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POLYPHENOL-RICH FRACTION OF THALASSIA HEMPRICHII: DUAL ENZYME INHIBITION, ANTIOXIDANT CAPACITY, AND ANTI-INFLAMMATORY ACTIVITY FOR DIABETES MANAGEMENT

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder marked by hyperglycemia and associated complications. Limitations of current therapies have driven interest in marine-derived bioactives, particularly polyphenols, for safer and multi-targeted interventions. *Thalassia hemprichii*, a tropical seagrass rich in phenolic compounds, remains underexplored for antidiabetic potential. **Methodology:** A polyphenol-rich fraction (PRF) was extracted from *T. hemprichii* leaves using sequential solvent extraction. FTIR spectroscopy was employed to identify functional groups. Total phenolic and flavonoid contents were quantified using spectrophotometric methods. In vitro antidiabetic activity was assessed by α-amylase inhibition (DNSA method) and α-glucosidase inhibition (pNPG assay), with acarbose as the reference, and anti-inflammatory and antioxidant assays were also performed. **Results and Discussion:** FTIR analysis confirmed the presence of hydroxyl, carbonyl, and aromatic groups, indicating the presence of phenolic compounds. PRF demonstrated dose-dependent inhibition of α-amylase (13.4–55.6%) and α-glucosidase (16.72–58.14%) at concentrations of 25–125 µg/mL, with IC_{50} values comparable to acarbose. The inhibitory profile showed stronger activity against α-glucosidase and moderate suppression of α-amylase. In addition, PRF exhibited significant antioxidant (49.40–78.23%) and anti-inflammatory (40.78–75.02%) effects in a concentration-dependent manner (50–200 µg/mL). **Conclusion:** The polyphenol-rich extract of *T. hemprichii* exhibits notable dual-enzyme inhibition, indicating its potential to manage postprandial hyperglycemia while reducing gastrointestinal side effects. Its cytoprotective properties, including antioxidant and anti-inflammatory activities, further support its role in preventing diabetes-related complications. These findings highlight *T. hemprichii* as a promising candidate for nutraceutical or phytopharmaceutical development, meriting further in vivo validation and safety evaluation.

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder characterized by persistent hyperglycemia due to deficiencies in insulin secretion, insulin action, or both. It stands as one of the pressing non-communicable health challenges that necessitates

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immediate focus in the twenty-first century, with the IDF estimating approximately 783 million cases worldwide by the year 2045. It is also a global metabolic disorder affecting millions of individuals, with prevailing management strategies predominantly relying on oral hypoglycemic agents [1,2].

The condition is associated with a spectrum of debilitating microvascular complications, including but not limited to: retinopathy, nephropathy, neuropathy, and macrovascular complications such as CAD and stroke. Collectively, these factors substantially affect morbidity, mortality, and healthcare expenditures [3]. Current therapeutic interventions, encompassing lifestyle modifications, oral hypoglycemic agents, and exogenous insulin, are effective in controlling blood glucose levels but are limited by side effects, reduced efficacy with prolonged use, and patient nonadherence. This has intensified the global search for novel, safe, and effective antidiabetic agents, particularly from natural sources, which can provide multi-targeted therapeutic effects and improved patient tolerability [4]. Marine ecosystems cover more than 70% of the Earth's surface and are integral to global biodiversity, providing a home to a broad range of organisms that produce distinctive bioactive secondary metabolites.

These compounds have evolved as adaptations to challenging environmental conditions-high salinity, intense light, variable hydrodynamic forces-and often display structural features not seen in terrestrial counterparts [5]. The seagrasses, which are marine flowering plants, represent one of the least-studied groups from a marine pharmacognosy perspective. We recognize their ecological roles, from nutrient cycling and sediment stabilization to the maintenance of diverse life forms. Still, their chemical bounty is only now being tapped. [6].Seagrasses are distributed from the mid-intertidal zone to depths greater than 50 m [7,8].

Seagrasses are known to biosynthesize an array of phenolic compounds, flavonoids, and tannins, many of which possess antioxidant, anti-inflammatory, antimicrobial & cytoprotective activities [9]. *Thalassia hemprichii* (Ehrenb.) Asch. is a tropical seagrass species that flourishes throughout the Indo-Pacific region. For a long time, this seagrass species has been used to manage certain health conditions, such as wound healing and inflammation, and to promote overall well-being. Phytochemical studies have established that *T. hemprichii* is exceptionally rich in polyphenolic compounds, including hydroxycinnamic acids, flavonols, and condensed tannins, which are implicated with a wide array of biological activities [10]. Subsequently, various polyphenols have been considered to play a role in the regulation of glucose metabolism through different mechanisms, including inhibition of carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase); stimulation of insulin secretion from pancreatic β-cells; enhancement of insulin sensitivity in peripheral tissues; and mitigation of β-cell dysfunction associated with oxidative stress [11]. Alpha-glucosidase, located in the brush-border membrane of intestinal epithelial cells, is a clinically validated approach for treating postprandial hyperglycemia by delaying the hydrolysis of dietary oligosaccharides and disaccharides [12]. Inhibitors of α-glucosidase, such as miglitol or acarbose, are effective oral hypoglycemic agents, but they often cause GI discomfort due to fermentation of unabsorbed carbohydrates.

