Current Botany 2023, 14: 41-48
doi: 10.25081/cb.2023.v14.8166
https://updatepublishing.com/journal/index.php/cb

Research Article

ISSN: 2220-4822

Screening of *Padina boergesenii* for pharmacological activities

Nirmala Mahendran[1], Priya Rajendran[1], Sasikumar Kandasamy[2], Gobianand Kuppannan[1], Muhammad Musthafa Poyil[1], Malarvizhi Arthanari[1]*

[1]Department of Microbiology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Namakkal-637205, Tamil Nadu, India, [2]Department of Integrative Biology, School of Biosciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India

ABSTRACT

Padina boergesenii is a distinctive small brown algae with rounded fronds growing to a length and diameter of 04 to 06 cm (1.6 to 2.4 in). *P. boergesenii* is widely present in the shallow water of tropical, subtropical and warm temperate areas. The present study aimed to investigate the anti-bacterial, anti-biofilm, antioxidant, anti-inflammatory and cytotoxicity activities of crude ethyl acetate extract of *P. boergesenii*. Anti-bacterial activity of crude ethyl acetate extract of *P. boergesenii* against Gram-positive and Gram-negative bacteria was determined using the well diffusion method. MIC of *P. boergesenii* against biofilm was carried out by the Resazurin method. Antioxidant potential was assessed by DPPH, FRAP, and the Hydrogen peroxide scavenging method. The anti-inflammatory activity was investigated using the albumin denaturation and heat-induced hemolysis method. Cytotoxicity activity of *P. boergesenii* against cell line L_{929} was analyzed by MTT assay. The maximum zone of inhibition obtained was 23 mm for *Staphylococcus aureus*, followed by 21 mm for *Escherichia coli*. Biofilm of *Enterococcus faecalis* showed higher resistance (MIC= 25.00±00.00 mg/mL). Biofilm of *Acinetobacter baumannii* was found to be most susceptible (MIC= 06.25±00.00 mg/mL). The IC_{50} value for the crude ethyl acetate extract *P. boergesenii* was 155.5 µg/mL for the DPPH method, 1567.18 µg/mL for the FRAP method, and 3098.27 µg/mL for the H_2O_2 method. The results of *in vitro* anti-inflammatory studies exhibited IC_{50}= 122.33 µg/mL and 2522.40 µg/mL for albumin denaturation assay and heat-induced hemolysis method respectively. The crude ethyl acetate extract of *P. boergesenii* showed cytotoxicity against the growth of the L_{929} cell line. The present study suggested that the crude ethyl acetate extract *P. boergesenii* has potent antibacterial, anti-biofilm, antioxidant, anti-inflammatory and cytotoxicity activities. The bioactive components present in the *P. boergesenii* extract can be a promising source for pharmaceuticals.

KEYWORDS: Brownalgae, Macroalgae, *Padina boergesenii*, Seaweeds

Received: December 19, 2022
Revised: March 13, 2023
Accepted: March 17, 2023
Published: March 28, 2023

***Corresponding author:**
Malarvizhi Arthanari
E-mail: pranavdharsh@gmail.com

INTRODUCTION

Macro algae are well known as a significant food source in several Asian countries. The importance of macroalgae as a valuable food source is expanding in the modern world (Mac MacArtain *et al.*, 2007). Approximately, 221 macroalgal species have been identified so far for use in commercial activity across the globe (Zemke-White & Ohno, 1999). Seaweed, often known as marine algae, is one of the natural resources required to keep the chemical and biological ecology of the oceans in appropriate balance. They are a source of agar, carrageenan, alginates, proteins, unsaturated fatty acids, vitamins, and minerals because they contain a variety of biologically active chemicals (Faulkner, 2001). Bioactive chemicals derived from macroalgae have been proven in numerous studies to have a wide range of biological activities, including cytotoxicity and the ability to cause apoptosis in cancer cells as well as antibiotics, antioxidants, antiviral and antitumor properties (Ibtissam *et al.*, 2009; Kim *et al.*, 2011).

Brown algae are abundant sources of several bioactive substances, such as polyphenols, carotenoids, and polysaccharides, which can have either beneficial or harmful physiological impacts on human health. Brown algae are a rich source of many different kinds of naturally occurring antioxidants, like polyphenols, which are crucial in avoiding the peroxidation of lipids (Amsler & Fairhead, 2005). The presence of fucoxanthin pigment and several Phaeophyceae tannins are responsible for the brown colour of the algae. Furthermore, brown algae offer a variety of biologically active substances, including uncommon secondary metabolites like phlorotannin, and many of them have particular biological functions (Wijesinghe & Jeon, 2012).

Padina is a widely distributed species found in a wide variety of habitats from subtidal to intertidal zones. It is quite simple to identify with its peacock tail-like structure. Geographically, *Padina* is found everywhere from tropical to cool temperate waters in South America, Southeast Asia and the region. So far,

43 different species of *Padina* have been identified (Ansari *et al.*, 2019). Many species of *Padina* can be used as biofertilizers, food, and fodder. The management and monitoring of the coastal marine ecosystems depend heavily on macro algae like *Padina*. They can serve as potential biomarkers and be used in phytoremediation to regulate toxins in coastal marine habitats (Samar *et al.*, 2022).

Padina boergesenii, which belongs to the class Phaeophyceae, is typically found in coastal areas near the continental regions. It has a fan-shaped plant body with a segmented and ruffled surface (Karthikeyan *et al.*, 2010). *P. boergesenii* is a common sight along India's Southeast coast in the Gulf of Mannar. It was reported to have high antioxidant activity and phenolic content (Kumar & Sudha, 2012). The present study was conducted to analyze the anti-bacterial, anti-biofilm, antioxidant, anti-inflammatory, and cytotoxicity activities of crude ethyl acetate extract *P. boergesenii*.

