, especially under various conditions (Sathya et al., 2017).

In current study, it was purposed to reveal chemical composition, cytotoxic, apoptotic antimicrobial and antioxidant potentials of oil extracted from *Dictyota dichotoma* collected from Antalya coast.

MATERIALS AND METHODS

The Collection of Macroalgae Samples

Dictyota dichotoma samples were collected from Antalya, Marina, from a depth of 15–22 m (36°53′1.14″ N 30°41′59.89″ E). Sampling studies were conducted with SCUBA divers. Images of the macroalgae materials were recorded using a camera (Supplementary Fig. S1). After the necessary observations were made, the study materials were placed in bags measuring 70 × 50 cm.

The collected samples were washed to remove residues and dried in a shaded place to be used in analysis. Some of the collected samples were preserved in 4–6% formaldehyde for identification. Identification studies were carried out using an Olympus brand microscope. While making taxonomic descriptions of the materials, their distinctive, anatomical and morphological features were taken into account.

Macroalgae Extraction Phase

Macroalgae were first powdered for better effect of chemicals, and 100 g of powder was used for each macroalgae extraction. Addition of methanol, chloroform and water (1 : 1 : 0.9 ratio) to the sample were performed as a two-step extraction. After centrifugation, first of all, the methanol-water layer was removed. The chloroform-oil layer at the bottom was removed and then the chloroform part was evaporated in the evaporator and made ready to be used in the current study. The ash and protein content of macroalgae extract were determined according to Vollenweider (1974) and Kjeldahl method (1883), respectively. In addition, lipid content and fatty acid analysis of extract were made according to the method described by Bligh and Dyer (1959) and Garches and Mancha (1993), respectively.

Cell Culture Analysis

Two different adenocarcinoma cell lines (Caco-2 and LnCap) and human embryonic kidney (HEK293) cells were obtained from a commercial company. While HEK293 and Caco-2 cells were maintained in DMEM medium, LnCap cells were maintained in RPMI medium. All mediums were supplied with 10% FBS and 1% Gibco. Cells were cultured at 37°C in a humidified incubator containing 5% CO_2 as described previously (Sen et al., 2015).

Cell Viability Assay

This assay was performed with MTT reagent. Ninety-six well plates were used to seed 1×10^3 cells/mL. Then, these plates were incubated for 24 h in a humidified atmosphere of 5% CO_2 at 37°C. Those cells were treated with different concentrations of *Dictyota dichotoma* oil (0.78, 1.56, 3.125, 6.25, 12.5, 25, and 50 μg/mL). The *Dictyota dichotoma* oil was solubilised in 100% DMSO. After 24 h, the cells were incubated with 10 μL of MTT solution (5 mg/mL) in a 100 μL medium for 3 h (Mutlu et al., 2022). After that, this cell medium was detracted and 50 μL of DMSO was put in 96 well plates. Then, spectrophotometric measurements were done at 590 nm (BioTek, USA). Finally, the viability of cells was calculated as compared with the control value (Konus et al., 2022).

Annexin V/PI Staining for Detection of Apoptosis

The Annexin V-FITC Apoptosis Assay Kit (Biovision, CA, USA) was used for the apoptosis analysis as described previously (Arslan et al., 2021). Annexin-V-FITC stained cells were analyzed by Arthur–Novel fluorescence cell counter (NanoEnTek, USA) to determine live, apoptotic and dead cell percentages.

The Evaluation of the Expression of Apoptosis Related Genes by qRT-PCR

LnCap and Caco-2 cell lines were treated with *Dictyota dichotoma* oil (at EC_{50} dose) for 24 h. After, RNA extraction was done by using the innuPREP RNA Mini Kit 2.0 (Analytik Jena, Germany). Total RNA measurements were done by spectrophotometrically at 260/280 nm with a Take-3 plate (Epoch, USA). OneScript® Plus cDNA Synthesis Kit (ABM, USA) was utilized for cDNA synthesis and *q*PCR was performed in triplicate as described previously (Kavak et al., 2022). Relative gene expression was quantified using the $2^{-\Delta\Delta CT}$ method and GAPDH, housekeeping gene, as described previously (Secme et al., 2023). All genes were listed in Supplementary Table S1.

