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In vitro antibiofilm, antibacterial, antioxidant, and antitumor activities of the brown alga *Padina pavonica* biomass extract

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ABSTRACT

The antibiofilm, antibacterial, antioxidant, and anticancer activities of the methanolic extract of *Padina pavonica* L. were determined. Results deduced that the algal extract had a high biofilm formation inhibitory action done via crystal violet (CV) assay, to 88–99%. The results showed a strong antibacterial against the identified bacteria species. *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis*, and the extract had moderate antibacterial activity against *Escherichia coli*, *Pseudomonas fluorescens* and *Streptococcus agalactiae*. The algal extract has a concentration-dependent DPPH radical scavenging activity (84.59%, with IC_{50} = 170.31 µg/ml). The inhibitory percent of *P. pavonica* methanolic extract *in vitro* antiproliferative activity was 1.79–98.25% with IC_{50} = 15.14 µg/ml against lung carcinoma. Phenols, terpenes, amino acids, alkaloids, flavones, alcohols, and fatty acids were among the metabolites whose biological actions were evaluated. In conclusion, for the first time, *P. pavonica* methanolic extract exhibited effective antibiofilm, antibacterial, antioxidant, and anticancer activities.

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KEYWORDS
Antibacterial; antibiofilm; *padina pavonica*; methanolic extract; lung cancer

Introduction

With more than 70% of the earth's surface, the oceans represent the largest habitat of the earth and a prolific resource of organisms with high biological and chemical diversity. Although most drugs are still derived from terrestrial sources, a considerable number of drugs, drug candidates, and other metabolites from marine organisms have been identified in recent years. About 30,000 compounds of marine origin are known and since 2008, more than 1,000 compounds have been newly discovered each year. They are often characterized by structural novelty, complexity, and diversity (Kiuru et al. 2014; Hu et al. 2015). Continuous annual reviews about novel marine natural products were published in Natural Products Report by Faulkner till 2002 and then by Blunt et al. Only the last one from 2016 is cited in this review (Blunt et al. 2016). Brown, green, blue-green, and red algae make up the marine flora. Brown algae, in particular, were employed as a source of secondary metabolites with various biological functions (Saadaoui et al. 2020). They are gaining much attention for creating chemically and pharmacologically new compounds with various biological potency.Shimizu (1996).

In the last three decades, seaweeds have piqued the scientific community's interest attributed of their ability to combat diseases such as biliary, gastrointestinal discomfort, allergies, melanoma, hepatic disorders, scabies, psoriasis, bronchitis, atherosclerosis, heart problems, lung disorders, and ulcers (El-Kassas and El-Sheekh 2014; Nabti et al. 2017). The pharmaceutical potency of such

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substances has been extensively researched for regenerative medicine. As a result, carotenoids, sugars, lipids, glycolipids, alkaloid compounds, terpenes, and many other compounds were discovered. Many of these compounds are undergoing clinical as well as preclinical testing (Saadaoui et al. 2020). Bacteria must adapt to changing environmental conditions by changing their growth pattern. A biofilm, found in approximately 90% of bacteria, is one favorable style. A three-dimensional bacterial aggregation is a biofilm within a matrix outside the bacterial cells. Bacterial cells adhere to surfaces by becoming sessile and secret polymeric substances outside the cells to safeguard themselves from any stresses (Shinde et al. 2021). Biofilm influences many chronic diseases, like bronchitis and immune disorders. The important quantification methods for biofilm inhibition detection are using the wells of microtiter plates as culture vessels, and the results were measured spectrophotometrically, as stated by Stepanovic´ et al. (2007). Treating infectious diseases resulting from biofilm formation is becoming more complicated due to multidrug resistance, which remains a serious public health issue. Dalia et al. (2020) estimated the antibiofilm activity of 3 seaweeds, *Sargassum vulgare* (Phaeophyceae), *Ulva lactuca (*formerly *Ulva fasciata)* (Chlorophyta), and *Jania rubens* (Rhodophyta) aqueous extract discovered that *J. rubens* showed the highest antibiofilm activity. Suganya et al. (2019) evaluated the antibiofilm activities of *Sargassum wightii* (Phaeophyceae) and *Halimeda gracilis* (Chlorophyta) ethanol extract and found that *S. wightii* ethanol extract has the strongest antibiofilm activity. In many studies, methanol is reported to be a more effective solvent (in terms of extracting bioactive metabolites) (Gupta et al. 2012; Al-Hazzani et al. 2014). Gadhi et al. (2018) used many solvents for secondary metabolite extraction and found that methanol was the best solvent for the extraction of antibiofilm metabolites from *Halimeda* sp. It is worth to say that antibiofilm activity is a novel application for *P. pavonica* methanol extract.

Cancer is a major global public health issue; lung cancer is a major cause of cancer-related deaths (WHO, 2020; Curran and Hussong 2003). Sadly, traditional therapeutic approaches, including surgical procedures, radiation therapy, and chemotherapy, were only partially effective. As a result, finding alternative therapies to slow the progression of lung cancer is critical.

Many biological properties have been demonstrated by algal products like carbohydrates, alkaloids, lipids, pigments, terpenes, and phenolics, including anticancer activity (Gheda et al. 2018; Saadaoui et al. 2020). These bioactive chemicals bind to various locations, restrict cell division, or induce apoptosis by stimulating specific cellular pathways (Blunt et al. 2015). The growing interest in employing macroalgae as a healthy, secure, reusable supplier of nutritional supplements and drugs targeting tumoral cells resulting an increase in associated papers. However, the emphasis was on specialized health applications, with a small number of articles devoted to anti-lung cancer research. Antioxidant activity is significant in various pharmacological processes, anticancer, and other important processes (Middleton et al. 2000; Lee et al. 2004; El Shafay et al. 2021).

Madhavi et al. (1995) demonstrated that most nutraceuticals and cosmeceuticals are said to have antioxidant action. However, many synthetic antioxidants are manufactured, but their toxicity is a worry. Natural compounds having antioxidant activity, such as those derived from algae, on the other hand, are used for human consumption due to their safety.