Natural polyphenolic inhibitors: Natural polyphenolic inhibitors have shown promise, potentially providing both glycemic control and additional benefits, such as antioxidant and anti-inflammatory activity. [13]. Marine-derived polyphenols, in particular, often exhibit greater polymerization, unique substitution patterns, and increased hydrophobicity, which may enhance their enzyme-inhibitory potency and radical-scavenging efficiency relative to terrestrial plant polyphenols [14]. Although there have been sporadic reports of enzyme inhibition and antioxidant activity in other seagrass species, such as *Enhalus acoroides* and *Halophila ovalis*, there is little literature on *Thalassia hemprichii* regarding its potential use in treating diabetes. The high polyphenol content and distinctive chemical structures of marine phenolics provide a solid foundation for work [15]. More importantly, oxidative stress is not merely a complication of hyperglycemia levels. Rather, it is a major mediator of complications in diabetes. This occurs through various mechanisms, including lipid peroxidation, protein glycation, and mitochondrial dysfunction. The role of polyphenols in managing diabetes lies in their ability to modulate redox signaling pathways by quenching oxidative stress. This provides a double benefit to patients with diabetes. This includes regulating acute glucose spikes and oxidative alterations in the pancreas and peripheral tissues [16]. In vitro bioassays can be used initially to screen natural samples for potential antidiabetic effects. α-Glucosidase and α-amylase inhibition assays are the most critical screening assays for

assessing the compound's potential to regulate blood glucose levels [17,18]. Given the growing need for novel, safe, and effective antidiabetic agents, this study aims to comprehensively investigate the *in vitro* antidiabetic potential of a polyphenol-rich extract derived from *T. hemprichii*.

Additionally, correlations between phytochemical composition and biological activity will be examined to establish structure–activity relationships. The objectives of this experiment are to determine the total polyphenol content of the extracts obtained using standard spectrophotometric techniques and to assess their ability to inhibit α-glucosidase and α-amylase, major enzymes responsible for the hydrolysis of carbohydrate molecules in food. *T. hemprichii* acts as a promising marine-derived candidate for the development of nutraceuticals or phytopharmaceuticals for glycemic control and the prevention of diabetes-related complications.

MATERIALS AND METHODS

Fresh specimens of *Thalassia hemprichii* were collected at low tide, carried on ice to the laboratory, and washed in seawater, then distilled water to eliminate epiphytes, sand, and salts. Rinsed seagrass leaves were chopped into small fragments and oven-dried at 40 ± 2 °C to a constant weight to avoid the breakdown of thermolabile polyphenols. The dried residue was ground into a powder (<0.5 mm) using a stainless-steel grinder, homogenized, and stored in amber bottles at −20 °C for subsequent extraction. Fifty grams of the powdered residue were initially defatted by 6 hours of stirring in n-hexane to extract lipids and pigments; the hexane extract was discarded. 70% ethanol was chosen as the extraction solvent because its balanced polarity effectively facilitates the recovery of marine polyphenols. The sample was subsequently extracted using 70% ethanol in water at a 1:10 ratio via sonication-assisted maceration (in an ultrasonic bath for 30 minutes), followed by orbital shaking at 25 °C for 24 hours to ensure efficient compound extraction. The extraction process was repeated twice, and the resulting extracts were filtered through Whatman filter paper. The filtrates were concentrated under reduced pressure at ≤40 °C using a rotary evaporator to remove ethanol, then lyophilized to yield the crude hydroalcoholic extract. The crude extract aqueous suspension was sequentially partitioned with n-hexane to expel remaining nonpolar contaminants & with ethyl acetate. The ethyl acetate-rich phenolic constituent fraction was collected, vacuum-dried & labeled as the polyphenol-rich fraction (PRF). PRF was kept at −20°C in amber vials. The total phenol content (TPC) of PRF was evaluated using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g extract. The total flavonoid content (TFC) was estimated using the aluminum chloride method (mg quercetin equivalents per g).