Agar-Plate Disc Diffusion Assay

The antimicrobial activity of *Dictyota dichotoma* was determined by Agar-plate disc diffusion assay on nine indicator strains (Konus et al., 2022). The zone diameters around discs were evaluated using Clinical

and Laboratory Standards Institute guidelines (CLSI) (Bonev et al., 2008).

Broth Microdilution Assay

For quantitative determination of *Dictyota dichotoma* antimicrobial activity, a broth microdilution assay was done as described by Yılmaz et al. (2022). In this experiment, Minimal Inhibitory Concentration (MIC) of *Dictyota dichotoma* oil against the same indicators used in the agar disc diffusion assay was determined. MIC data evaluation was carried out by CLSI recommendations (Kurt-Kızıldoğan et al., 2022). The evaluation of MIC for bacteria and fungi is different. MIC represents the lowest concentration without turbidity for the respective bacterial strain. However, antifungal activity was evaluated through MIC_{50} (a value that inhibits 50% of growth compared to the control). Control reactions were also included (Konus et al., 2022). In addition, minimum bactericidal/fungicidal concentration (MBC/MFC) was detected as previously described (Kurt-Kızıldoğan et al., 2022).

ABTS Assay

ABTS method is dependent on the determination of the % RSA by measuring the colour change of ABTS radical cation ($ABTS^{\cdot+}$) with a spectrophotometer. The antioxidant capacity of *Dictyota dichotoma* oil was determined according to the modified form of the ABTS method (Re et al., 1999). Potassium persulfate (2.45 mM) and ABTS (7 mM) were used to prepare ABTS stock solution. Then, this solution was kept in a shaking incubator in a brown bottle for 12–16 h.

Dictyota dichotoma oil and trolox standard were dissolved with 2-propanol. Dilutions were made using 2-propanol at five different concentrations. While concentrations in the range of 1.4–90 μg/mL were prepared for *Dictyota dichotoma* oil, trolox concentration range was 0.31–5 μg/mL.

In order to start experiment, the stock solution was diluted with ethanol to obtain working solution. Then, standards and substances were mixed with ABTS working solution in equal proportions in 0.5 mL tubes and incubated in dark for half an hour at room temperature.

After incubation, they were transferred to a ninety six-well plate and measured at 734 nm wavelength. The % radical scavenging activity (% RSA) was calculated as indicated in Eq. (1) and compared with trolox.

$$\%\mathrm{RSA} = \frac{(\mathrm{ABS_{Blank}} - \mathrm{ABS_{Sample}})}{\mathrm{ABS_{Blank}}} \times 100. \qquad (1)$$

DPPH Assay

DPPH is based on the principle of measuring the colour change at 517 nm, which occurs when an antioxidant is added to the $DPPH^{\cdot}$ solution. The radical scavenging capacity of the tested *Dictyota dichotoma* oil was determined by modifying the DPPH method of Blois (BLOIS, 1958). DPPH was dissolved with pure 2-propanol at a concentration of 400 μM in a brown bottle. For complete dissolution, DPPH was left in a sonicator for 30 min. Concentrations in the range of 5.62–90 μg/mL were prepared by diluting the tested *Dictyota dichotoma* oil with 2-propanol. Furthermore, concentration ranges between 0.9375–15 μg/mL was used for trolox. Blank and each sample were incubated in dark half an hour in 0.5 mL tubes (containing100 μM DPPH) at room temperature. After incubation, they were transferred to a ninety six-well plate and measured at 517 nm. The % radical scavenging activity (%RSA) was calculated as stated in Eq. (1).

Statistical Analysis

GraphPad Prism 9 was used to perform statistical analyses. Differences between control and oil treated samples were analysed by one-way ANOVA. *p* values less than 0.05 were considered statistically significant.