MATERIALS AND METHODS

Sample Collection

The algal species used in this study were collected from Rameshwaram - odakarai, Mandapam - Thonithurai near Pamban Bridge, Gulf of Mannar and Tamil Nadu. The collected sample was washed thoroughly with seawater followed by freshwater to remove debris. Then it was shade dried, pulverized into a fine powder, and stored in air-tight containers for extraction.

Extraction

The fine powder of *Padina boergesenii* (20 g) was extracted using Hexane, Chloroform, Ethyl acetate and Methanol using a Soxhlet apparatus. The extraction was carried out many times to get a substantial amount of extract. Then, it was concentrated at low pressure and the obtained residues were stored for further studies. All extracts were tested, the positive results were highlighted.

Antibacterial Activity

Bacterial strains

Six different bacterial strains were used for the antibacterial activity assay. Gram-positive bacteria: *Staphylococcus aureus*, and *Enterococcus faecalis*. Gram-negative bacteria: *Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. All the test organisms were obtained from Genolites Research and Development Laboratory, Coimbatore, and were maintained at 4°C in Mueller–Hinton Agar and were subcultured every month.

Antibacterial assay

The antibacterial activity of crude ethyl acetate extract *Padina boergesenii* was determined using the well diffusion method (Zain *et al.*, 2012). Petri plates containing 20 mL of Muller Hinton agar medium were seeded with 01-03 days cultures of bacterial inoculums. Wells of 6 mm in diameter were cut off from agar and the different concentrations (0.125, 0.25, 0.5, and 1.0 g) of algal extracts were added and incubated at 37°C for 24-48 hrs. The diameter resulting zone of inhibition around the well was measured in millimeters and the experiment was performed in duplicates.

Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of crude ethyl acetate extract of *P. boergesenii* was determined using the broth dilution method (CLSI, 2012). The serially diluted crude ethyl acetate extract *P. boergesenii* was prepared in sterile 96-well plates containing Mueller-Hinton broth medium. Then, 50 µL of bacterial inoculums with a density of 10^5 CFU mL^{-1} were seeded to each well and incubated at 37°C overnight. The lowest concentration of extract which inhibits the growth of test organisms was recorded as MIC.

Anti-Biofilm Activity

The anti-biofilm activity of crude ethyl acetate extract of *P. boergesenii* against the test organisms was carried out by the Resazurin method (Elshikh *et al.*, 2016). The Muller Hinton Broth was added to a 96-well microplate and inoculated with a 50 mL overnight culture of the test organisms. Different concentrations of crude ethyl acetate extract of *P. boergesenii* were added to the wells and it was incubated at 35°C under a 20% CO_2 atmospheric condition for 24 h. After the period of incubation, 30 µL resazurin (0.015 %) was added to all wells and further incubated for 02-04 h for the observation of colour change. The optical density was measured at 600 nm.

Time Kill Assay

Time kill assay was performed to determine the potential of the crude ethyl acetate extract *P. boergesenii* to inhibit the growth of bacteria overtime. The 100 µL of diluted overnight Cultures of test organisms (1010 CFU mL^{-1}) was added to each well-containing Muller Hinton along with different concentrations of *Padina boergesenii* extracts with appropriate controls. Plates were incubated at 37°C with orbital shaking at 120 rpm. The optical density was measured at 600 nm for every half hour from 0 to 72 hrs (Zhou *et al.*, 2012).

Antioxidant Activity

DPPH method

The DPPH assay was carried out as per the procedure recommended by Burits and Bucar (2000). 1 mL of crude ethyl acetate extract *P. boergesenii* was added to 1 mL of methanolic DPPH reagent (0.002%w/v). The absorbance was measured at 517 nm using a spectrophotometer after the incubation for 30 min in the dark at room temperature. Ascorbic acid was used as a control. Inhibition (%) = Absorbance of Control – Absorbance of Sample/ Absorbance of Sample×100.

Ferric Reducing Antioxidant Power Assay

The Ferric reducing antioxidant power (FRAP) of the extract was evaluated according to the method of Benzie and Strain (1996). FRAP solution consists of 300 mM sodium acetate buffer, 10 mM 2,4,6-Tri-(2-pyridyl)-5-triazine) (TPTZ), and 20 mM $FeCl_3 \bullet 6H_2O$ in the ratio of 10:1:1. FRAP solution (900 µL) was mixed with 30 µL of *P. boergesenii* extract and it was incubated for 30 mins at room temperature in the dark. Then, the absorbance was measured spectrophotometrically at 593 nm.

Hydrogen peroxide scavenging activity

The ability of the crude ethyl acetate extract *P. boergesenii* to scavenge hydrogen peroxide was determined as per the protocol described by Govindarajan *et al.* (2003) and Gulcin *et al.* (2004). 1 mL of the *P. boergesenii* extract (0.25 mg) was added to 2 mL of hydrogen peroxide solution buffered with phosphate (10 mM, pH 7.4) and incubated for 10 mins at 37°C. Then, the absorbance was measured at 230 nm using a UV spectrophotometer. Inhibition (%) = Absorbance of Control – Absorbance of Sample/Absorbance of Sample×100.