α-Amylase inhibition (DNSA method)

In vitro α-amylase inhibitory activity of *the Thalassia hemprichii polyphenol-rich fraction (PRF) was assessed using the 3,5-dinitrosalicylic acid (DNSA) protocol, with porcine pancreatic α-amylase (EC 3.2.1.1) as the enzyme source*. PRF was dissolved in ≤1% DMSO and further diluted with 0.02 M phosphate buffer at pH 6.9 containing 6 mM NaCl to a final concentration of 1-1000 $\mu g \cdot mL^{-1}$. In each reaction tube, 250 µL of phosphate buffer, 250 µL of α-amylase solution (0.5 $mg \cdot mL^{-1}$), and 250 µL of PRF were incubated at 37°C for 10 min. The reaction was initiated by adding 250 µL of 1% (w/v) soluble starch, followed by incubation at 37°C for 10 min. To terminate the enzymatic reaction, use 500 µL of DNSA reagent.

α-Glucosidase Inhibitory Activity (pNPG assay)

The α-glucosidase inhibitory activity was assessed with yeast α-glucosidase (EC 3.2.1.20) and p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate. In brief, the enzyme (0.1 U/mL) in 0.1 M phosphate buffer (pH 6.8) was pre-incubated with varying concentrations of polyphenol-rich fraction (PRF) (500 µg/mL) at 37 °C for 10 min. The reaction was started by the addition of 5 mM pNPG and incubated for 20 min at 37 °C. The reaction was quenched by adding 0.1 M Na_2CO_3, which also intensified the yellow color of the released p-nitrophenol. Absorbance at 405 nm was measured using a spectrophotometer. Acarbose was used as the positive control, and a blank without inhibitor served as the negative control. Inhibition percentage was determined from the absorbance difference between the test and control samples.

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay: The radical-scavenging activity of a test sample was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The materials were suspended in DMSO at a concentration of 10 µg/mL, and various concentrations of the extract (10, 20, 30, 40, and 50 µg/mL) were added to the DPPH solution. After 30 min of incubation at 37°C in the dark, the absorbance of the DPPH solution will be measured at 517 nm. Ascorbic acid will be used

as a positive control, and the percentage of scavenging activity inhibition will be calculated as follows:

$$DPPH\ scavenging\ effect = \frac{A0\ -\ A1}{A0} \times 100$$

where A0 is the absorbance of the control, and A1 is the absorbance of the sample.

Anti-inflammatory activity

Protein denaturation assay: The reaction mixture of 1% bovine albumin, 5 mL of PBS (pH 6.4), and the samples was suspended in 10 µg/mL DMSO. It was combined and incubated in a 37°C water bath for 15 minutes, then heated to 70°C for 5 minutes. After cooling, turbidity at 660 nm was measured using a UV/VIS spectrometer. A phosphate buffer served as the control. The protein denaturation inhibition percentage was calculated using the following calculation-

$$\%\ inhibition\ of\ denaturation = \frac{1\ -\ A2}{A1} \times 100$$

where A1 = absorption of the control sample and A2 = absorption of the test sample f. Figure 1 provides an overview of the experimental workflow and the biological activities associated with the polyphenol-rich extract of T. hemprichii.

Data analysis

Statistical comparisons were performed using one-way ANOVA with Tukey's post hoc test; $p < 0.05$ was considered significant.