RESULTS

Macroalgae Extract Analysis

The percentages of ash, lipid and protein content of macroalgae extract were 27.34 ± 0.72, 5.43 ± 0.23 and 8.70 ± 0.25, respectively. Lipids were determined in the chloroform phase after phase separation. In addition, *Dictyota dichotoma* oil extract fatty acid content was determined and listed in supplementary Table S2. The percentages of saturated, monounsaturated, and polyunsaturated fatty acids that were highest were: palmitic acid (16:0) 29.37 at %; palmitoleic acid (16:1n7) 5.02 at %; and eicosatetraenoic acid (20:4n3) 11.78 at %, respectively.

Cytotoxic Effects of Dictyota dichotoma Oil

MTT assays were performed to evaluate the cytotoxicity of *Dictyota dichotoma* oil on Caco-2, LnCap and HEK293. The experimental results were shown in Fig. 1. *Dictyota dichotoma* oil showed a dose-dependent inhibition of cell survival for Caco-2 and LnCap cells compared to the HEK293 cells. The EC_{50} values of *Dictyota dichotoma* oil were found 8.83 and 10.86 μg/mL for Caco-2 and LnCap cells, respectively. *Dictyota dichotoma* oil was also tested on noncancerous (HEK293) cells and doses of 0.78–25 μg/mL did not show significant viability effect on the HEK293 cells with an EC_{50} value of 26.92 μg/mL (Fig. 1). These results showed that the *Dictyota dicho-*

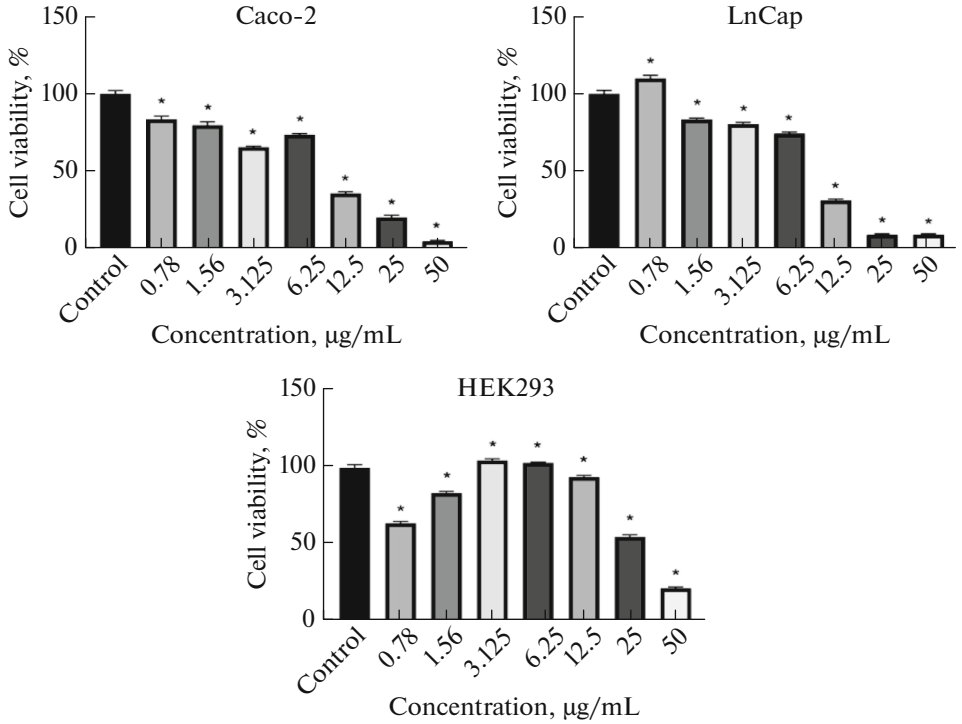


Fig. 1. Cytotoxic effects of *Dictyota dichotoma* oil on Caco-2, LnCap, and HEK293 cell lines, after 24 h of incubation. Results are mean ± SD values for three independent experiments. * $p < 0.05$.

toma oil showed better cytotoxic activity towards Caco-2 and LnCap compared to HEK293 cells.