Anti-Inflammatory Activity

Albumin denaturation assay

The albumin denaturation inhibition assay was estimated according to the method of Mizushima and Kobayashi (1968). The different concentrations of the sample (25-1000 µg/mL) were mixed with 1% of the aqueous solution of albumin and the pH of the reaction mixture was adjusted with 1N hydrochloric acid. Then, it was incubated at room temperature for 20min followed by heating at 51°C for 20 min and it was allowed to cool. The absorbance was read at a wavelength of 660 nm by spectrophotometer. Inhibition (%) = Absorbance of Control - Absorbance of Sample/Absorbance of Sample×100.

Heat induced hemolysis assay

The reaction mixture consists of 1 mL of different concentrations of crude ethyl acetate extract *P. boergesenii* and 1 mL of 10% RBCs suspension. The reaction mixtures were kept in the water bath for 30 mins at 56°C. After the incubation, the tubes were allowed to cool under running tap water. The test tubes were centrifuged at 3000 rpm for 5 min and the supernatant was collected. The absorbance of the supernatants was measured at 560 nm using a spectrophotometer. Inhibition (%) = Absorbance of Control – Absorbance of Sample/Absorbance of Sample×100 (Shinde *et al.*, 1999; Sakat *et al.*, 2010).

Cytotoxicity Activity

MTT assay

MTT assay was used to determine the Cytotoxicity of crude ethyl acetate extract *P. boergesenii* against the L_{929} (mouse lung fibroblast) cell line. It was carried out in South Indian Textile Research Association (SITRA), Coimbatore.

10 µL of MTT solution (5 mg/mL in PBS) was mixed with 90 µL DMEM medium in the wells of microtitre plate. Different concentration of crude ethyl acetate extract *P. boergesenii* was added to the microtitre plate and it was incubated at 37°C. The optical density of each well was measured using a micro plate reader at 540 nm (Loosdrecht *et al.*, 1994).

RESULTS

Anti-Bacterial Assay

All the investigated concentrations of crude ethyl acetate extract of *P. boergesenii* exhibited potent antibacterial activity against both Gram positive as well as Gram negative bacteria. The results obtained in the evaluation of the antibacterial activity of the crude ethyl acetate extract marine macro-algae *P. boergesenii* using a well diffusion method and minimum inhibition concentration (MIC) are shown in Table 1. The antibacterial activity of *P. boergesenii* extract was determined by measuring zone of inhibition and MIC (Minimum Inhibitory Concentration) values against all tested bacterial strains. The maximum zone of inhibition obtained for Gram positive bacteria was 23 mm for *Staphylococcus aureus* at the concentration of 1 mg/mL whereas the zone of inhibition was 21 mm for *Escherichia coli* (Gram negative bacteria) at the same concentration. The minimum zone of inhibition was obtained for Gram positive bacteria was *Enterococcus faecalis* (9 mm) and for Gram negative bacteria was *Klebsiella pneumonia* (11 mm). No inhibitory effect was observed for *Enterococcus faecalis* and *Klebsiella pneumonia* at the concentration of 0.125, and 0.25 mg/mL.

The results revealed that *Enterococcus faecalis* (MIC=100.00±00.00 mg/mL) was the most resistant against the crude ethyl acetate extract *P. boergesenii*, followed by *Klebsiella pneumoniae* (MIC = 50.00±00.00 mg/mL), *Staphylococcus aureus* (MIC= 09.37±03.12 mg/mL), *Escherichia coli* (MIC= 09.37±03.12 mg/mL), and *Pseudomonas aeruginosa* (MIC= 6.25±00.00 mg/mL). Among the tested bacterial strains, *Acinetobacter baumannii* was the most sensitive bacteria with the MIC value of 04.68±02.20 mg/mL.

Anti-Biofilm Activity

The crude ethyl acetate extract *Padina boergesenii* showed anti-biofilm activity against all tested organisms. The MIC of crude

Table 1: Anti-bacterial activity and Minimum Inhibitory concentration (MIC) of crude ethyl acetate extract *P. boergesenii*

Name of the microorganisms	Inhibition Zone Concentrations (g)				MIC (mg/mL)
	0.125	0.25	0.5	01	
Staphylococcus Aureus	12	15	19	23	09.37±03.12
Enterococcus Faecalis	-	-	06	09	100.00±00.00
Acinetobacter baumannii	09	14	17	20	04.68±02.20
Escherichia Coli	11	15	18	21	09.37±03.12
Pseudomonas aeruginosa	12	15	17	19	06.25±00.00
Klebsiella pneumoniae	-	-	08	11	50.00±00.00

ethyl acetate extract *Padina boergesenii* against biofilm was given in Table 2. *Acinetobacter baumannii* was found to be most susceptible to the crude ethyl acetate extract *Padina boergesenii* (MIC=06.25±00.00 mg/mL), followed by *Pseudomonas aeruginosa* (MIC= 07.81±04.68 mg/mL). Biofilm of *Enterococcus faecalis* showed higher resistance to the crude ethyl acetate extract *Padina boergesenii* with the MIC value of 25.00±00.00 mg/mL. Then the biofilm of *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus* were also displayed significant anti-biofilm activity with the MIC value of 18.75±06.25 mg/mL, 15.62±09.37 mg/mL, and 14.06±10.93 mg/mL respectively.

Time Kill Assay

Time Kill assay was performed over a period of 72 hrs with different strains of Gram positive (*Staphylococcus aureus*, and *Enterococcus faecalis*), and Gram negative bacteria (*Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*). The killing kinetics of crude ethyl acetate extract *P. boergesenii* on all tested bacterial strains was time-dependent. However, the killing efficacy of crude ethyl acetate extract *P. boergesenii* varied against all tested bacterial strains due to the different MIC. The results of the Time Kill assay were given in Figures 1-6.

