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Bioactive Extracts from *Padina boryana* Thivy from Phu Quoc Island, Vietnam: *In vitro* Antioxidant, Anticancer, Alpha-glucosidase inhibitory, Anti-inflammatory, Antimicrobial, and Hepatoprotective Activities

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ABSTRACT

The brown alga *Padina boryana* Thivy has been studied for its bioactive properties. This study aimed to evaluate the antioxidant, anticancer, alpha-glucosidase inhibitory, anti-inflammatory, antimicrobial, and hepatoprotective activities of *Padina boryana* extracts. The antioxidant activity was investigated using DPPH and ABTS radical scavenging, and ferric reducing power assays. The anticancer activity was determined via cytotoxic activity on human lung carcinoma (A549) and human embryonic kidney (HEK 293) cancer cell lines. Alpha-glucosidase inhibitory activity was determined using standard method, anti-inflammatory activity was assessed via nitric oxide (NO) inhibition assay, antimicrobial activity was assessed using the broth microdilution assay, and the hepatoprotective activity was evaluated using CCl_4-induced hepatotoxicity in HepG2 cells. Results showed that the algal extracts have low DPPH scavenging activity, but effective ABTS scavenging activity with EC_{50} value of 52.09 µg/mL for the ethyl acetate (EA) extract. The EA extract had the highest ferric-reducing power with EC_{50} of 61.7 µg/mL. The petroleum ether (PE) extract had the highest cytotoxic effect with EC_{50} values of 38.19 µg/mL and 33.28 µg/mL against A549, and HEK 293 cells, respectively. All extracts showed significant alpha-glucosidase inhibitory activity, with EA being the most potent (IC_{50} = 3.01 µg/mL). The PE extract showed notable NO inhibitory activity with IC_{50} of 50.91 µg/mL. No significant antimicrobial activity was observed except for EtOH and PE extracts, which inhibited *Staphylococcus aureus* and *Bacillus subtilis*. The extracts did not demonstrate significant hepatoprotective effect. These findings highlight the potential of *Padina boryana* as a valuable source of natural therapeutic agents.

Keywords: Antioxidant, Hepatoprotective, Alpha-glucosidase, *Padina boryana*.

Introduction

Marine algae have garnered significant scientific interest due to their rich reservoir of bioactive compounds, which hold potential applications in pharmaceuticals, nutraceuticals, and cosmeceuticals.[1,2] Brown algae, in particular, have been extensively studied for their diverse secondary metabolites, such as polysaccharides, polyphenols, terpenoids, and fatty acids, at the same time exhibit a vast amount of biological characteristics, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties.[1,2] The brown alga *Padina boryana* Thivy (Figure 1), a species commonly found in tropical and subtropical regions, represents a promising candidate for such bioactive compounds.

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Recent studies have highlighted the potential of various *Padina* species.[3-5]

For instance, research on *Padina pavonica* has revealed significant antioxidant properties linked to its high phenolic content. *Padina gymnospora* has demonstrated notable antimicrobial activities against various bacterial strains.[6-8] Additionally, *Padina boergesenii* has been shown to possess anti-inflammatory, and anticancer properties. These findings underscore the therapeutic potential of this genus.[9]

Despite these promising findings, there is a paucity of detailed studies on *Padina boryana*, particularly those harvested from the unique ecological environment of Phu Quoc Beach, Vietnam. This region has distinct marine ecosystem, characterized by high biodiversity and unique water chemistry, which may influence the chemical composition and potency of the algae's bioactive compounds. Therefore, this study aims to fill this gap by comprehensively evaluating the antioxidant, anti-glucosidase, anti-inflammatory, antimicrobial, and anticancer activities of various extracts of *Padina boryana*. By comparing the present findings with the findings from previous research on other *Padina* species, it is expected that a better understanding of the potential therapeutic applications of *Padina boryana* will be obtained, and this will contribute to the growing body of knowledge on marine algal bioactive compounds.

Materials and Methods

Collection and extraction of algal material

Padina boryana was collected from Phu Quoc Island (Figure 1), Vietnam, and identified by standard taxonomic methods. The dried algal material was ground into a fine powder and extracted sequentially with petroleum ether (PE), chloroform ($CHCl_3$), ethyl acetate (EA), ethanol (EtOH), and n-butanol (n-BuOH) using a Soxhlet apparatus. Each sample was concentrated under reduced pressure using a rotary evaporator and stored at 0-5°C.