The Effects of Dictyota dichotoma Oil on Apoptosis

Treatment of the Caco-2 and LnCap cells with *Dictyota dichotoma* oil for 24 h showed the induction of apoptosis in both cell lines. Besides, hydrogen peroxide was used as a positive control in this assay. The percentages of apoptotic cells after treatment were as 18 and 20% for Caco-2 and LnCap cells, respectively as shown in Fig. 2. Results revealed that *Dictyota dichotoma* oil significantly induced cell death/apoptosis of Caco-2 and LnCap cells, suggesting that *Dictyota dichotoma* oil might have a beneficial therapeutic effect in these cancer cells.

The Changes in Gene Expression by Dictyota dichotoma Oil

The effects of *Dictyota dichotoma* oil on caspases (3, 8 and 9), P53, Bcl-2 and Bax genes were determined by using *q*PCR with SYBR Green detection chemistry. Yılmaz et al. (2022) showed that the caspases and Bcl-2 family are the most important genes in the control of apoptosis (Yılmaz et al., 2022). Bcl-xs, Bid, Bad and Bax belonging to the Bcl-2 family stimulate apoptosis, but not Bcl-xl and Bcl-2.

In our study, in Caco-2 cells, while it was determined decrease in Bcl-2 expressions (1.08 fold), it was determined increase in the expression of P53 (2.09 fold), Bax (1.21 fold), caspase 3 (1.31 fold), caspase 8 (1.09 fold) and caspase 9 (1.68 fold) genes. Similarly, the related gene expressions of LnCap cells were investigated, while Bcl-2 expression was decreased 1.85 fold and it was found that expression of P53, Bax, caspase 3, caspase 8 and caspase 9 genes were increased 2.42, 1.59, 1.18, 3.04 and 3.08 fold, respectively (Fig. 3).

Antimicrobial Potential of Dictyota dichotoma Oil

Regarding bioassay data, *Dictyota dichotoma* slightly inhibited the growth of the *B. subtilis* ATCC 6633 strain by causing an average zone diameter of 8 mm (Table 1, Fig. S2). We further evaluated the antimicrobial activity of the compound by applying a broth microdilution assay to obtain detailed information. The MIC results were given in Table 2.

Exposure of gram-negative bacteria to *Dictyota dichotoma* oil was resulted in a MIC value of 256 μg/mL. Only *P. aeruginosa* ATCC 27853 showed a detectable MBC value (512 μg/mL) (Fig. S3), while the remaining two probably have higher MBC concentrations exceeding our scale. *E. faecalis* ATCC 29212 was the most sensitive bacterial strain against *Dictyotadichotoma*, with a 170.6 μg/mL MIC value. However, its MBC was higher (>512 μg/mL). Interestingly, *B. subtilis* ATCC 6633 provided the same MIC and MBC value of 256 μg/mL. Although, *S. aureus* ATCC 25923 had a MIC value of