Antioxidant Activity

DPPH method

The crude ethyl acetate extracts *P. boergesenii* exhibited DPPH radical scavenging activity in a concentration dependent manner. The maximum scavenging activity was 68.62% for 1000 µg/mL of crude extract whereas the lowest scavenging activity obtained was 4.5% for 25 µg/mL. The IC_{50} value for crude ethyl acetate extracts *P. boergesenii* was 155.5 µg/mL. The result of the DPPH method were given in Table 3 and Figure 7.

Table 2: MIC of crude ethyl acetate extracts of *P. boergesenii* against biofilm

Name of the bacteria strains	Minimun Inhibitory Concentration (mg/mL)
Acinetobacter baumannii	06.25±00.00
Escherichia coli	15.62±09.37
Staphylococcus aureus	14.06±10.93
Pseudomonas aeruginosa	07.81±04.68
Enterococcus faecalis	25.00±00.00
Klebsiella pneumoniae	18.75±06.25

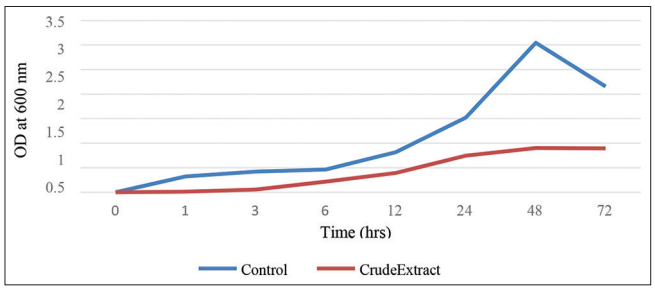

Figure 1: Time Kill assay of crude extract against *Escherichia coli*

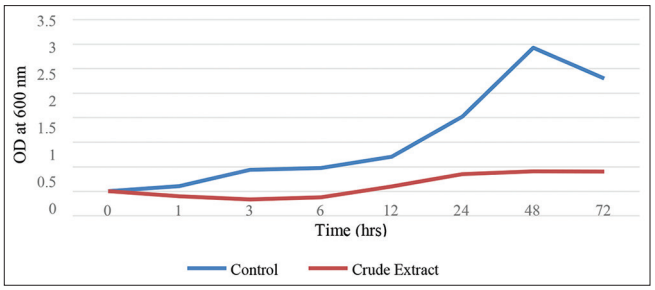

Figure 2: Time Kill assay of crude extract against *Staphylococcus aureus*

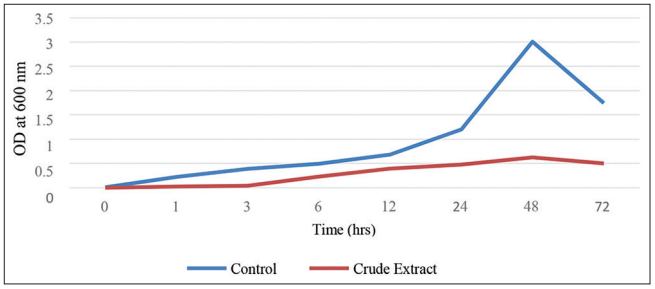

Figure 3: Time Kill assay of crude extract against *Acinetobacter baumannii*

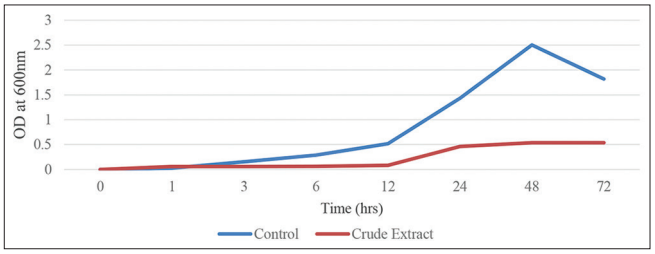

Figure 4: Time Kill assay of crude extract against *Pseudomonas aeruginosa*

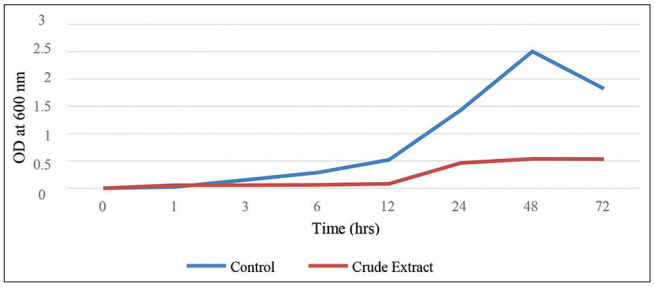

Figure 5: Time Kill assay of crude extract against *Klebsiella pneumonia*

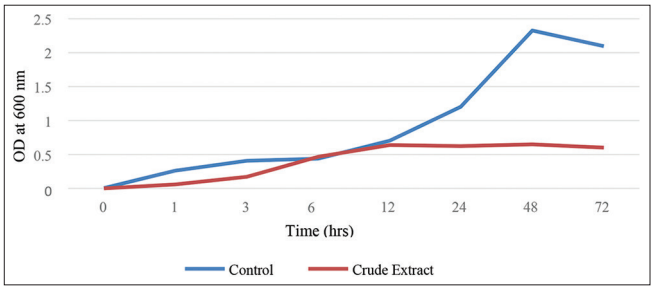

Figure 6: Time Kill assay of crude extract *Enterococcus faecalis*

FRAP assay

Ferric Reducing Antioxidant Power (FRAP) values of crude extracts of *P. boergesenii* were depicted in Table 3 and Figure 8. The data showed the maximum FRAP value of *P. boergesenii* crude extract was 62.56% for 1000 µg/mL and the IC_{50} value obtained was 1567.18 µg/mL.