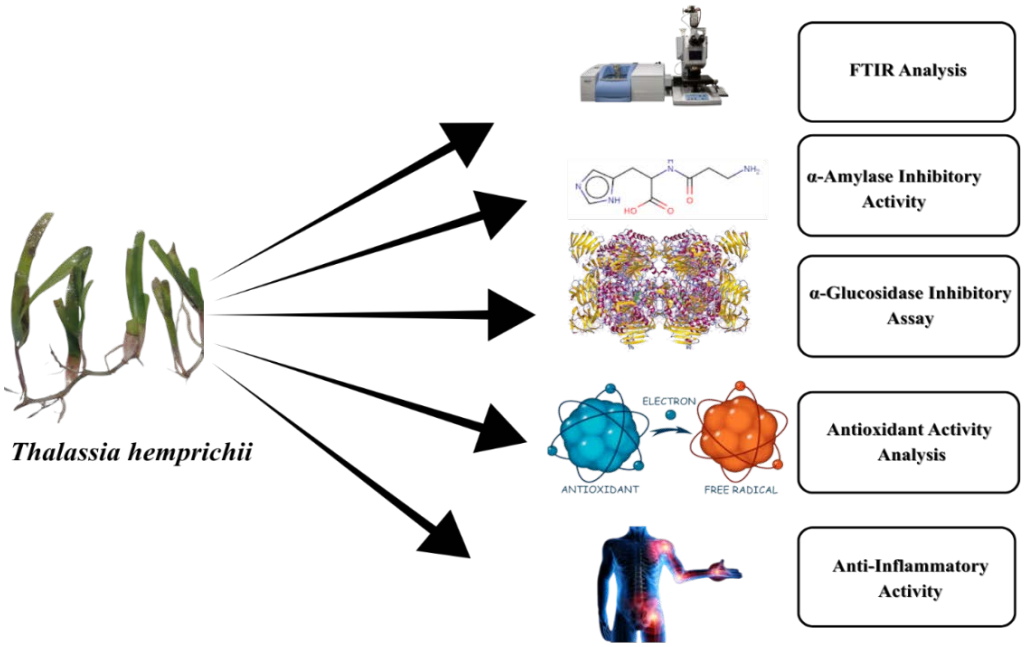

Figure 1: Overview of the experimental workflow and biological activities of the polyphenol-rich extract of *T. hemprichii*.

RESULT AND DISCUSSION

Results

FTIR Analysis

The FTIR spectrum in Figure 2 of the polyphenol-rich fraction of *Thalassia hemprichii* showed a broad band at 3374 cm^{-1}, typical of the O–H stretching vibration of hydrogen-bonded hydroxyl groups in phenolic compounds. A band at 2935 cm^{-1} was due to C–H stretching of the aliphatic CH_2 group. The band at 1622 cm^{-1} was attributed to C=O stretching of conjugated carbonyl groups, either from flavonoids or other polyphenols, blended with aromatic C=C stretching. Bands at 1340 and 1101 cm^{-1} were signs of O–H bending and C–O stretching vibrations, respectively. The bands at 829 and 780 cm^{-1} were assigned to aromatic C–H out-of-plane bending, thereby proving the presence of aromatic rings, and the band at 615 cm^{-1} was due to aromatic ring skeletal vibrations. These findings collectively demonstrate the presence of hydroxyl-rich aromatic compounds in the extract, consistent with its polyphenolic nature.

Quantitative Estimation of Total Phenolic and Flavonoid Content

The polyphenol-rich fraction (PRF) derived from *Thalassia hemprichii* was quantitatively analyzed for its total phenolic and flavonoid content. The results demonstrated that the PRF contains a Total Phenolic Content (TPC) of 2.57 ± 0.28 mg GAE/g extract and a Total Flavonoid Content (TFC) of 1.96 ± 0.031 mg QE/g extract. The relatively higher TPC compared to TFC suggests that phenolic compounds constitute a major proportion of the extract's bioactive constituents. These findings indicate the potential antioxidant capacity of the PRF, as

phenolics and flavonoids are well known for their free-radical-scavenging properties. All values are expressed as mean ± standard deviation (SD) of three independent determinations, indicating good reproducibility and reliability of the experimental measurements.

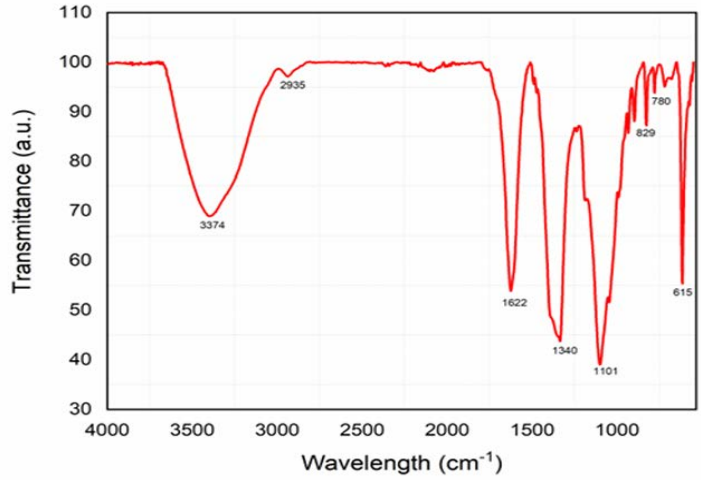

Figure 2: FTIR spectrum of polyphenol-rich compounds isolated from *Thalassia hemprichii*

Table 1: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Polyphenol-Rich Fraction (PRF) from *Thalassia hemprichii*.

Parameter	Value (Mean ± SD)
Total Phenolic Content (TPC)	2.57 ± 0.28 mg GAE/g extract
Total Flavonoid Content (TFC)	1.96 ± 0.031 mg QE/g extract

Values are means + SD for 3 determinations.