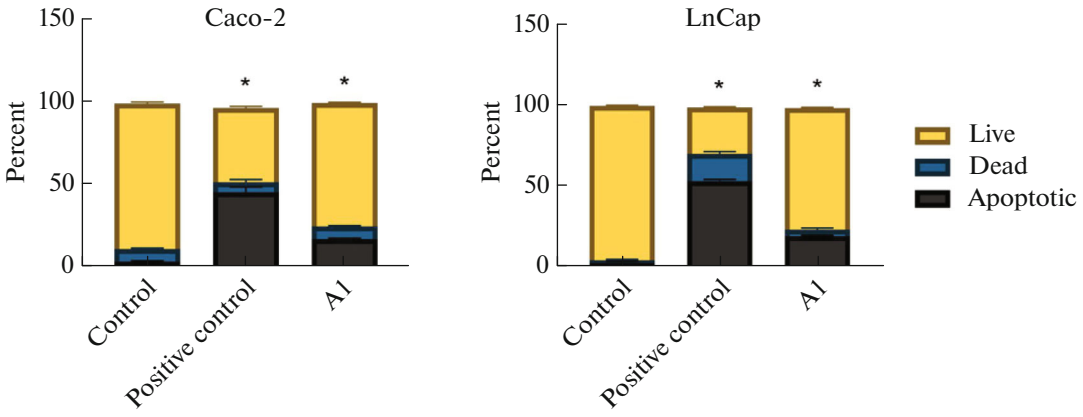


Fig. 2. Apoptosis analysis of *Dictyota dichotoma* oil in cell lines. Percentages of live, dead, and apoptotic cells were measured by Arthur image cytometer 24 h after exposure to EC_{50} concentration of *Dictyota dichotoma* oil. At least three independent experiments were considered in presented data. H_2O_2 was the positive control. Significantly different with respect to the respective control value (* $p < 0.05$).

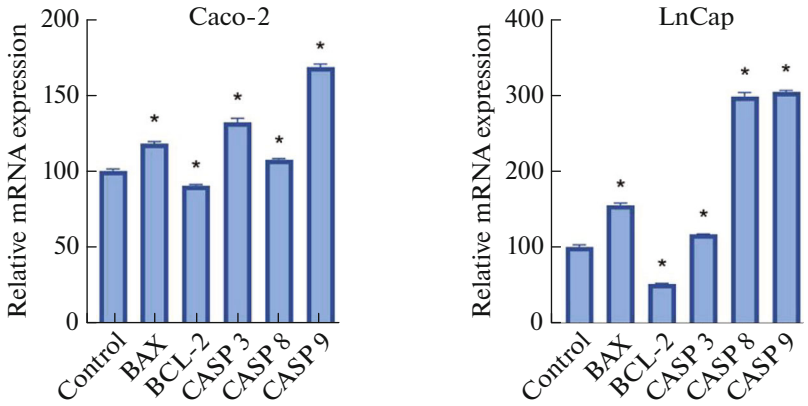


Fig. 3. Relative mRNA levels of P53, Bcl-2, Bax, Caspase 3, Caspase 8, and Caspase 9 in Caco-2 and LnCap cell lines after 24 h exposure to *Dictyota dichotoma* oil as determined by real-time PCR. The relative expression was normalized as a percentage of GAPDH mRNA by using the comparative threshold cycle by the $2^{-\Delta\Delta CT}$ method, * $p < 0.05$.

256 μg/mL, its MBC doubled (512 μg/mL). *C. albicans* did not have a high MIC value (256 μg/mL), and its MBC concentration was still higher than our microdilution test's highest concentration. Strikingly, *Dictyota dichotoma* showed remarkable inhibitory activity against *A. niger* ATCC 10231 with a MIC value of 32 μg/mL. However, its fungicidal concentration was very high (>512 μg/mL).

Antioxidant Potential of Dictyota dichotoma Oil

The amount of antioxidant substance that inhibits 50% of the radical is expressed as the EC_{50} value. In ABTS assay, while EC_{50} value of trolox, used as a standard, was calculated as 1.84 μg/mL, it was determined 8.83 μg/mL for *Dictyota dichotoma* oil. In DPPH assay, the EC_{50} value of trolox used as a standard was calculated as 6.64 μg/mL. However, even at the highest concentration of 67.5 μg/mL (final concentration) used for *Dictyota dichotoma* oil, the EC_{50} value could not be calculated since it inhibited only 18% of the DPPH radical.

DISCUSSION

Chemotherapy is the main treatment method for cancer. However, drug resistance and efficacy are the two major problems for chemotherapy. Therefore, many research groups have been searching new active compounds and extracts having less side effects and more efficient properties to treat the different types of cancer (Kavak et al., 2022). Recently, marine macroalgae have drawn a lot of interest as a potential source of new, inexpensive, safe and powerful agents for cancer treatment (Guedes et al., 2013). Therefore, it was assessed the anticancer, antimicrobial and antioxidant activity potential of *D. dichotoma* oil throughout in this study.