Seaweed extracts have been demonstrated to have strong antioxidant effects (Ibrahim et al. 2016; Mohy El-Din and El-Ahwany 2016; El-Shenody et al. 2019; Ismail et al. 2020; Al-Araby et al. 2020; El-Sheekh et al. 2020; Makhlof et al. 2022). Bioactive components such as dietary fibers, carotenoids, lipids, and amino acids, are abundant within marine macroalgae. Seaweed has long been used as a food source in several Asian countries, including Korea and China (Wijesekara et al. 2012; Barba et al. 2017; Lopez-Santamarina et al. 2020). A few populations of *Padina* have traditionally been utilized as a source of food in numerous places and are recognized as jelly candy (Robledo and Freile-Pelegrin 1997). *Padina pavonica* body contains two parts: the thallus, which is divided into 8, and sometimes more, whitish to brownish color fronds, and the holdfast, consisting of flexible rhizoids for surface attachment (Aisha and Shameel, 2010). The fronds are fan or ear-shaped and can reach up to 15 cm in length in the summertime, becoming narrower towards the base (Benita

et al. 2018). The motivation behind this work is to look at the biochemical content of *P. pavonica*, which is widely found in Alexandria's Rocky Bay of Abu Qir. The antibacterial, antibiofilm, antioxidant, and anti-lung cancer activities of the examined seaweed extract were estimated, confirming the seaweed's pharmacological, therapeutic, and nutritional applications which were done for the first time.

Material and methods

Algal sampling

Padina pavonica was obtained recently in October from Alexandria, Egypt, from Rocky Bay of Abu Qir. The samples were thoroughly cleansed with seawater, then distilled water, and transported to the laboratory in an icy state. The seaweed was powdered and kept in tightly sealed pages for further investigation after being dried in the shade by air, followed by oven drying at 60°C. A fraction of the alga was kept in 3–4% formalin in distilled water for taxonomic identification. The algae were identified by Prof. Dr. Mohamed Saad Abd El-Kareem, Professor of Phycology, Botany and Microbiology Department, Faculty of Science, Alexandria University, Egypt. According to Aleem (1993), seaweed was identified (Jha et al. 2009; Kanaan and Belous 2016). Second, double-check the identification and habitat information by the Algae Base website (Guiry and Guiry 2019).

Algal extract preparing

The methanol extract was carried out by putting the sample (brown powder) within solvent with a ratio of 1:30 w/v as 10 gm algal powder was extracted using 300 ml ethanol in a Perkin Elmer flask and was sealed. The mixtures were kept for 3 days at room temperature in a shaking incubator. After 3 days, the samples were centrifuged at 7212 g for 20 min. The extract was evaporated with a rotary evaporator at 40°C with decreased pressure (72 mbar) and then lyophilized. To prevent microbiological contamination, the crude algal extract was resuspended in methanol with 5 mg/mL concentration, filtered through a sterilized bacterial syringe filter (CHM CA syringe filter 0.22 um pore size, Chem lab group), and then stored at −20 °C in a sealed tube, the extraction yield % of the methanolic extract was estimated according to (Maisuthisakul et al. 2007).

Extraction yield%= ($Weight_1$/$Weight_2$)*10.

Where $Weight_1$ is the weight of dried crude extract, and W_2 is the weight of the sample before extraction (10 gm).

Evaluation of antibiofilm and antibacterial activities

Bacterial strains

The study presented here encompasses a collection of eight pathogenic bacterial isolates of Gram-positive and Gram-negative strains: *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29,212), *Pseudomonas aeruginosa* (ATCC 9027), *Klebsiella pneumonia* (ATCC 13,883), *Pseudomonas fluorescens* (ATCC13525) and *Streptococcus agaloctiae* (ATCC13813).

Evaluation of the antibacterial activity of Padina pavonica methanolic extract

P. pavonica extract was tested for antibacterial activity by the disc diffusion method in plates using Gentamicin as a reference drug. 6 mm diameter sterile paper discs were saturated by 25 µl of the sample, placed on the agar medium surface (Mueller-Hinton Agar, pH 7.4 0.2 at 25°C) earlier loaded with pathogenic bacterial cells, incubation was done at 37℃ for 24 hours. The radius of inhibitory halos of the growth of the bacteria around the disc is measured in millimeters, and the findings are presented in millimeters. As a negative control, absolute methanol without algal extract

was employed (Zbakh et al. 2012). Experiments were repeated three times, with the findings shown as the average inhibition zones (mm) and standard deviation.

Biofilm measurement using quantitative absorbance (crystal violet method)

Carneiro et al. (2020) method has been applied with some changes to evaluate the inhibitory effect of the methanolic *Padina pavonica* extract on pathogenic bacterial biofilm formation. The algal methanolic extract was applied to a suspension of bacterial cells (2×106 (CFU) mL^{-1}) in polystyrene plates with 96-well at concentrations ranging from 0.0625 to 0.75 g mL^{-1}. The plates were incubated for 24 h at 37°C. After that, total biomass measurement was used to gauge the development of biofilms. Crystal violet staining was used to determine the total biomass. The biofilms were fixed for 15 min in 200 µL of methanol, after which the polystyrene plate was left to become dry at 25°C; then, wells' staining was done for 5 min by using 200 µL c.v (1%, v/v), the surplus stain was eliminated, and distilled water was used to clean the plate.

Measuring the optical density of each well was done at 540 nm (OD540) by a microplate reader after 200 µL of glacial acetic acid (33%, v/v) was added to dissolve the crystal violet stain (Erbalisa Scan). All of the results were triple-checked and graphically depicted. These data were then utilized to calculate the percentage of biofilm inhibition using the equation provided in the study (Shinde et al. 2021).

Inhibition% = [(OD C – OD S)/OD C] × 100

ODC: is the optical density of the control

ODS: is the optical density of the sample

Antioxidant activities estimation

Determination of DPPH (2, 2-Diphenyl-1-picryl hydrazyl) radical scavenging activity:

Antioxidant-acting molecules prevented the oxidation of DPPH, which tested the free radical scavenging properties. One hundred microlitres of DPPH were combined with 100 microlitres of algal extract at various concentrations ranging from 0 to 1280 µg, 100 µL of standard (gallic acid), or 100 µL of methanol (control). The reaction mixture was vortexed completely before being left at the temperature of the room for 30 min. The mixture's absorbance was measured using a spectrophotometer at 517 nm (Blois 1958). The following equation was used to calculate the DPPH scavenging activity percent:

The percentage of DPPH scavenging activity = (Abs C – Abs S/Abs C) x 100

Abs C is the absorbance of the DPPH value, and Abs S is the sample and DPPH absorbance value. Using the plotted graph of inhibition (%) against extract conc., the extract concentration providing 50% inhibition (EC50) was computed. As a positive control, the ascorbic acid methanolic solution was employed. Increased reducing power was associated with higher absorbance. The reducing power of DPPH was measured in mg ascorbic acid equivalents per gram of crude extract (Yen and Duh 1994).