α-Amylase Inhibitory Activity

The α-amylase inhibitory activity of the *Thalassia hemprichii* polyphenol-rich fraction was evaluated over a concentration range of 25–125 µg·mL⁻¹ (Figure 3). The inhibitory activity increased in a concentration-dependent manner, with inhibition rising from 13.42% at 25 µg·mL⁻¹ to 57.83% at 125 µg·mL⁻¹. Intermediate inhibition values of 24.68%, 30.21%, and 46.89% were observed at concentrations of 50, 75, and 100 µg·mL⁻¹, respectively. The IC_{50} value of the test sample was calculated to be approximately 107 µg·mL⁻¹. In comparison, the standard drug acarbose exhibited an IC_{50} value of approximately 136 µg·mL⁻¹. A comparative dose–response analysis demonstrated a consistent increase in enzyme inhibition as concentration increased.

α-Glucosidase Inhibitory Assay

"The polyphenol-rich extract of *Thalassia hemprichii* exhibited concentration-dependent inhibitory activity against both α-amylase and α-glucosidase enzymes (Figure 4). In the α-glucosidase inhibition assay, the percentage inhibition increased progressively from 13.42% at 25 µg/mL to 57.83% at 125 µg/mL. Intermediate inhibition values of 24.68%, 30.21%, and 46.89% were observed at concentrations of 50, 75, and 100 µg/mL, respectively. The IC_{50} value of the test sample was approximately 107 µg/mL. In comparison, the standard drug acarbose exhibited an IC_{50} value of approximately 136 µg/mL. A comparative dose–response analysis demonstrated the inhibitory profiles of both the test sample and acarbose, with a consistent increase in enzyme inhibition as concentration increased. The inhibitory potency was more pronounced at higher concentrations in both assays, indicating that the extract's bioactive polyphenolic constituents may contribute significantly to the suppression of carbohydrate-hydrolyzing enzymes. This dual inhibitory potential suggests a possible role for *T. hemprichii* extract in modulating postprandial hyperglycemia, thereby supporting its potential application in the management of type 2 diabetes mellitus

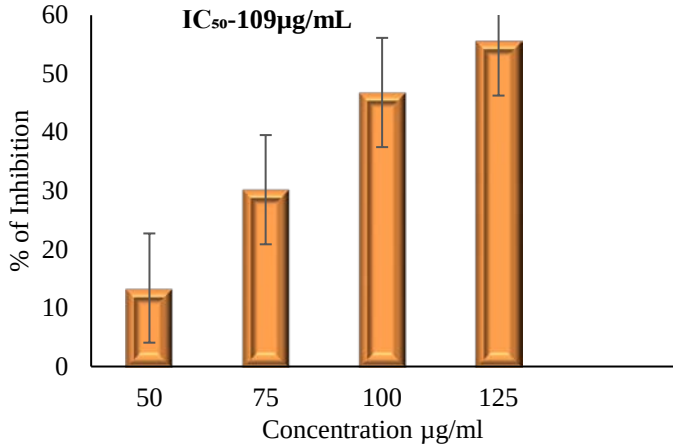

Figure 3: α-Amylase Inhibitory Activity of polyphenol-rich compounds isolated from *Thalassia hemprichii*

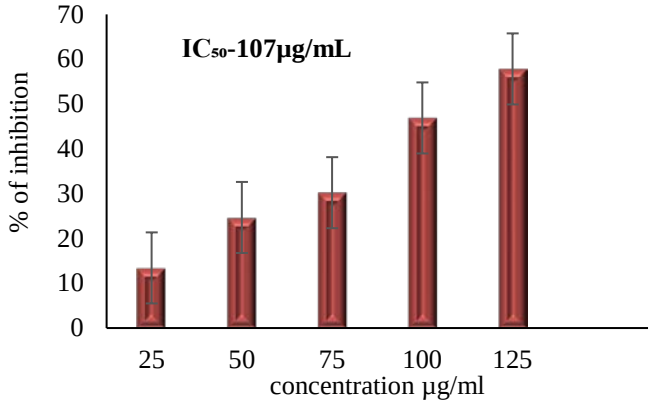

Figure 4: α-Glucosidase Inhibitory Assay of polyphenol-rich compounds isolated from *Thalassia hemprichii*

Antioxidant activity

In Figure 5, the antioxidant potential of the polyphenol-rich fraction of *Thalassia hemprichii* also increased in a concentration-dependent manner. At 50 µg/mL, the inhibition was 49.40%, increasing slightly to 50.65% at 100 µg/mL. However, a marked increase was observed at 150 µg/mL with 67.68% inhibition, reaching a maximum of 78.23% at 200 µg/mL. This trend highlights the compounds' strong free-radical scavenging capacity, which is particularly pronounced at higher concentrations, underscoring their role as potent natural antioxidants.