Hydrogen peroxide scavenging activity

Hydrogen peroxide (H_2O_2) scavenging activity of crude extracts of *P. boergesenii* was depicted in Table 3 and Figure 9. The data showed the maximum hydrogen peroxide scavenging value of *P. boergesenii* crude extract was 43.74% for 1000 µg/mL and the IC_{50} value obtained was 3098.27 µg/mL.

Anti-Inflammatory Activity

Protein denaturation assay

The crude ethyl acetate extract of *P. boergesenii* exhibited protein denaturation in a concentration dependent manner. In this current study, the in vitro anti-inflammatory activity of crude ethyl acetate extract *P. boergesenii* was determined by the inhibitory activity against protein albumin denaturation. The highest inhibitory activity against albumin denaturation was observed in the concentration of 6.5 mg/mL of crude extract. The maximum inhibition of protein denaturation was exhibited at 1000 µg/mL (79.35%). The IC_{50} value calculated for the crude ethyl acetate extract *P. boergesenii* was 122.33 µg/mL. The results of the protein denaturation assay of crude ethyl acetate extract P.boergesenii were given in Table 4 and Figure 10.

Table 3: Antioxidant potential of crude ethyl acetate extract of *P. boergesenii*

Concentration (µg/mL)	% of Inhibition		
	DPPH	FRAP	H_2O_2
25	4.5	1.59	0.3
50	6.5	8.93	2.1
75	10.37	13.83	6.28
100	22.54	25.61	13.47
250	48.83	38.13	21.22
500	53.21	45.01	29.93
750	59.01	58.91	35.18
1000	68.62	62.56	43.74
IC_{50}	155.57	1567.18	3098.27

Table 4: *In vitro* anti-inflammatory activities of crude ethyl acetate extract *P. boergesenii*

Concentration (µg/mL)	% of Inhibition	
	Albumin Denaturation	Heat induced hemolysis
25	19.28	0.98
50	21.65	3.73
75	32.9	4.98
100	45.85	12.42
250	58.67	18.64
500	69.01	38.23
750	72.59	43.02
1000	79.35	56.91
IC_{50}	122.33	2522.40

Heat induced hemolysis

The anti-inflammatory activity of crude ethyl acetate extract *P. boergesenii* was examined by membrane stabilization of human erythrocytes at various concentrations. All the tested concentrations were significantly inhibiting the heat-induced hemolysis and these results contribute additional evidence for their anti-inflammatory effect. The maximum percentage inhibition was recorded as 56.91 and the minimum percentage as 0.98 at the concentrations of 1000 µg/mL and 25 µg/mL of crude extract respectively. The IC_{50} value was found to be 2522.40 µg/mL. The results demonstrated its effective membrane stabilizing activity by preventing the lysis of erythrocytes induced by heat. The results of the heat induced hemolysis of crude ethyl acetate extract P. boergesenii were given in Table 4 and Figure 11.

Cytotoxicity Activity

The effects of crude ethyl acetate extract *P. boergesenii* on the growth of the L_{929} (mouse lung fibroblast) cell line was examined by the MTT assay. The cell line was treated with increasing concentrations (25, 50, and 100 µg/mL) for 24 h. The percentage of growth inhibition was elevated in a concentration dependent manner. The IC_{50} value obtained for crude ethylacetate extract *P. boergesenii* on the L_{929} cell line was 114.90 µg/mL against the L_{929} cell line. The highest percentage of cytotoxicity was obtained at the concentration of 100 µg/mL (36.73%). The results of the cytotoxicity activity of crude ethyl acetate extract *P. boergesenii* were given in Table 5.

DISCUSSION

P. boergesenii is a brown macroalgae. The members of brown algae were enriched with bioactive compound, which contribute immense pharmacological activities (Rajamani & Thirugnanasambandan, 2018). The extract of the genus *Padina* has hypolipidemic, antioxidant, anti-inflammatory and hepatoprotective activity (Ahmed *et al.*, 2016).

Padina sp. was capable of producing bactericidal compounds against various human as well as animal pathogens (Ismail *et al.*, 2016). The Gram positive bacteria were more susceptible to algal extracts than Gram negative bacteria due to the variations in the composition of the cell wall (Taskin *et al.*, 2007). The Gram negative bacteria possess a hydrophobic outer membrane, which gives protection against different substances which doesn't allow the active agents (Berber *et al.*, 2015).

According to the study done by El-Sheekh *et al.* (2020), the Methanolic extract of *P. boergesenii* exhibited the highest zone

Table 5: Cytotoxicity activities of crude ethyl acetate extract *P. boergesenii*

Concentration (µg/mL)	Crude ethyl acetate extract of *P. boergesenii* Cytotoxicity (%)
25	8.17
50	12.26
100	36.73