Evaluation of cytotoxic effects against A-549 cells (lung carcinoma)

Cell lines of mammalian

A-549 cells were donated via ATCC Culture Collection (lung cancer). Dimethyl sulfoxide (DMSO), crystal violet, and trypan blue dye were all given by Sigma (St. Louis, Missouri, USA). Fetal Bovine Serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin, and 0.25% Trypsin-EDTA were all supplied by Lonza. 0.5% (w/v) crystal violet and 50% methanol are combined to make a 1% crystal violet stain, which is then filtered through Whatman No. 1 filter paper.

Propagation of cell line

The cells were raised in Dulbecco's modified Eagle's medium (DMEM), which also contained HEPES buffer, 50 g/mL gentamycin, 10% heat-inactivated foetal bovine serum, and 1% L-glutamine. All cells were subcultured twice each week at 37°C in a humid atmosphere with 5% CO_2.

Using viability assay in cytotoxicity evaluation

Cancer cells were planted on a plate with 96-well with a cell concentration of 1×10^4 cell/well in 100 µl of nutrient media for the cytotoxicity experiment. A fresh medium containing varied dosages of the test substance was introduced after 24 h of seeding. Confluent cell monolayers placed into 96-well, flat-bottomed microtiter plates were given repeated two-fold dilutions of the investigated chemical component using a multichannel pipette (Falcon, NJ, USA). The microtiter plates have incubation for 24 h at 37°C with CO_2 at conc 5% in a moist environment. For each concentration, the test substance was split into three wells. Control cells were cultured with or without DMSO in the absence of a test material. The modest amount of DMSO present in the wells was demonstrated to have no impact on the experiment (maximum 0.1%). After the cells were cultured at 37°C for 24 h, the viable cell yield was assessed using a colorimetric technique. In brief, the media were aspirated during incubation, and each well received a 30-min application of the 1% crystal violet solution. The plates were cleaned with tap water to remove the stain to get rid of any remaining discoloration. After shaking the plates gently, the absorbance was measured on a microplate reader (TECAN, Inc.) using a test wavelength of 490 nm, adding glacial acetic acid (30%) to the wells with thorough mixing. Background absorbance detected in wells without additional stain was adjusted for in all results. Without the investigated substances, samples with treatment were compared to the control cells. The experiments were all carried out twice. The cytotoxic impact of every substance on cells was calculated. Using a microplate reader to measure the optical density, the number of live cells was ascertained (SunRise, TECAN, Inc., USA), and viability (%) was calculated as $(OD_{test}/OD_{control})$ x100%, where OD_{test} is the read of wells treated with sample and $OD_{control}$ is the read of untreated wells. The curve of survival per cancer cell line following adding the specified chemical is displayed utilizing the association between surviving cells and drug concentration. The 50% inhibitory concentration (IC50) needed to cause negative effects in 50% of intact cells was computed using Graphpad Prism software (San Diego, CA, USA) (Mosmann, 1983; Gomha et al., 2015).

Gas-liquid chromatography of P. pavonica methanolic extract

Instrumentation and chromatographic conditions

The system of GC/MS: To analyze, the *P. pavonica* methanolic extract Gas Chromatograph (Thermo Scientific TRACE 1310) attached with ISQ LT single quadrupole M. S. was used. Acquisition parameters: the initial temperature of the oven was 40°C/3 min that ramped from 5°C/min to 280°C, wait 5 min, the temperature of the injector = 200°C, source temperature = 300°C, transfer temp = 250°C, column (DB5-MS, 30 m; 0.25 mm ID (J&W Scientific)). Scan = 50–500 Da, the ratio of splitting = 20:1, Injection volume = 1.0 µL of *P. pavonica* sample, the used gas as the carrier was Helium of flow (1 ml per minute). Solvent delay = 5.00 min.

Characterization of the methanolic extract by FTIR spectra

The methanolic extract was characterized by FTIR spectroscopy (a Magna-FTIR 560 (USA) instrument). B.W. Souza et al. 2012 crushed 5 mg of *P. pavonica* methanolic extract residue with KBr (spectroscopic grade) powder and compressed it into a 1 mm pellet to make FTIR measuring at wavelength 400–4000 cm^1. The FTIR spectrum was examined using spectral consistency data from three KBr powder samples taken from the seaweed.

Result and discussion

Collected seaweed taxonomic description

The seaweed was identified using the literature, with synonyms and the most recent approved names checked, and the alga linked to its systematic groupings and described. *Padina pavonica* (Linnaeus) Thivy formerly *Padina pavonia* (Linnaeus) J.V.Lamouroux this class Phaeophyceae, was the species collected.

Extraction yield

As there are numerous valuable molecules with antioxidant activity, methanol was used to extract the crude extract, which was then utilized to test the antioxidant and anti-lung cancer actions. Furthermore, the extraction solvent's polarity index was: methanol (5.1). Because methanol is a polar solvent, it was chosen. In red and brown algae, there was a trend toward higher polar solvent yields than non-polar solvent yields, with the brown alga remaining the most excellent yielder (El-Sheekh et al. 2020). This result agrees with that of (Truong et al. 2019), who found that in *Atalantia buxifolia*, formerly *Severinia buxifolia (Angiosperm, Rutaceae)*, more polar solvents have a higher extraction efficiency. This could be because this plant extract contains many polar chemicals soluble in polar solvents like water, methanol, and ethanol. El-Sheekh et al. (2020) investigated the antioxidant capability of bioactive components in the extract to understand the solvent's effect on the extraction yield. The polarity of different components found in the seaweeds and species – species variances could explain the substantial discrepancies in extraction yield seen among seaweed species Agregán et al. (2018). (For the reasons stated above, we used methanol as the solvent in this work. *P. pavonica* yielded (5.1%) with methanol extract).