Table 1. Agar disc diffusion assay results of *Dictyota dichotoma* oil

Indicator strains	*D. dichotoma* (194 μg)	Amikacin (100 μg)	Ketoconazole (100 μg)	Amikacin (AK30 μg)/Flucanozole (FLU25 μg)
ATCC 700603 (*K. pneumoniae*)	Not determined	25 ± 1.41	Not determined	≥15/12–14/≤ 11
ATCC 25922 (*E. coli*)	Not determined	24.5 ± 0.7	Not determined	≥17/15–16/≤14
ATCC 25923 (*S. aureus*)	Not determined	31.5 ± 0.7	Not determined	≥17/15–16/≤ 14
ATCC 6633 (*B. subtilis*)	8 ± 0	26.5 ± 0.7	Not determined	≥16/15–16/≤ 15
ATCC 27853 (*P. aeruginosa*)	Not determined	25.5 ± 0.7	Not determined	≥17/15–16/≤14
ATCC 10231 (*C. albicans*)	Not determined	Not determined	16 ± 0.0	≥19/15–18/≤ 14
ATCC 16404 (*A. niger*)	Not determined	Not determined	16.5 ± 0.0	≥18/14–18/≤ 14
ATCC 29212 (*E. faecalis*)	Not determined	23.5 ± 0.7	Not determined	≥16/13–15/≤12

Zone diameter (mm): obtained by subtracting the zone diameters of DMSO.

Table 2. MBC and MIC values of *Dictyota dichotoma* oil against indicator strains

Indicator strains	*D. dichotoma*, μg mL^{-1}		* A ** B *** C	CLSIS/I/R**
	MIC/MIC_{50} for fungi	MBC/MFC		
P. aeruginosa ATCC 27853	256 ± 0.0	512 ± 0.0	1 ± 0.0	≤16/32/≥64
E. coli ATCC 25922	256 ± 0.0	>512	2 ± 0.0	≤16/32/≥64
S. aureus ATCC 25923	256 ± 0.0	512 ± 0.0	0.375 ± 0.17	≤16/32/≥64
B. subtilis ATCC 6633	256 ± 0.0	256 ± 0.0	0.375 ± 0.17	≤16/32/≥64
E. faecalis ATCC 29212	170.6 ± 73.9	>512	32 ± 0.0	≤64/128/≥256
K. pneumoniae ATCC 700603	256 ± 0.0	>512	0.5 ± 0.0	≤4/–/>32
C. albicans ATCC 10231	256 ± 0.0	>512	256 ± 0.0	≤14 ≥ 19
A. niger ATCC 16404	32 ± 0.0	512 ± 0.0	256 ± 0.0	4–16

** S/I/R: Sensitive/Intermediate/Resistant, * A: MIC for standards (μg mL^{-1}), ** B: Amikacin for bacteria, *** C: Flucanozole for fungi.

These results showed that the *Dictyota dichotoma* had cytotoxic activity towards colorectal adenocarcinoma (Caco-2) and human prostate adenocarcinoma (LnCap) cells. Lezcano et al. (2023) showed that different extracts from brown algae, including *D. dichotoma*, have cytotoxic activity against human cancer cell (Lezcano et al., 2023).

El-Shaibany et al. (2020) demonstrated that *D. dichotoma* extracts showed dose-dependent cytotoxicity especially for MCF-7 and prostate cancer cell lines. Moreover, moderate antitumor activity was observed after treatment of *Dictyota dichotoma* extracts against MCF-7 and HepG2 cancer cells (Alaidroos et al., 2022). *Dictyota dichotoma* showed strong cytotoxicity against the Daudi, Jurkat and K562 cancer cell lines (Zubia et al., 2009). Also, Go et al. (2010) and Ermakova et al. (2011) demonstrated that other brown algae such as *Eclonia cava*, *Costaria costata*, *Laminaria japonica* and *Sargassum hornery* showed cytotoxicity in human colon cancer cells (Go et al., 2010; Ermakova et al., 2011). The cytotoxic activity of the *Dictyota dichotoma* oil can be attributed to different mechanism such as apoptosis.