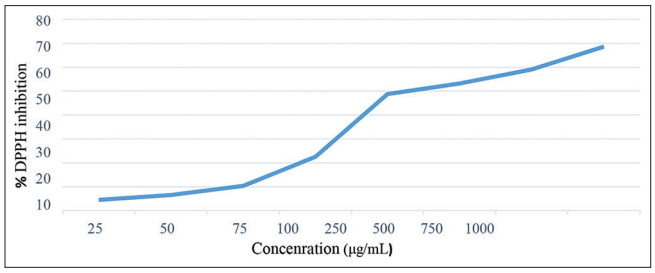

Figure 7: DPPH Assay

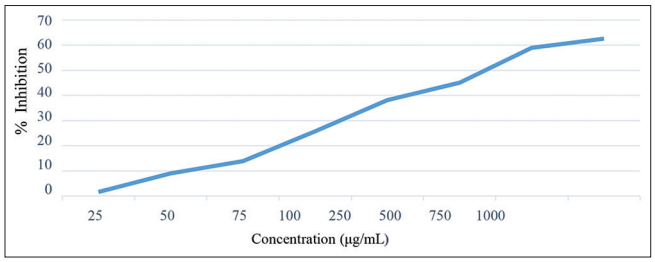

Figure 8: FRAP Assay

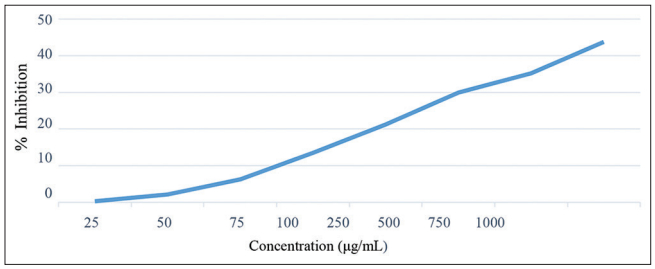

Figure 9: Hydrogen Peroxide Assay

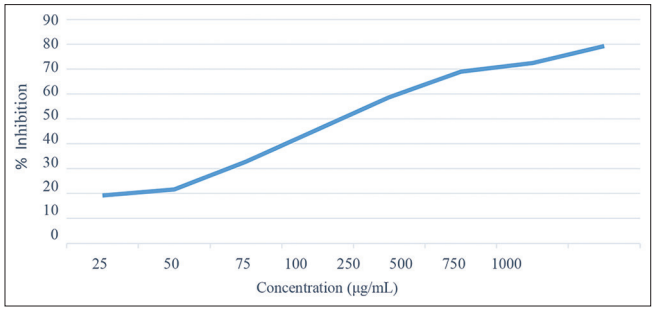

Figure 10: Albumin Denaturation Assay

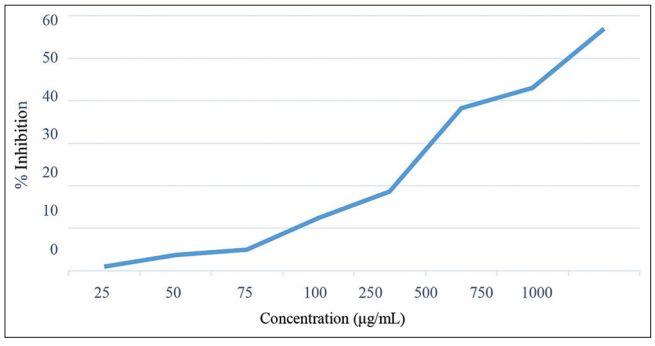

Figure 11: Heat Induced Hemolysis Assay

of inhibition (2.03 cm) for *Shigella flexneri*. Contrarily, the methanolic extract of *P. boergesenii* didn't show any inhibition zone against *Streptococcus pyogenes*, *Enterobacter aerogenosa*, and *Escherichia coli* up to the concentration of 200 mg/mL. The ethyl acetate fraction of *P. boergesenii* was reported with the lowest MIC value. The methanolic and aqueous extract of *P. boergesenii* showed moderate bactericidal activity in all tested bacterial strains (El-Fatimy & Said, 2011; Ragunath *et al.*, 2020).

The ethyl acetate extract of *P. boergesenii* inhibited the growth of *Staphylococcus aureus* and *Enterococcus faecalis* with MIC value of 3.75 and 7.5 mg/mL respectively (Mashjoor *et al.*, 2016). Methanolic extract of *P. boergesenii*, collected from the backwaters of Muttukadu, Chennai exhibited higher antibacterial potency against *Xanthomonas oryzae pv. oryzae* are the same species collected from coastal waters (Kumar & Rengasamy, 2000).

The antioxidant activity investigated using the ABTS method exhibited the IC_{50} value of 06.53±0.22 and 04.27±00.12 mg/mL for methanol and aqueous extract of *P. boergesenii* respectively (Ramezanpour *et al.*, 2021). The study done by Kanagarajjeevitha *et al.* (2014), demonstrated the antioxidant activity of methanol extract of brown alga *P. boergesenii* using DPPH assay. It was found that the *P. boergesenii* extract exhibited the radical scavenging capability in a dose-dependent manner and the IC_{50} was 59.70 ± 00.21% at 1000 µg/mL.

The antioxidant potential of crude ethyl acetate extract *P. boergesenii* evaluated using the DPPH method showed an EC_{50} value of 3.16±0.13 (El-Manawy *et al.*, 2019). At a concentration of 500 µg/mL of aqueous extracts of *P. boergesenii* showed DPPH radical scavenging activity (75.92±0.3%), superoxide radical scavenging activity (102.5±0.02%), and FRAP radical scavenging activity (0.45±0.01) (Kumar & Sudha, 2012). The study of Naeem *et al.* (2022) revealed that the DPPH radical scavenging activity of crude polysaccharides of *P. boergesenii* was 84.33±0.88%.