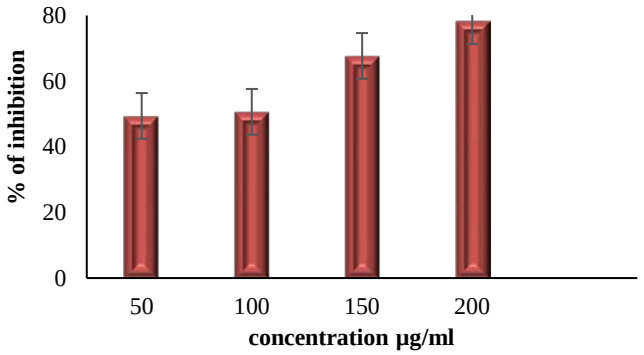

Figure 5: Antioxidant activity of polyphenol-rich compounds isolated from *T. hemprichii* at different concentrations.

Anti-Inflammatory activity

In Figure 6, the polyphenol-rich compounds of *Thalassia hemprichii* exhibited a clear dose-dependent anti-inflammatory effect. At the lowest tested concentration of 50 µg/mL, the percentage inhibition was 40.78%, which progressively increased to 55.69% at 100 µg/mL and 63.66% at 150 µg/mL. The maximum inhibition of 75.02% was observed at 200 µg/mL. These findings indicate that the extract's anti-inflammatory efficacy increases with concentration, suggesting strong potential to suppress inflammatory responses at higher doses.

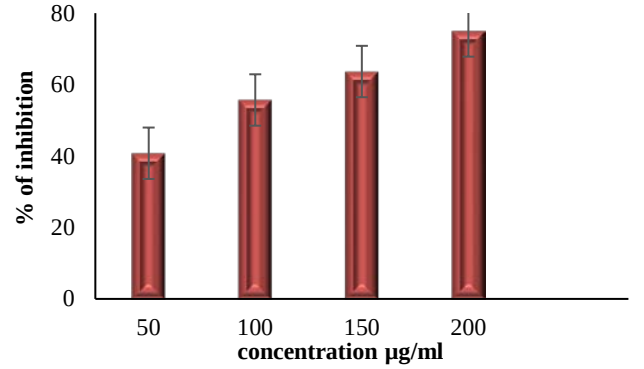

Figure 6: Anti-inflammatory activity of polyphenol-rich compounds isolated from *T. hemprichii* at different concentrations.

Discussion

The present investigation provides new insights into the *in vitro* antidiabetic potential of a polyphenol-rich extract from the seagrass *T. hemprichii*, supported by structural characterization using FTIR spectroscopy and enzymatic inhibition assays against α-amylase and α-glucosidase. FTIR spectral analysis of the *T. hemprichii* extract revealed characteristic absorption bands corresponding to functional groups commonly associated with phenolic and flavonoid compounds. Prominent peaks in the range of 3200–3500 cm^{-1} were indicative of hydroxyl (–OH) stretching vibrations, a hallmark of polyphenolic hydroxylation which confers strong hydrogen-donating antioxidant potential. Absorptions near 1600–1650 cm^{-1} corresponded to aromatic C=C stretching, suggesting the presence of conjugated phenolic rings. Additional bands at ~1700 cm^{-1} were attributed to carbonyl (C=O) stretching, possibly from flavonoid ketones or phenolic acids, while peaks in the 1000–1300 cm^{-1} range were assigned to C–O stretching of alcohols, esters, or glycosidic linkages. These spectral features align with earlier phytochemical reports on marine angiosperms, reinforcing the extract's polyphenolic richness and potential bioactivity. The chemical profile inferred from FTIR supports the likelihood of both hydrophilic and moderately hydrophobic phenolic constituents, which may influence enzyme-binding affinity and biological activity [19,20].

A more detailed interpretation of the FTIR fingerprint suggests the possible presence of specific classes of marine polyphenols previously reported in *Thalassia hemprichii* and related seagrasses. The broad O–H stretching band observed at ~3374 cm^{-1} is characteristic of extensively hydrogen-bonded phenolic hydroxyl groups, typical of hydroxycinnamic acid derivatives such as caffeic acid, ferulic acid, and p-coumaric acid, which have been documented in marine angiosperms [9,15,21]. The absorption around ~1622 cm^{-1}, corresponding to aromatic C=C stretching conjugated with carbonyl groups, is consistent with flavonol structures such as luteolin, quercetin, and kaempferol derivatives, which possess a C2=C3 double bond conjugated with a 4-oxo group. The C=O stretching region (~1700–1620 cm^{-1}) further supports the presence of flavonoids and phenolic acid esters, while strong C–O stretching bands (1100–1300 cm^{-1}) suggest glycosylated phenolics. Additionally, aromatic C–H bending vibrations (780–830 cm^{-1}) indicate substituted benzene rings typical of condensed tannins and polymerized phenolics reported in marine angiosperms [14,22]