As stated by Jin and El-Deiry (2005), the process of apoptosis occurs in two pathways: mitochondrial pathways and death receptor. Salvesen and Dixit (1997) reported that caspases -3, -8 and -9 are important in the apoptosis signalling pathway, and caspase-8 can lead to caspase activation cascade signalling (Salvesen and Dixit, 1997). Monla et al (2020) revealed that the Bcl 2 family is a regulator of mitochondrial permeability and can suppress the activation of caspases and therefore the release of cytochrome c and many factors. Our results showed that *Dictyota dichotoma* oil significantly induces apoptosis in Caco-2 and LnCap cells, suggesting it may have a beneficial therapeutic effect in these cancer cells. These results confirmed by *q*PCR studies (Fig. 3). Apoptosis related gene expressions of Caco-2 cells showed that decreased Bcl-2 expressions. However, mRNA levels of Bax and all tested caspase genes (3, 8 and 9) were increased in Caco-2 cells. In addition, similar expression pattern were obtained in LnCap cells. These

results revealed that *Dictyota dichotoma* oil seems to play a role in induction of apoptosis in human cancer cells, but the extent to which the oil induces apoptosis may differ among cancer cells. Moreover, intrinsic and extrinsic pathways might be involved in *Dictyota dichotoma* oil induced apoptosis.

Dictyota dichotoma oil extracts showed a moderate antibacterial and antifungal activity against *E. faecalis* ATCC 29212 and *A. niger* ATCC 10231, respectively. The first is a gram-positive and nosocomial pathogen found in animal intestines (Kim et al., 2012). The latter is a member of genus causing severe diseases and allergic reactions in indoor environments (Mousavi et al., 2016). Ambreen et al. (2012) reported that brown macroalgae have high antimicrobial activity, and among brown macroalgae species, the highest antibacterial activity was detected in Dictyota species.

Ibraheem et al. (2017b) and Demirel et al. (2009) determined that extracts obtained from *Dictyota dichotoma* species showed antimicrobial effects on gram-negative and gram-positive bacteria. Alaidroos et al. (2022) stated that extracts obtained from *Dictyota dichotoma* and *Halimeda tuna* showed the highest antimicrobial activity against *Streptococcus pneumonia* and *Staphylococcus aureus*, and that the combined extract mixture was effective for all bacteria tested in the study. When compared with the literature data our results indicate that oil obtained from *D. dichotoma* provides a promising antimicrobial activity. However, its potency might be further improved by structural and molecular analysis and in vitro structural modifications.

It is observed that the TEAC method shows a higher activity in antioxidant tests performed for the oil extracted from *Dictyota dichotoma*. This relatively high antioxidant capacity measured in oil sample, with a potency of approximately 4.8 fold less EC_{50} value compared to the trolox standard, is in line with some other studies on brown algae of this species and the same Genus (Miranda-Delgado et al., 2018; Mekinić et al., 2021; Imran et al., 2023; Martić et al., 2023).

CONCLUSIONS

It is well established that macroalgae are good source of secondary bioactive metabolites responsible for important biological activities. According to our findings, this study may serve as a starting point for future research aimed at exploiting new natural compounds, particularly for anticancer applications. In conjunction with antimicrobial activity against fungi (*A. niger*) and bacteria (*E. faecalis*), it possesses sufficient antioxidant capacity to be enhanced. This oil could be potential use in the pharmaceutical industry. But, further studies including fractionation of oil, isolation and characterization of new molecules will be required.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1134/S1062359024608814.

Table S1. Sequences of the primers used for the real-time PCR

Table S2. Fatty acid composition (%) of the macroalgae *Dictyota dichotoma* oil

Fig. S1. *Dictyota dichotoma*.

Fig. S2. Inhibition zone diameters of the indicator strain against *Dictyota dichotoma* oil.

Fig. S3. MBC results of *Dictyota dichotoma* oil. The numbers on agar plate indicate the number of wells in the MIC plates from which the samples were taken; 1: 512 μg/mL, 2: 256 μg/mL, 3: 128 μg/mL, 4: 64 μg/mL, 5: 32 μg/mL.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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