Antibiofilm activity of the algal methanolic extract

Padina pavonica methanolic extract had a strong and promising antibiofilm inhibitory effect on all tested pathogenic bacteria, where the antibiofilm % increased gradually by rising the algal extract concentrations, Figure 1(a,b). Algal methanolic extract exhibit the strongest antibiofilm % towards *Pseudomonas aeruginosa ATCC 9027*, where the result could reach 99% with the highest concentration of 0.75 µg/ml of extract, followed by 96% antibiofilm inhibition towards *Bacillus subtilis* ATCC 6633, then the % reached 93.97% against *Escherichia coli* ATCC 8739, and 93.26% against *Staphylococcus aureus* ATCC25923, then 93.12% antibiofilm activity recorded from the *Padina pavonica* extract towards *Pseudomonas fluorescens* ATCC13525, the antibiofilm % decreased to be 92.24% against *Klebsiella pneumonia ATCC 13,883*, and 92.15% achieved against *Enterococcus faecalis* ATCC 29,212; finally, the lowest activity was 88.44% against *Streptococcus agalactiae* ATCC13813; we did all experiments three times and represented graphically as (mean ±SD) as shown in Figure 2(a-h).

Similarly, Ba-Akdah (2018) found that the extract of *Padina sp*. has strong biofilm inhibitory effects against *Vibrio harveyi*, implying that *Padina sp*. could be employed as a potential marine source for discovering bioactive chemicals for the creation of natural product antibiofilm compounds such as Morphinone, an alkaloid detected in the Gc analysis of the tested alga. Also, according to Ba-Akdah (2018), seaweed extracts (*Padina* sp.) influenced the generation of

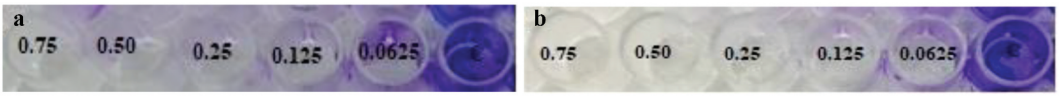

Figure 1. (a and b) The microtiter plates for antibiofilm quantification CV assay with different concentrations of *Padina pavonica* methanolic extract in each well; (a): *Enterococcus faecalis* ATCC 29,212 and (b) *Bacillus subtilis* ATCC 6633.

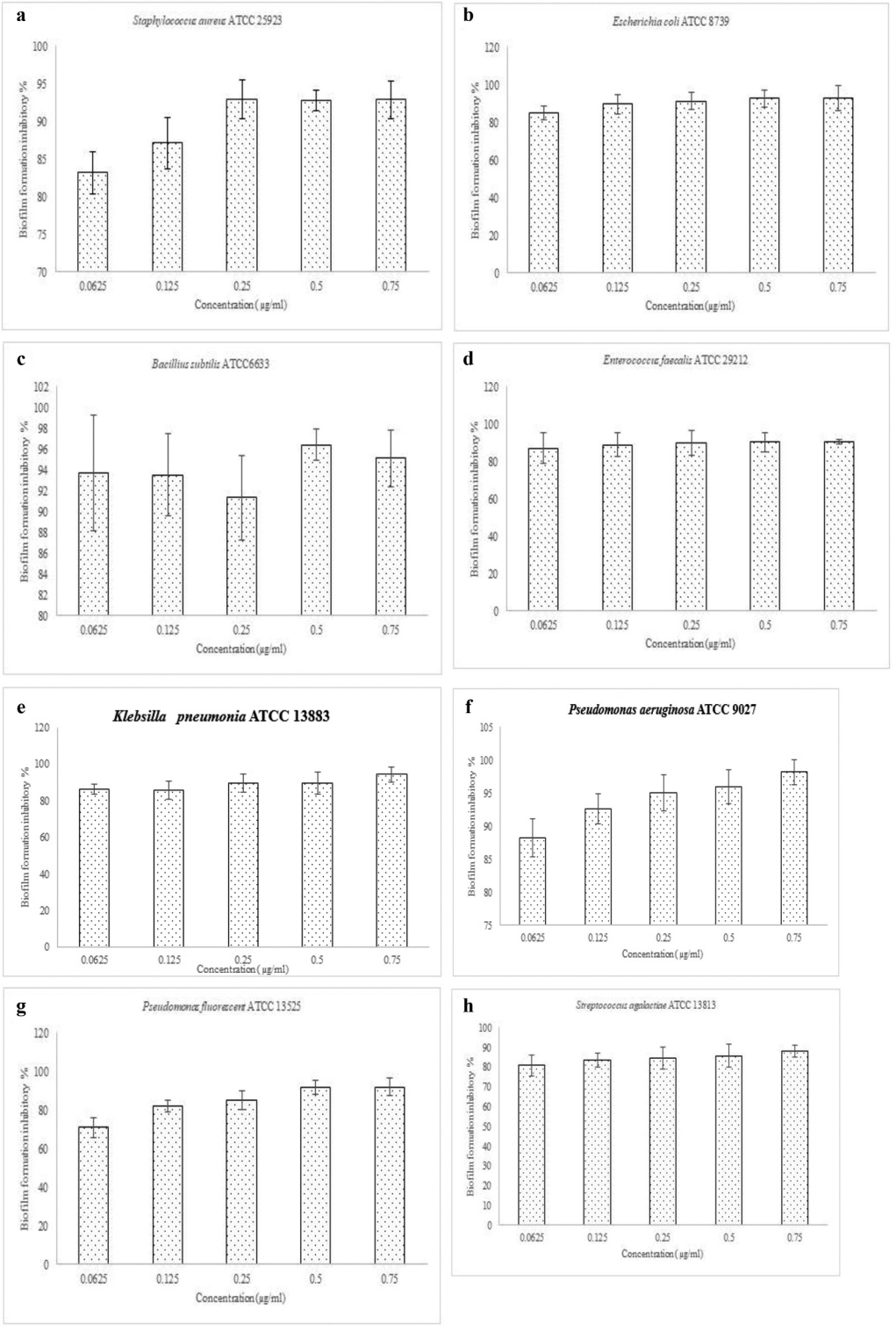

Figure 2. (a to h). Antibiofilm activity of *Padina pavonica* methanolic extract against (a): *Staphylococcus aureus* ATCC25923, (b): *Escherichia coli* ATCC 8739, (c): *Bacillus subtilis* ATCC 6633 (d) *Enterococcus faecalis* ATCC 29,212, (e): *Pseudomonas aeruginosa ATCC 9027*, (f*): Klebsiella pneumonia ATCC 13,883*, (g) *Pseudomonas fluorescens* ATCC13525, and (h): *Streptococcus agalactiae* ATCC13813. The antibiofilm activity was measured using microtiter plate assay. Experiments were repeated in triplicate. The

exopolysaccharides in bacterial strains, which is an important stage in preventing biofilm formation. Kenny et al. (2009) and Arsic et al. (2012) detected that increased cell disruption and release of proteases were detected when methicillin-resistant *S. aureus* (MRSA) was grown with 50 µM linoleic acid. It has been reported that both oleic and linoleic acid inhibit the fatty acid synthesis of S. aureus (Zheng et al. 2005) and these fatty acids were detected in the Gc report (Table 1) of the tested weed