Figure 1: *Padina boryana* Thivy from Phu Quoc Island, Vietnam

Determination of antioxidant activity

DPPH radical scavenging assay: Briefly, various concentrations of the extracts were mixed with 0.1 mM DPPH solution in methanol. The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm using a spectrophotometer. The EC_{50} value, representing the concentration required to scavenge 50% of DPPH radicals, was calculated.

$$\% \text{ RSA} = \frac{\text{OD}_c - \text{OD}_t}{\text{OD}_c} \times 100 \quad \text{(Eq. 1)}$$

Where;
%RSA: Percentage radical scavenging activity
OD_c: Optical density of the control sample.
OD_t: Optical density of the test sample.

ABTS radical scavenging assay

The ABTS radicals were generated by reacting ABTS stock solution with potassium persulfate. The resulting $ABTS^{\bullet+}$ solution was diluted with ethanol until an absorbance of 0.70 ± 0.02 at 734 nm was obtained. Various concentrations of the extracts were added to the $ABTS^{\bullet+}$ solution, and the absorbance was measured after 6 minutes. The EC_{50} values were determined based on the reduction in absorbance at 734 nm using Eq.1 above.

Ferric reduction capacity

The iron-reducing capacity of the extracts was determined based on its ability to reduce Fe^{3+} in the complex $Fe(CN_6)^{3-}$ to Fe^{2+} in the complex $Fe(CN_6)^{4-}$ in the presence of an antioxidant (extract). Subsequently, the complex $Fe(CN_6)^{4-}$ reacts with Fe^{3+} in $FeCl_3$ to form the $Fe[Fe(CN)_6]$ complex, which has a green colour and exhibit the maximum absorbance at a wavelength of 700 nm.[10,11] Briefly, a stock solution of the extract (10 mg/mL) was prepared by dissolving 10 mg of the extract in 1 mL of methanol. The stock solution was diluted with methanol to various concentrations in µg/mL. A 500 µL solution of phosphate buffer (pH 6.6) was added to 5000 µL of the diluted extract, followed by the addition of 5000 µL of 1% $K_3Fe(CN)_6$. The mixture was incubated at 50°C for 20 min. Thereafter, 5000 µL of 10% of trichloroacetic acid (CCl_3COOH) was added before centrifuging at 3000 rpm for 10 min. The supernatant (5000 µL) was diluted with 5000 µL of distilled water, followed by the addition of 1000 µL of 0.1% $FeCl_3$. The absorbance of the resulting solution was measured at a wavelength of 700 nm. Gallic acid was used as a positive control, while methanol was used as the negative control. The experiment was repeated three times, and the ferric reduction capacity was calculated using the formula as shown in equation Eq.1.

Determination of anticancer activity

Cell lines and culture: Human lung carcinoma (A549) and human embryonic kidney (HEK293) cell lines were supplied by the American Type Culture Collection (ATCC). They were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% glutamine. Cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

MTT assay

The cytotoxic activity of the extracts was evaluated using the MTT assay. Cells were seeded in 96-well plates at a density of 5×10^3 cells/well. They were allowed to adhere overnight. The cells were then treated with various concentrations of the extracts for 24 hours. After the treatment, 20 μL of MTT solution at 5 mg/mL concentration was added to each well and incubated for 4 hours. The formazan crystals collected after incubation were dissolved in DMSO. The absorbance was computed at 570 nm using a microplate reader. The EC_{50} values were calculated for each extract.

Determination of alpha-glucosidase inhibitory activity

The study followed a protocol described in previous research.[11,12] The α-glucosidase enzyme inhibition was conducted in 100 mM sodium phosphate buffer at pH 6.8. A mixture containing 60 µL of sample solution and 50 µL of phosphate buffer containing α-glucosidase solution (0.2 U/mL) was incubated in a 96-well plate at room temperature for 20 minutes. Subsequently, 50 µL of *p*-nitrophenyl-α-D-glucopyranoside solution, prepared in phosphate buffer, was added to each well, and the wells were further incubated for 10 minutes. The reaction was then terminated by adding 160 µL of 0.2 M Na_2CO_3. The absorbance was computed at 405 nm using a Multiplate Reader.