The α-amylase inhibitory assay demonstrated that the *T. hemprichii* extract exerted a dose-dependent suppression of starch hydrolysis. α-Amylase catalyzes the endo-hydrolysis of α-1,4-glycosidic bonds in polysaccharides, releasing maltose and dextrins, which are subsequently converted to glucose by α-glucosidase [23]. Excessive α-amylase activity leads to rapid postprandial glucose surges, a major glycemic challenge in the management of type 2 diabetes. The inhibitory effect observed in this study is consistent with prior reports on phenolic-rich plant extracts, wherein hydroxylated aromatic structures interact with the active site of α-amylase, forming hydrogen bonds and hydrophobic contacts that hinder substrate binding [24].

Although synthetic inhibitors such as acarbose achieve similar effects, their gastrointestinal side effects necessitate exploration of alternative sources. The comparatively moderate α-amylase inhibition by *T. hemprichii*, compared with potent synthetic agents, may be advantageous, as partial enzyme inhibition can attenuate glycemic spikes without provoking excessive colonic fermentation [25]. Several studies have indicated that the structure–activity relationship of marine-derived phenolics extends beyond the abundance of hydroxyl groups. Increased hydroxylation enhances hydrogen bonding with catalytic residues of α-glucosidase, while conjugated aromatic systems promote π–π stacking within the enzyme pocket. Notably, the higher degree of polymerization commonly observed in marine phenolics (e.g., condensed tannins or phlorotannin-like compounds) enables multivalent binding to multiple subsites of α-glucosidase, thereby improving inhibitory stability and potency. Such structural features have been reported to enhance inhibition of carbohydrate-hydrolyzing enzymes by polyphenols [14,26,27].

The α-glucosidase inhibitory activity of the extract was notably higher, indicating a stronger interaction with this brush-border enzyme. α-Glucosidase catalyzes the terminal step in carbohydrate digestion, liberating glucose for intestinal absorption [27]. Inhibition at this stage is a clinically validated approach to reducing postprandial hyperglycemia, as evidenced by the current therapeutic use of α-glucosidase inhibitors. Polyphenols are known to competitively or non-competitively bind to α-glucosidase, with inhibitory potency influenced by the degree of polymerization and substitution of hydroxyl groups [26]. The strong α-glucosidase inhibition observed here aligns with literature on marine-derived phenolics, which often exhibit higher binding affinities due to unique structural configurations, such as additional methoxy substitutions or halogenation in some marine metabolites. These results suggest that *T. hemprichii* phenolics may exert their antidiabetic effect primarily during intestinal carbohydrate hydrolysis, thereby modulating glucose influx more effectively than starch breakdown alone [28].

Selective inhibition of α-glucosidase with only moderate suppression of α-amylase is considered therapeutically advantageous in managing postprandial hyperglycemia. Strong α-amylase inhibition can lead to excessive amounts of undigested starch reaching the colon, where bacterial fermentation produces gases such as hydrogen and methane, resulting in gastrointestinal side effects including flatulence, abdominal discomfort, and diarrhea. In contrast, preferential α-glucosidase inhibition delays terminal carbohydrate digestion at the brush-border level without markedly increasing colonic starch load, thereby improving glycemic control while minimizing gastrointestinal intolerance [12,13,27].

So the comparative inhibition profile between α-amylase and α-glucosidase suggests a therapeutic advantage of selective α-glucosidase inhibition with moderate α-amylase suppression is considered optimal in minimizing gastrointestinal discomfort while effectively controlling postprandial glycemia. This dual yet balanced inhibitory pattern mirrors the pharmacodynamic target profile for next-generation enzyme inhibitors in diabetes management [29,30].

From a mechanistic standpoint, the antidiabetic effects of polyphenols extend beyond enzyme inhibition. Their antioxidant capacity, supported here by FTIR evidence of hydroxyl-rich aromatic systems, may protect pancreatic β-cells from oxidative stress-induced apoptosis, improve insulin receptor signaling by attenuating oxidative modifications, and suppress pro-inflammatory pathways linked to insulin resistance. The coexistence of enzymatic inhibition and antioxidant potential thus positions *T. hemprichii* as a promising candidate for functional food or nutraceutical development [31,32]

Previous studies on other seagrasses, such as *Enhalus acoroides* and *Halophila ovalis*, have reported similar enzyme inhibitory activities. Still, the current findings expand the chemotaxonomic and bioactivity data to include *T. hemprichii*, a relatively

underexplored species [22,33]. The combination of FTIR-derived structural insights with functional bioassays provides a robust framework for understanding its therapeutic relevance. However, it should be acknowledged that *in vitro* assays, while valuable for preliminary screening, do not fully replicate the complexity of gastrointestinal digestion, bioavailability, and metabolic transformation in vivo. The phenolic compounds detected here may undergo extensive conjugation, degradation, or microbiota-mediated transformation, potentially modulating their efficacy in human systems [34].