Antibacterial activity evaluation of the algal methanolic extract

Table 2 illustrates the strong and remarkable antibacterial activity of *Padina pavonica* methanol extract against all tested Gram-positive and Gram-negative bacterial pathogens, negative results were obtained when using absolute methanol only without adding the algal extract, is shown in. No activity (-: diameter inhibition 10 mm), low (+: diameter inhibition 10–15 mm), moderate (++: diameter inhibition 15–20 mm), and high activity (+++: diameter inhibition up to 20 mm) were used to classify antibacterial activity. Figure 3 shows one of the remarkable antibacterial actions of our extract against *Bacillus subtilis* (ATCC 6633). However, the inhibition zones made by *Padina pavonica* extract against all tested pathogens were promising. The inhibitory zones were measured in millimeters, and the standard deviation was calculated (SD). The highest antibacterial activity levels were reported to be against *Klebsiella pneumonia* (ATCC 13,883), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC 29,212), and *Bacillus subtilis* (ATCC 6633) by mean I.Z. and SD of (26.5 ± 2.6457, 24.666 ± 2.7537, 24 ± 2, 23.666 ± 2.7537 and 20.833 ± 2.5166), respectively. This might be in the same view of Sameeh et al. (2016) that the reduction in growth possibly occurred due to interference by active compounds in the extract. This was in agreement with Awad et al. (2009), who discovered that the *P. pavonica* volatile fractions had a substantial antibacterial effect against *Bacillus cereus*.

Tüney et al. (2006) used 11 Turkish marine seaweeds to investigate their susceptibility to pathogenic bacteria. When tested against *Escherichia coli* and *Staphylococcus aureus* germs, *Padina* sp. had a significant impact, especially when methanolic extracts were used, where methanolic extracts of *Padina sp*. could only show maximum activity from 25% to 30% against bacterial cells (Rajasulochana et al. 2009). Also, Kamenarskaa et al. (2002) studied the antibacterial activity of *P. pavonica* methanolic extract and found that; this extract showed a considerable action against gram+ve bacteria *Staphylococcus aureus*. Ozdemir et al. (2006) found that active compounds from *Dictyopteris polypodioides* (formerly *Dictyopteris membranacea*) membranaceous and *Gongolaria barbata* (formerly *Cystoseira barbata*) (Phaeophyceae) did not significantly limit the growth of the microbes in their investigation.

The current results can have the same explanation suggested by (Sonbol et al. 2021). Extracts of *P. boryana* are also rich in biologically active compounds including compounds with high phenolic content, including flavonoids and tannins, proteins and steroids with antimicrobial activities. Sulfolane, a phenolic compound detected by Gc in our sample, is a good antimicrobial agent already used in drug manufacturing (Zarovnaya et al. 2014).

Sameeh et al. (2016), reported that; the ability of algae to produce antimicrobial substances could be used not only as a defense agent (against pathogens) but also as pharmaceutical bioactive natural compounds. Though much is known about the chemistry and the antimicrobial action of several phytochemicals, very few reports are available on the possible mechanism of action. Algal

percentage of inhibition with different concentrations of *Padina pavonica* methanolic extract against different pathogenic bacteria indicated the efficiency of this algal extract in biofilm inhibition. Error bars represent standard deviations from the mean ($n = 3$).

Table 1. GC analysis of *Padina pavonica* methanolic extract.

RT	Compound	Common name	Molecular formula	Peak area %	M. WT	Chemical group
9.25	Thiophene, tetrahydro-, 1,1dioxide	Sulfolane	$C_4H_8O_2S$	1.78	120	Phenols
16.56	2(1 H)-Naphthalenone, Octahydro-1-Methyl-1-(2-Propenyl)-, (1à,4Aá,8Aà)-	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	0.23	206	
22.07	3-Buten-2-ONE, 4-(6,6-Dimethyl-1-Cyclohex en-1-YL)-	Propofol	$C_{12}H_{18}O$	0.88	178	
28.12	Phenol, 4,4′-methylenebis-	2-(Benzyloxy)phenol	$C_{13}H_{12}O_2$	1.54	200	
13.01	Isobornyl thiocyanoacetate	Thanite	$C_{13}H_{19}NO_2S$	0.18	253	terpenes
13.01	3-Thujanol	Geraniol	$C_{10}H_{18}O$	0.18	154	
23.60	Neophytadiene	Neophytadiene	$C_{20}H_{38}$	2.76	278	
23.60	2-Hexadecen-1-OL, 3,7,11,15-Tetramethyl-,	Icosanal	$C_{20}H_{40}O$	2.76	296	
23.88	8-Phenyloctanoic acid	Butibufen	$C_{14}H_{20}O_2$	0.45	220	
24.45	Phytol	-	$C_{20}H_{40}O$	2.17	296	
24.60	Isochiapin B	Gibberellic acid	$C_{19}H_{22}O_6$	0.24	346	
30.55	cis-5,8,11,14,17-Eicosapentaenoic acid	Abietic acid	$C_{20}H_{30}O_2$	0.86	302	
30.72	Cis-2-Phenyl-1, 3-Dioxolane-4-Methyl Octadec-9, 12, 15-Trienoate	Suillin	$C_{28}H_{40}O_4$	3.98	440	
34.07	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	Dammarenediol	$C_{30}H_{52}O_2$	0.35	444	
40.72	10-Acetoxy-2-hydroxy-1,2,6a,6b,9, 9,12a-heptamethyl-1,3,4,5,6,6a,6b,7, 8,8a,9,10,11,12,12a,12b,13,14b-oc tadecahydro-2 H-picene-4a-carboxyl ic acid, methyl ester	Pachymic acid	$C_{33}H_{52}O_5$	2.08	528	
13.01	Isobornyl acetate	Geranyl acetate	$C_{12}H_{20}O_2$	0.18	196	Alcohol
24.09	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	0.58	312	
25.11	1-Heptatriacotanol	1-Heptatriacotanol	$C_{37}H_{76}O$	0.33	536	
16.80	*N*-(4-[2(DimeT\thylamino)Et hoxy]Bnnzyl)-3,4,5 Trimethoxybenzamide #	Ramiprilat	$C_{21}H_{28}N_2O_5$	0.20	388	Amino acid
21.43	1-(3-Methyl-2-butenyl)-3,6-diazahomoadamantan-9-ol	1-Amino-3-[(1,1-dimethylethyl) (phenylmethyl) amino]propan-2-OL	$C_{14}H_{24}N_2O$	0.36	236	
21.43	Octanal,(2,4-dinitrophenyl)hydrazone	L-Arginine, N2-[(phenylmethoxy) carbonyl]-	$C_{14}H_{20}N_4O_4$	0.36	308	
18.01	Pentadecanoic acid	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.44	242	fatty acid
18.01	9-Octadecenoic acid (Z)-	Oleic acid	$C_{18}H_{34}O2$	0.44	282	
18.01	Tetradecanoic acid	Myristic acid	$C_{14}H_{28}O_2$	0.44	228	
18.01	Dodecanoic acid	Lauric acid	$C_{12}H_{24}O_2$	0.44	200	
20.10	9,10-Secochola-5,7,10(19)-trien-24-al, 3-hydroxy-,(3á,5Z,7E)-	Ethyl docosahexaenoate	$C_{24}H_{36}O_2$	0.16	356	
24.09	17-Octadecynoic acid	Linoleic acid	$C_{18}H_{32}O_2$	0.58	280	
23.60	Phytol, acetate	Cetoleic acid	$C_{22}H_{42}O_2$	2.76	338	
24.91	Cyclopropanedodecano acid, 2-octyl-, methyl ester	Nervonic acid	$C_{24}H_{46}O_2$	0.19	366	
25.87	9-Hexadecenoic acid	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	0.96	254	
26.86	Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$	13.50	256	
27.36	10-Heptadecen-8-ynoic acid, methylester, (E)-	Linolenic acid	$C_{18}H_{30}O_2$	0.44	278	
29.12	Octadecanoic acid, Methyl ester	Methyl stearate	$C_{19}H_{38}O_2$	0.76	298	
29.32	Linoleic acid ethyl ester	Ethyl linoleate	$C_{20}H_{36}O_2$	0.17	308	
29.32	E,E,Z-1,3,12-Nonadecatriene5,14-diol	Methyl linolelaidate	$C_{19}H_{34}O_2$	0.17	294	
30.36	Octadecanoic acid	Stearic acid	$C_{18}H_{36}O_2$	3.02	284	
30.55	3à,17á-dihydroxyestr-4-ene	Stearidonic acid	$C_{18}H_{28}O_2$	0.86	276	