Determination of anti-inflammatory activity

The ability of the extracts to inhibit nitric oxide (NO) production was assessed using lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages.[13] Cells were seeded in 96-well plates at a density of 1×10^5 cells/well and allowed to adhere overnight. The cells were then treated with various concentrations of the extracts in the presence of LPS (1 µg/mL) for 24 hours. The NO levels in the culture supernatant were measured using the Griess reagent. The absorbance was read at 540 nm, and IC_{50} values were calculated.

Determination of antimicrobial activity

The antimicrobial activity of the extract was tested against six bacterial strains; *Staphylococcus aureus, Bacillus subtilis, Lactobacillus fermentum, Salmonella enterica, Escherichia coli,* and *Pseudomonas aeruginosa* using the broth microdilution method. The susceptibility of the organisms to the extracts was evaluated by incubating the organisms with various concentrations of the extract at 37ºC for 24 hours. The turbidity of the medium is indicative of growth of the organisms, and the extent of turbidity was measured using a spectrophotometer. The minimum inhibitory concentration (MIC) and IC_{50} of the extracts were determined. The MIC values were determined as the lowest concentration of the extracts that completely inhibited the growth of the microorganisms. The IC_{50} values were determined as the concentration of the extracts that resulted in 50% growth inhibition of the organisms based on the turbidity measurements.

Carbon tetrachloride-induced Hepatotoxicity Assay

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The hepatoprotective effect of the extracts was evaluated on HepG2 cells treated with carbon tetrachloride (CCl_4).[14] Cells were spread in 96-well plates at a density of 5×10^4 cells/well and then allowed to adhere overnight. The cells were then treated with various concentrations of the extracts for 24 hours. CCl_4 (1%) was added to induce hepatotoxicity. Cell viability was computed using the MTT assay, and the EC_{50} values were determined.

Statistical analysis
All the measurements were performed in triplicate. Results were presented as mean ± standard deviation (SD).

Results and Discussion

Antioxidant activity
The DPPH radical scavenging assay revealed that all extracts had EC_{50} values greater than 1000 µg/mL, indicating low free radical scavenging activity (Table 1). This suggests that the extract has little or low antioxidant activity. Previous studies have shown that brown algae possess moderate to high DPPH radical scavenging activity, which may be attributed to the presence of polyphenols, and other antioxidant compounds. The low activity observed in this study could be due to the extraction methods or the specific chemical composition of *Padina boryana*.
The ABTS radical scavenging assay demonstrated that the EA and n-BuOH extracts had significant radical scavenging activity with EC_{50} values of 52.09 µg/mL and 55.7 µg/mL, respectively (Table 1). This indicates that these extracts contain potent antioxidant compounds capable of neutralizing ABTS radicals. The difference in activity between the DPPH and ABTS assays may be due to the different mechanisms of action of the antioxidants present in the extracts. ABTS radicals are more sensitive to hydrophilic antioxidants, which may explain the higher activity observed in the EA and n-BuOH extracts.
The ferric-reducing capacity assay revealed that the EA extract had the highest reducing power with an EC_{50} value of 61.7 µg/mL, followed by the n-BuOH extract (Table 1). This finding suggests that the EA extract contains compounds with strong electron-donating abilities, which can reduce ferric ions to ferrous ions. The high reducing power of the EA extract is consistent with its significant ABTS radical scavenging activity, indicating the presence of potent antioxidants.

Table 1: Antioxidant activity of fractionated *Padina boryana* Thivy extracts

Extracts	DPPH assay		ABTS assay		Ferric reducing power
	%RSA[b]	EC_{50} (µg/mL)	%RSA[c]	EC_{50} (µg/mL)	EC_{50} (µg/mL)
Ethanol	48.6	>1000	2.87	>1000	>1000
Petroleum ether	38.22	>1000	16.42	>1000	196.7
Ethyl acetate		>1000	89.76	52.09	61.7
Chloroform	49.3	>1000	28.25	245.9	232.0
n-Butanol		>1000	64.93	55.7	161.9
Water		>1000	22.56	198.5	765.0
Quercetin[a]		10.69	78.62[c]	21.9	21.0

RSA: Radical scavenging activity; [a]Control. [b]%RSA at 1000 µg/mL; [c]RSA at 62.5 µg/mL