The methanol-ethyl acetate fraction of the crude methanol extract of *P. boergesenii* inhibited the proliferation of human renal adenocarcinoma cell line A498 (EC_{50}=22.73 mg) and ACHN (EC_{50}=26.43) in dose-dependent manner (Rajamani & Thirugnanasambandan, 2018). *P. boergesenii* extract treated with breast adenocarcinoma (MCF7), monkey kidney (Vero) cells and HeLa cells showed reduction in viability after 24-48 hrs in a dose-dependent manner (Mashjoor *et al.*, 2016). According to the study done by Soleimani *et al.* (2012), the ethyl acetate fraction of *P. boergesenii* exhibited antioxidant activity against DPPH free radicals. Moreover, it also protects the HaCaT human keratinocytes from UVB-induced Cytotoxicity.

CONCLUSION

Macro algae are gaining attention all over the world due to its various biological activities. The present study suggested that the crude ethyl acetate extract *P. boergesenii* is a potent source of

antibacterial, anti-biofilm, antioxidant, and anti-inflammatory agents. The extracts revealed significant bactericidal activity against selected Gram-positive and Gram-negative strains. Furthermore, it also has effective antioxidant properties and free radical scavenging activities. *P. boergesenii* extract possesses significant cytotoxicity activity against mouse lung fibroblast cell line L_{929}. Hence, the result of the present study suggests that the crude ethyl acetate extract *P. boergesenii* has bioactive components, which can be a promising source for pharmaceutical applications.

REFERENCES

Ahmed, H. H., Hegazi, M. M., & Fahim, C. B. (2016). Cystoseira myrica and *Padina pavonica*: A potential natural hope against hepatic injury in animal model. *Der Pharmacia Lettre, 8*(4), 161-172.

Amsler, C. D., & Fairhead, V. A. (2005). Defensive and sensory chemical ecology of brown algae. *Advances in Botanical Research, 43*, 1-91. https://doi.org/10.1016/S0065-2296(05)43001-3

Ansari, A. A., Ghanem, S. M., & Naeem, M. (2019). Brown Alga *Padina:* A review. *International Journal of Botany Studies, 4*(1), 01-03.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry, 239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Berber, I., Avşar, C., & Koyuncu, H. (2015). Antimicrobial and antioxidant activities of *Cystoseira crinita* Duby and *Ulva intestinalis* Linnaeus from the coastal region of Sinop, Turkey. *Journal of Coastal Life Medicine, 3*(6), 441-445.

Burits, M., & Bucar, F. (2000). Antioxidant activity of Nigella sativa essential oil. *Phytotherapy Research, 14*(5), 323-328. https://doi.org/10.1002/1099-1573(200008)14:5%3C323:AID-PTR621%3E3.0.CO;2-Q

CLSI. (2012). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition*. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute.

El-Fatimy, E. S., & Said, A. A.-M. (2011). Antibacterial activity of methanolic extract of dominant marine alga (*Padina pavonia*) of Tolmeta Coasts, Libya. *Journal of American Science, 7*, 745-751.

El-Manawy, I. M., Nassar, M. Z., Fahmy, N. M., & Rashedy, S. H. (2019). Evaluation of proximate composition, antioxidant and antimicrobial activities of some seaweeds from the Red Sea coast, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries, 23*(1), 317-329. https://doi.org/10.21608/ejabf.2019.30541

El-Sheekh, M. M., Mousa, A. S. H., & Farghl, A. A. M. (2020). Antibacterial efficacy and phytochemical characterization of some marine brown algal extracts from the red sea, Egypt. *Romanian Biotechnological Letters, 25*(1), 1160-1169. https://doi.org/10.25083/rbl/25.1/1160.1169

Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M., Marchant, R., & Banat, I. M. (2016). Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotechnology Letters, 38*, 1015-1019. https://doi.org/10.1007/s10529-016-2079-2

Faulkner, D. J. (2001). Marine natural products. *Natural Product Reports, 18*, 1R-49R. https://doi.org/10.1039/B006897G

Govindarajan, R., Rastogi, S., Vijayakumar, M., Shirwaikar, A., Rawat, A. K. S., Mehrotra, S., & Pushpangadan, P. (2003). Studies on the antioxidant activities of *Desmodium gangeticum*. *Biological and Pharmaceutical Bulletin, 26*(10), 1424-1427. https://doi.org/10.1248/bpb.26.1424

Gulcin, I., Sat, I. G., Beydemi, S., & Kufrevioglu, O. I. (2004). Evaluation of the in vitro antioxidant properties of extracts of Broccoli (*Brassica oleraceal*). *Indian Journal of Food Science, 16*(1), 17-30.

Ibtissam, C., Hassane, R., Jose, M.-L., Francisco, D. S. J., Antonio, G. V. J., Hassan, B., & Mohamed, K. (2009). Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. *African Journal of Biotechnology, 8*(7), 1258-1262.

Ismail, A., Ktari, L., Ahmed, M., Bolhuis, H., Boudabbous, A., Stal, L. J., Cretoiu, M. S., & El Bour, M. (2016). Antimicrobial Activities of Bacteria Associated with the Brown Alga *Padina pavonica. Frontiers in Microbiology, 7*, 1072. https://doi.org/10.3389/fmicb.2016.01072

Kanagarajjeevitha, Damahe, J., Das, S., Chowdhury, T. R., & Khora, S. S. (2014). In vitro antioxidant and cytotoxic activity of brown alga *Padina boergesenii*. *International Journal of Drug Development and Research, 6*(2), 110-119.