(*Continued*)

Table 1. (Continued).

RT	Compound	Common name	Molecular formula	Peak area %	M. WT	Chemical group
21.43	9-Oximino-2,7-diethoxyfluorene	Morphinone	$C_{17}H_{17}NO_3$	0.36	283	alkaloid
28.12	2,2′-Bipyridine, 6,6′-Dimethyl-, 1-oxid	Harmalol	$C_{12}H_{12}N_2O$	1.54	200	
21.76	Hi-Oleic Safflower oil	Flavanomarein	$C_{21}H_{22}O_{11}$	0.34	450	flavone
33.32	4 H-1-Benzopyran-4-one, 2-(3,4-Dimethoxyphenyl)-5, 7-Dihydroxy-	Cirsimaritin	$C_{17}H_{14}O_6$	1.59	314	
35.95	4 H-1-Benzopyran-4-one,2-(3,4Dimethoxyphenyl)-3,5-Dihydroxy-7-Methoxy-	Eupatorin	$C_{18}H_{16}O_7$	0.31	344	

Table 2. Antibacterial activity of *Padina pavonica* methanol extract was against bacterial pathogens.

Bacterial strain	Inhibition activity level	Mean of I.Z. diameter (mm) ± SD	Standard drug (*Gentamicin*)
Staphylococcus aureus ATCC25923	+++	24 ± 2	19.9 ± 0.3
Escherichia coli ATCC 8739	++	16.66 ± 2.0816	22.3 ± 0.1
Bacillus subtilis ATCC 6633	+++	20.833 ± 2.5166	32.4 ± 0.3
Enterococcus faecalis ATCC 29,212	+++	23.666 ± 2.7537	25.4 ± 0.1
Pseudomonas aeruginosa ATCC 9027	+++	24.666 ± 2.7537	19.6 ± 0.3
Klebsilla pneumonia ATCC 13,883	+++	26.5 ± 2.6457	23.7 ± 0.1
Pseudomonas fluorescens ATCC13525	++	17.166 ± 1.5275	22.9 ± 0.5
Streptococcus agalactiae ATCC13813	++	18.66 ± 1.8929	19.01 ± 0.2

Each value represents the mean of inhibition zones(mm) of three replicates± SD (Standard deviation).

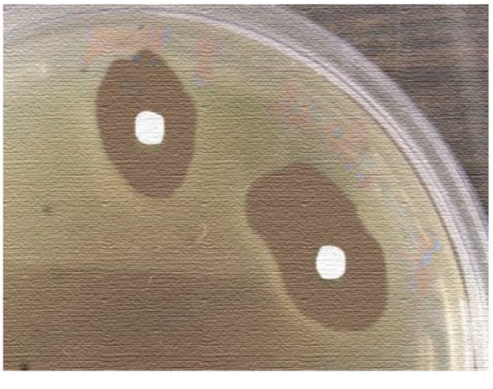

Figure 3. Strong antibacterial inhibition zones formed by *Padina pavonica* methanolic extract against *Bacillus subtilis* ATCC 6633.

antimicrobial activity has been recognized based on the existence of compounds belonging to numerous chemical classes, including phenols, fatty acids, indoles, and terpenes.