Anticancer activity
The cytotoxicity assays on A549 and HEK293 cell lines revealed that the PE extract had the lowest EC_{50} values (38.19 µg/mL and 33.28 µg/mL for A549 and HEK293 cells, respectively), indicating that this extract have the highest anticancer activity (Table 2). At 256 µg/mL, the EtOH, PE, EA, $CHCl_3$, and n-BuOH extracts exhibited over 80% toxicity to both cell lines. In particular, PE extract showed toxicity of 61.5% (A549) and 66% (HEK293) at a concentration of 64 µg/mL. These findings suggest that the PE extract contains potent cytotoxic compounds that can induce cell death in cancer cells. The difference in cytotoxicity between the cell lines may be due to the varying sensitivity of the cells to the compounds present in the extracts. Further studies are needed to isolate and identify the specific bioactive compounds responsible for the observed cytotoxic effects.

Table 2: Cytotoxic activity of *Padina boryana* Thivy extracts

Sample	EC_{50} (µg/mL)	
	A549	Hek293
Ethanol	100.1 ± 5.79	101.45 ± 3.60
Petroleum ether	38.19 ± 1.21	33.28 ± 2.72
Ethyl acetate	128.60 ± 4.47	123.23 ± 2.90
Chloroform	102.73 ± 4.26	70.86 ± 3.23
n-Butanol	137.53 ± 5.78	138.16 ± 3.73
Water	>256	>256
Ellipticine	0.44 ± 0.02	1.54 ± 0.04

Ellipticine as positive control.

Alpha-glucosidase inhibitory activity
The alpha-glucosidase inhibitory activity assays demonstrated that all extracts had significant inhibitory effects on glucosidase enzyme activity (Table 3), with the EA extract showing the most potent inhibition (IC_{50} = 3.01 µg/mL). The alpha-glucosidase inhibitory activity of the EA extract was significantly higher than that of acarbose (IC_{50} = 156 µg/mL) by 52-fold. At a 4 µg/mL concentration, the inhibition of α-glucosidase was 71%, increasing to 92% at 16 µg/mL concentration (Figure 2). These results suggest that the EA extract contains compounds with strong alpha-glucosidase inhibitory activity, which could be beneficial in reducing postprandial hyperglycemia in diabetic patients. The higher activity of the EA extract may be due to the presence of specific polyphenols or other bioactive compounds that can inhibit glucosidase enzymes.

Table 3: Alpha-glucosidase inhibitory activity of *Padina boryana* Thivy extracts

Extracts	IC_{50} (µg/mL)
Ethanol	40.00 ± 0.09
Petroleum ether	12.71 ± 0.06
Ethyl acetate	3.01 ± 0.01
Chloroform	10.62 ± 0.19
n-Butanol	11.84 ± 0.03
Water	>256
Acarbose	156.16 ± 5.43

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Acarbose as control.

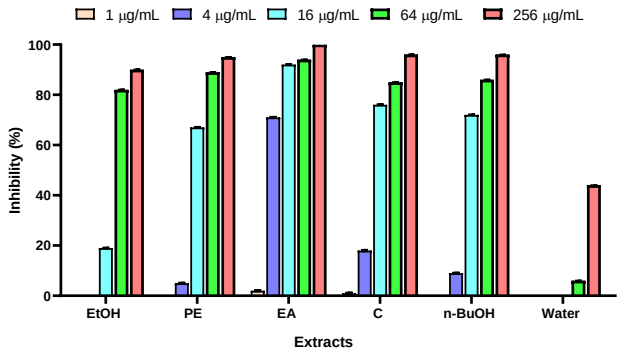

Figure 2: Alpha-Glucosidase inhibitory activity of fractionated *Padina boryana* Thivy extracts

Anti-inflammatory activity

The NO inhibition assay showed that the PE extract had the highest NO inhibitory activity with an IC_{50} of 50.91 µg/mL (Table 5). Despite this, the PE extract also exhibited cytotoxicity at 256 µg/mL, indicating a need for further investigation to balance efficacy and safety. The inhibition of NO production is an important anti-inflammatory mechanism, as excessive NO production can lead to tissue damage and inflammation. The high NO inhibitory activity of the PE extract suggests the presence of compounds that can modulate inflammatory responses. However, the observed cytotoxicity highlights the importance of determining safe and effective doses for potential therapeutic applications.