Karthikeyan, R., Somasundaram, S. T., Manivasagam, T., Balasubramanian, T., & Anantharaman, P. (2010). Hepatoprotective activity of brown alga *Padina boergesenii* against CCl4 induced oxidative damage in Wistar rats. *Asian Pacific Journal of Tropical Medicine, 3*(9), 696-701. https://doi.org/10.1016/S1995-7645(10)60168-X

Kim, S.-K., Thomas, N. V., & Li, X. (2011). Anticancer compounds from marine macroalgae and their application as medicinal foods. *Advances in Food and Nutrition Research, 64*, 213-224. https://doi.org/10.1016/B978-0-12-387669-0.00016-8

Kumar, K. A., & Rengasamy, R. (2000). Evaluation of antibacterial potential of seaweeds occurring along the coast of Tamil Nadu, India against the plant pathogenic bacterium *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Dye. *Botanica Marina, 43*(5), 409-415. https://doi.org/10.1515/BOT.2000.042

Kumar, P. S., & Sudha, S. (2012). Evaluation of Antioxidant Activity and Total Phenolic Content of *Padina boergesenii* from Gulf of Mannar. *Drug Invention Today, 4*(12), 635-639.

Loosdrecht, A. A. V. de, Beelen, R. H. J., Ossenkoppele, G. J., Broekhoven, M. G., & Langenhuijsen, M. M. A. C. (1994). A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. *Journal of Immunological Methods, 174*(1-2), 311-320. https://doi.org/10.1016/0022-1759(94)90034-5

MacArtain, P., Gill, C. I. R., Brooks, M., Campbell, R., & Rowland, I. R. (2007). Nutritional value of edible seaweeds. *Nutrition Reviews, 65*(12), 535-543. https://doi.org/10.1111/j.1753-4887.2007.tb00278.x

Mashjoor, S., Yousefzadi, M., Esmaeili, M. A., & Rafiee, R. (2016). Cytotoxicity and antimicrobial activity of marine macro algae (Dictyotaceae and Ulvaceae) from the Persian Gulf. *Cytotechnology, 68*, 1717-1726. https://doi.org/10.1007/s10616-015-9921-6

Mizushima, Y., & Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology, 20*(3), 169-173. https://doi.org/10.1111/j.2042-7158.1968.tb09718.x

Naeem, E., El-Ghoneimy, A., & Ahmed, H. (2022). Anti-inflammatory activities of a sulfated polysaccharide isolated from the brown seaweed *Padina boergesenii* (Phaeophyceae, Dictyotaceae). *SVU- International Journal of Veterinary Sciences, 5*(1), 83-101. https://doi.org/10.21608/svu.2022.105385.1158

Ragunath, C., Kumar, Y. A. S., Kanivalan, I., & Radhakrishnan, S. (2020). Phytochemical screening and GC-MS analysis of bioactive constituents in the methanolic extract of *caulerpa racemosa* (Forssk.) j. agardh and *Padina boergesenii* allender & kraft. *Current Applied Science and Technology, 20*(3), 380-393. https://doi.org/10.14456/cast.2020.24

Rajamani, K., & Thirugnanasambandan, S. S. (2018). Polyphenols from brown alga*, Padina boergesenii* (Allendar & Kraft) decelerates renal cancer growth involving cell cycle arrest and induction of apoptosis in renal carcinoma cells. *Environmental Toxicology, 33*(11), 1135-1142. https://doi.org/10.1002/tox.22619

Ramezanpour, Z., & FatemeGhanbariPirbasti, & RasouliDogaheh, S. (2021). Bioactivity potential of *Gracilaria salicornia, Padina boergesenii, Polycladia myrica*: antibacterial, antioxidant and total phenol assays. *Journal of Phycological Research, 5*(1), 597-615.

Sakat, S. S., Juvekar, A. R., & Gambhire, M. N. (2010). In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences, 2*(1), 146-155.

Samar, J., Butt, G. Y., Shah, A. A., Shah, A. N., Ali, S., Jan, B. L., Abdelsalam, N. R., & Hussaan, M. (2022). Phycochemical and Biological Activities fom Different Extracts of *Padina antillarum* (Kützing) Piccone. *Frontiers in Plant Science, 13*, 929368. https://doi.org/10.3389/fpls.2022.929368

Shinde, U. A., Kulkarni, K. R., Phadke, A. S., Nair, A. M., Mungantiwar, A. A., Dikshit, V. J., & Saraf, M. N. (1999). Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara (*Roxb.) Loud Wood Oil. *Indian Journal of Experimental Biology, 37*(3), 258-261.

Soleimani, S., Yousefzadi, M., Nezhad, S. B. M., Pozharitskaya, O. N., & Shikov, A. N. (2022). Utilization of the ethyl acetate fraction of *Padina*

boergesenii as a natural UV filter in sunscreen cream formulation. *Research Square, Preprint,* 1-17. https://doi.org/10.21203/rs.3.rs-2032182/v1

Taskin, E., Ozturk, M., Taskin, E., & Kurt, O. (2007). Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *African Journal of Biotechnology, 6*(24), 2746-2751.

Wijesinghe, W. A. J. P., & Jeon, Y.-J. (2012). Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: a review. *Carbohydrate Polymers, 88*(1), 13-20. https://doi.org/10.1016/j.carbpol.2011.12.029

Zain, M. E., Awaad, A. S., Al-Outhman, M. R., & El-Meligy, R. M. (2012). Antimicrobial activities of Saudi Arabian desert plants. *Phytopharmacology, 2*, 106-113.

Zemke-White, W. L., & Ohno, M. (1999). World seaweed utilization: an end-of-century summary. *Journal of Applied Phycology, 11*, 369-376. https://doi.org/10.1023/A:1008197610793

Zhou, Y., Kong, Y., Kundu, S., Cirillo, J. D., & Liang, H. (2012). Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and *bacillu*s Calmette-Guérin. *Journal of Nanobiotechnology, 10*, 19. https://doi.org/10.1186/1477-3155-10-19