Thus, the antimicrobial activity of the tested *Padina pavonica* might be attributed to phenolic and flavonoid content. In this concern, a positive relationship between antimicrobial activity potential and the amount of phenolic compounds in the crude extracts was reported in the study of (Sameeh et al. 2016). Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than n-hexane and ethyl acetate. Using organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activities compared to water-based methods

Determination of DPPH radical scavenging activity

According to Table 3, *P. pavonica* methanolic extract could be considered an antioxidant. Where this extract makes activation for DPPH scavenging activity, and the activity (%) increased in a concentration-dependent manner, but its efficacy does not exceed vitamin C (reference drug) in its antioxidant properties. According to Duh et al. (1992), reducing qualities are often associated with the presence of reductions. Reductions also limit the generation of peroxide by reacting with peroxide precursors. The results show that the tested seaweed extract's extraordinary antioxidant activity was attributable to its reducing power. The free radical chain reaction may be efficiently stopped by some seaweed components acting as reductions, which donate electrons and interact with free radicals to convert them to more stable molecules (El-Sheekh et al. 2020; Farghl et al. 2021). Several authors studied the activity of antioxidants of certain phenolic and alkaloid compound types. Chlorogenic acid (5-caffeoylquinic acid or 5-CQA), discovered by Luis et al. (2007), is a hydrophilic phenolic compound with antioxidant properties, and the simple phenolic acid with a single aromatic ring gallic acid widely distributed in angiosperms and also found in some algae (Waterman and Mole 1994), distinguished as a favour antioxidant (Nakatani et al. 1992; Madsen and Bertelsen 1995; Makhlof et al. 2022). Geraniol (an alkaloid compound), detected in the tested extract, has also been shown to have antimicrobial, anti-inflammatory, antioxidant, anticancer, and neuroprotective properties. Many types of cancer, such as the prostate, have all been found to respond favorably to it (Seema et al. 2013; National Center for Biotechnology Information 2022). The GC screening in this study (Table 1) proves the presence of phenols, alkaloids, flavonoids, fatty acids, terpenes, amino acids, and alcohols that enhance the antioxidant activity of this algae.

Cytotoxicity of the methanolic extract against human lung carcinoma cell line

Cancer is an unregulated cell growth capable of invading, metastasizing, and spreading to distant locations (El-Kassas and Attia 2014). With an estimated 5-year survival rate of 18%, lung cancer is the biggest cause of cancer-related death worldwide (Rebecca et al. 2016). Parallel to the development of new technology (e.g. molecular profiling and chemical plasticity), new biological therapies that focus on various tumor components have been made possible by developments in cancer biology. The natural history of several molecular subtypes of lung cancer has already been impacted by these treatments, and it is anticipated that this trend will persist in the coming years (Zugazagoitia et al. 2018). Table 4 illustrates the effect of *P. pavonica* methanolic extract on the viability of the lung carcinoma cell line, revealing that the algal extract had an anticancer effect against the lung carcinoma cell line at all its concentrations except at the first concentration (1 µg/mL) there was no effect on the

Table 3. The effect of different concentrations of *Padina pavonica* methanolic extracts on DPPH radical scavenging activity.

Algal extract concentrations (µg/ml)	DPPH scavenging activity%	
	Algal extract	Vit C (reference drug)
0	0	0
2.5	0.11 ± 0.05	34.57 ± 0.79
5	0.25 ± 0.13	40.36 ± 0.82
10	0.98 ± 0.46	48.52 ± 2.64
20	3.17 ± 1.25	71.38 ± 1.39
40	10.28 ± 0.94	83.09 ± 1.95
80	24.46 ± 1.28	90.25 ± 0.41
160	47.08 ± 2.46	92.31 ± 0.87
320	60.31 ± 2.63	95.64 ± 1.22
640	77.23 ± 1.95	97.83 ± 0.39
1280	84.59 ± 1.23	98.91 ± 0.74

Note: The data are expressed in the form of mean ± SD (Standard deviation).

Table 4. Evaluation of cytotoxicity of different concentrations of *P. Pavonica* methanolic extracts against lung carcinoma cell line.

Algal extract concentration (µg/ml)	Cell viability%	Inhibitory %
0	100	0
1	100	0
2	98.21 ± 0.73	1.79
3.9	94.03 ± 1.29	5.97
4.8	79.56 ± 3.12	20.44
15.6	48.12 ± 2.76	51.88
31.25	31.74 ± 1.38	68.26
62.5	17.96 ± 0.42	82.04
125	10.23 ± 0.11	89.77
250	4.67 ± 0.25	95.33
500	1.75 ± 0.39	98.25

The data are expressed in the form of mean ±.

viability of lung carcinoma cell line, with IC_{50} = 15.14 ± 1.08 µg/mL, and by the increasing algal extract concentration, the carcinoma cells' viability decreases until it reaches (1.75%) at the highest algal extract concentration (500 µg/mL); this goes in harmony with Mofeed et al. (2021), who found that algal extracts exhibited significantly dose-dependent anticancer activity.

Growing evidence demonstrates that bioactive substances derived from algae exhibit cytotoxic activity via various mechanisms, including suppression of cancer in all its stages and triggering cancer cells' apoptosis (Farooqi et al. 2012). The mitochondrial-mediated (intrinsic) or the death receptor-mediated (extrinsic) mechanism can both trigger apoptosis (Brenner and Mak 2009; Mellier et al. 2010).

Caspase activation is involved in each process, leading to apoptosis (Park et al. 2012). The existence of several medicinal chemicals that can trigger apoptosis through diverse pathways and molecular mechanisms could be linked to the cytotoxic ability of the examined alga (Moghadamtousi et al. 2014). As a result, natural substances such as carbohydrates, alcohols, lipids, alkynes, amines, sulphur compounds, and sulphates (according to Gc report Table 1) are present in the methanol extract of *P. pavonica* could be responsible for A549 cancer cell lines' cytotoxic properties.

Identification of bioactive compounds in the P. pavonica extract

GC analysis of P. pavonica methanolic extract

GC-MS of *P. pavonica* methanolic extract is presented in Figure 4. The phytocomponents detected in the GCMS analysis are shown in Table 1. Phytochemical compounds were identified using the peak area, retention time, molecular weight, and molecular formula.

FTIR characterization of P. pavonica methanolic extract

The FT-IR results of the crude seaweed (Figure 5) revealed the existence of several chemical groups in this investigation. The presence of alcohols is indicated by the high absorption bands at 3677–3408 cm^{-1} in the seaweed methanolic extract showing O-H stretching (Silva et al. 2014; Hu et al. 2016). The CH_2 anti-symmetric stretch of methyl groups in lipids caused weak absorption bands at 2977–2852 cm^{-1} in the seaweed methanolic extract (Lu and Rasco 2012). By condensation processes, Jóźwiak et al. (2020) discovered various fatty acids with current anticancer medicines and heterocyclic moieties. In vivo and *in vitro* investigations, such conjugations, improved tissue selectivity and may have enhanced the effectiveness and safety of chemotherapy.