In addition to its α-amylase and α-glucosidase inhibitory activity, *Thalassia hemprichii* extract, which is abundant in polyphenols, also exhibits high antioxidant and anti-inflammatory activity, thereby contributing to its antidiabetic activity. Polyphenols are recognized as free-radical scavengers that donate a hydrogen atom or an electron to inactivate reactive oxygen species (ROS), thereby quenching oxidative damage to lipids, proteins, and DNA, which contributes to β-cell failure and insulin resistance [35]. In extracts of seagrasses, higher total phenolic and flavonoid content generally corresponds to higher radical-scavenging activity (e.g., DPPH and ABTS assays), metal chelation, or ferric reducing ability (FRAP). It suggests multiple modes of action by which ROS are inhibited/neutralized in vitro [21].On the anti-inflammatory side, polyphenols inhibit major signaling pathways and the synthesis of pro-inflammatory mediators. For instance, in seagrasses like *Thalassia hemprichii,* lipopolysaccharide (LPS) or other inflammatory inducers increase cytokine levels of TNF-α, IL-1β, and IL-6; phenolic extract treatment reduces cytokine synthesis, inhibits NF-κB activation, and down-regulates COX-2 or iNOS expression [36].

Significantly, by diminishing oxidative stress, polyphenols also mitigate the oxidative activation of inflammatory pathways (such as ROS-mediated activation of MAPKs and NF-κB), thereby linking antioxidant effects to anti-inflammatory properties [35]. In our extract, characteristic FTIR features — namely, abundant hydroxyl groups, aromatic rings, and potentially carbonyls — are consistent with strong antioxidant activity and the ability to interact with proteins or enzymes involved in inflammation. With antioxidant and anti-inflammatory mechanisms in tandem, it might be possible to protect islet β-cells from oxidative injury, reduce inflammation-induced injury in tissues (such as the pancreas, liver, and gut), and enhance insulin signal transduction, thereby improving glycemic control independently of enzyme inhibition alone. This balanced profile suggests a dual mechanism in controlling postprandial hyperglycemia while protecting β-cells from oxidative and inflammatory damage, highlighting its potential as a nutraceutical candidate for diabetes management.

CONCLUSION

This study showed that the polyphenol-rich fraction (PRF) of *Thalassia hemprichii* exhibits significant in vitro antidiabetic activity. FTIR spectral analysis of the PRF revealed the presence of hydroxyl, carbonyl, and aromatic functional groups, likely indicating a high phenolic content responsible for its bioactivity. PRF showed a significant, clear, concentration-dependent inhibition of both α-amylase and α-glucosidase comparable to that of standard acarbose. This concurrent inhibition of enzymes indicates that the extract can efficiently retard carbohydrate hydrolysis and glucose absorption, thereby modulating postprandial hyperglycemia and exerting antioxidant and anti-inflammatory activity, which supports the treatment and prevention of diabetes. Such an activity highlights the potential of *T. hemprichii* as a natural source of bioactive compounds for the management of type 2 diabetes mellitus. Given its balanced dual-enzyme inhibition and cytoprotective properties, the polyphenol-rich fraction of *Thalassia hemprichii* holds potential for development as a standardized phytopharmaceutical preparation to target postprandial hyperglycemia. Alternatively, given its natural origin and antioxidant profile, it may be explored as a functional food ingredient or nutraceutical supplement to support glycemic control and maintain metabolic health. Future research should focus on formulation optimization, dose standardization, bioavailability enhancement, and in vivo validation to determine its most suitable therapeutic application.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Mohammed Asif Hussein and Dilipan Elangovan conceptualized the study and secured the funding. Dilipan Elangovan was responsible for data collection and statistical analysis. Navni Rohatgi and Vasugi Suresh interpreted the data.

Navni Rohatgi and Mohammed Asif Hussein prepared the first draft of the manuscript. Dilipan Elangovan edited and critically reviewed the manuscript. Vasugi Suresh supervised the project administration. All authors read and approved the final version of the manuscript.

DECLARATION OF USE OF AI TOOLS

The authors used AI-assisted tools solely for language editing and to improve the readability of the manuscript. All scientific content, analysis, interpretations, and conclusions were developed, reviewed, and verified by the authors. The authors take full responsibility for the originality, accuracy, and integrity of the work.

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