Antimicrobial activity

The antimicrobial assays indicated that the extracts had no significant activity against gram-negative bacteria (MIC >256 μg/mL). However, the EtOH and PE extracts inhibited the growth of gram-positive bacteria, specifically *Staphylococcus aureus* and *Bacillus subtilis*, with MIC value of 256 µg/mL for both organisms. These results suggest that the extracts contain selective antibacterial compounds that are more effective against gram-positive bacteria. The lack of activity against gram-negative bacteria may be due to the presence of an outer membrane that acts as a barrier to the bioactive compounds. The observed antibacterial activity against gram-positive bacteria highlights the potential of these extracts as natural antimicrobial agents.

Hepatoprotective activity

The extracts did not demonstrate significant hepatoprotective activity against CCl4-induced liver damage, with EC_{50} values exceeding 100 µg/mL. The CHCl3 extract provided 31.94% protec
tion at the highest concentration tested, but showed no significant activity at lower concentrations. These results suggest that the extracts may not contain potent hepatoprotective compounds or that the concentrations used in this study were insufficient to observe significant effects. Further studies are needed to investigate the potential hepatoprotective activity of the extracts at higher concentrations or with different experimental models.

Table 4: Antimicrobial activity of *Padina boryana* Thivy extracts

Extract	Values (µg/mL)	Gram (+)			Gram (-)		
		Staphylococcus aureus	*Bacillus subtilis*	*Lactobacillus fermentum*	*Salmonella enterica*	*Escherichia coli*	*Pseudomonas aeruginosa*
Ethanol	IC_{50}	160.00 ± 9.69	>256	>256	>256	>256	>256
	MIC	256 ± 0.00	>256	>256	>256	>256	>256
Petroleum ether	IC_{50}	160.00 ± 10.09	160.00 ± 8.96	>256	>256	>256	>256
	MIC	256 ± 0.00	256 ± 0.00	>256	>256	>256	>256
Ethyl acetate	IC_{50}	>256	>256	>256	>256	>256	>256
	MIC	>256	>256	>256	>256	>256	>256
Chloroform	IC_{50}	>256	>256	>256	>256	>256	>256
	MIC	>256	>256	>256	>256	>256	>256
n-Butanol	IC_{50}	>256	>256	>256	>256	>256	>256
	MIC	>256	>256	>256	>256	>256	>256
Water	IC_{50}	>256	>256	>256	>256	>256	>256
	MIC	>256	>256	>256	>256	>256	>256
Ampicillin	IC_{50}	0.02 ± 0.005	3.62 ± 0.15	1.03 ± 0.07	-	-	-
	MIC	0.125 ± 0.0	32 ± 0.0	32 ± 0.0	-	-	-
Cefotaxime	IC_{50}	-	-	-	0.43 ± 0.05	0.007 ± 0.002	4.34 ± 0.15
	MIC	-	-	-	32 ± 0.0	0.5 ± 0.0	8.0 ± 0.0

RSA: Radical scavenging activity; [a]Control. [b]%RSA at 1000 µg/mL; [c]RSA at 62.5 µg/mL

Table 5: Anti-inflammatory (*In-vitro* NO inhibitory) activity of *Padina boryana* Thivy extracts

Extract	Cell viability[b]	IC_{50} (µg/mL)
Ethanol	80	149.71 ± 6.21
Petroleum ether	46	50.91 ± 2.56
Ethyl acetate	86	98.91 ± 3.35
Chloroform	83	128.00 ± 7.03
n-Butanol	88	203.03 ± 12.5
Water	89	>256
L-NMMA[a]	89[c]	11.91 ± 0.7

[a]Control. [b]at 256 µg/mL. [c]at 128 µg/mL.

Conclusion

This study highlights the diverse bioactive properties of extracts from *Padina boryana* Thivy, with notable antioxidant, anticancer, alpha-glucosidase inhibitory, anti-inflammatory, and selective antimicrobial activities. The EA and PE extracts, in particular, showed promising results in multiple assays, suggesting their potential as sources of therapeutic compounds. The extracts' significant alpha-glucosidase inhibitory and NO inhibitory activities indicate their potential for managing diabetes and inflammatory conditions, respectively. However, the observed cytotoxicity and lack of hepatoprotective activity need further investigation to ensure their safety and efficacy. Overall, the findings from this study contribute to the growing body of

knowledge on the bioactive properties of marine algae and highlight the potential of *Padina boryana* as a valuable source of natural therapeutic agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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