C = C phenyl compounds stretching and C = O of aromatic amide I stretching (proteins and peptides) are shown by the bands at 1739–1638 cm^{-1} of the spectra, respectively (Demir et al. 2015).

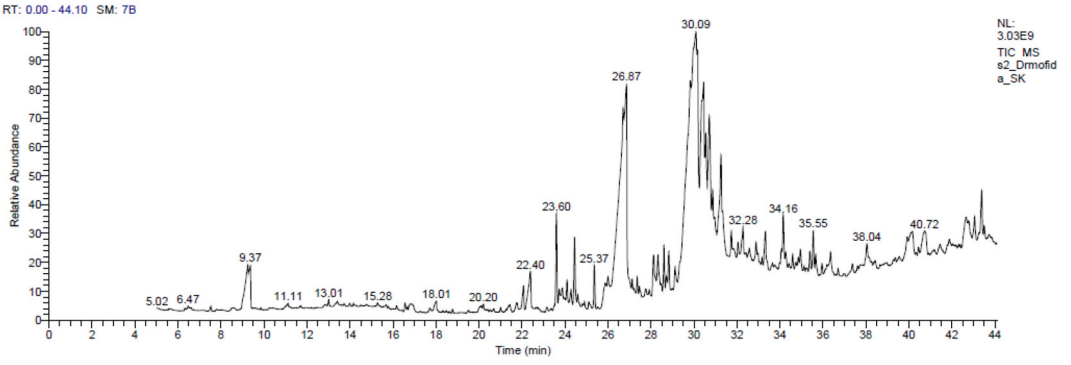

Figure 4. GC graph of *Padina pavonica* methanolic extract.

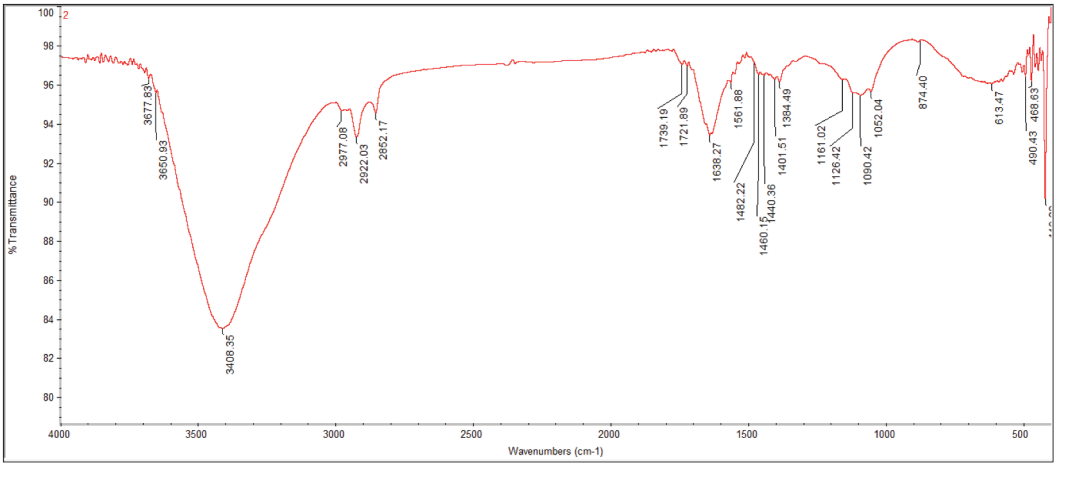

Figure 5. FTIR graph of *Padina pavonica* methanolic extract.

The presence of carboxylic acid (O-H bending) is indicated by the absorption band at 1482–1401 cm $^{-1}$ Younger (2014). Seaweed was known to contain a large amount of carboxylic acids, particularly fatty acids. Deyab et al. (2012) illustrated the *in vitro* anticancer effects of several fatty acids (oleic and palmitic acids) extracted from seaweed. C-O carbohydrates stretching, such as pectin and starch, was represented in the absorption band at 1090 cm^{-1} Singh et al. (2016). The ACH_2OH groups of carbohydrates are responsible for the absorption band at 1126 cm^{-1} (Mordechai et al. 2001).

Previous studies have demonstrated that the sulfated polysaccharide fucoidan, which is present in brown seaweeds such as *Fucus* sp., *Ascophyllum nodosum*, and *Undaria pinnatifida* (Phaeophyceae), inhibits the proliferation of colon cancer cells as well as a variety of tumor cells (Kim et al. 2010; Yang et al. 2013). Amines in this seaweed are indicated by the medium absorption bands at 1052 cm^{-1} related to C-N stretching. According to Pereira et al. (2013) *Padina pavonica* FTIR spectra showed alginate M/G ratio which was tentatively estimated from the 1030/1080 cm^{-1} band ratio in infrared spectra, their results suggesting higher values of guluronic than mannuronic acid blocks in *Padina pavonica*. Also, the absorption bands at wavelengths 874 cm^{-1} & 613 cm^{-1} correspond to C-H bending & C = S stretching, respectively, confirming the presence of sulphides in the tested seaweeds (Younger 2014).

Conclusion

This study concluded that the extract of *Padina pavonica* has products of great value which inhibit the pathogenic bacteria growth and their biofilm formation (*Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29,212), *Pseudomonas aeruginosa* (ATCC 9027), *Klebsiella pneumonia* (ATCC 13,883), *Pseudomonas fluorescens* (ATCC13525) and *Streptococcus agalactiae* (ATCC13813). The obtained results revealed a significant effect in increasing the inhibition zone diameter against pathogenic strains, this result confirms the promising activity of this extract as an antibacterial and antibiofilm agent. The extract also showed promising antioxidant and antitumoral activity. Furthermore, as these biological supports are natural sources that can be exploited in the environmental and medicinal domains, we also suggested that compounds such as Geranoil, Sulfolane, and other active components detected in the studied seaweed by Gc could be used in drug manufacturing after certain medical trials, the current study on *Padina pavonica* yielded a result that can be appreciated in other research endeavors. As a suggestion, because each tool used for the extraction of secondary metabolites has its own characteristics, optimizing lots of factors like pH, algal: solvent ratio concentration, and incubation time could increase obtaining more active compounds.

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Authors contribution

Mofida Makhlof and Abeer EL-Sayed carried out the experiments, constructed the figures and tables, and wrote the first draft of the manuscript. Mostafa El-Sheekh, shared the idea of the research, wrote, edited the manuscript, and revised the data and whole work.

Data availability statement

All data and materials are supplied in the manuscript

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