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Evaluation of Phytochemical Constituents, Antioxidant & Anti-Diabetic Activity of Seaweed Turbinaria Decurrens Collected from Gulf of Mannar, Tamil Nadu, India

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ABSTRACT

The genus *Turbinaria* stands out as a prominent and widely distributed member of the brown algae. This species is characterized by distinct morphology, ecological importance, and biochemical properties. In the present study, taxonomical, molecular identification along with antioxidant and anti-diabetic activity of the different extracts (acetone, aqueous & methanol) of *T.decurrrens* was determined. The preliminary phytochemical components were investigated qualitatively and quantitatively by using standard methods and confirmed the presence of variety of phytoactive components such as coumarins, glycosides, phenols tannins, terpenoids and saponins. The collected seaweed sample was identified by molecular approaches using QIAGEN DNA isolation kit and the BLAST program at the NCBI website that showed se quences similarities with existing seaweed rbcL gene sequences up to 99.21%. "The antioxidant activity of three crude extracts was evaluated by various antioxidant assays viz DPPH and ABTS." The findings revealed potent scavenging activity of ABTS, with a minimum inhibitory concentration (IC_{50}) of 1.06mg/ml for the methanolic extract while the DPPH assay indicated an IC_{50} value 1.56mg/ml. The investigation into the antidiabetic properties of seaweed extract revealed that the methanolic extract of *T.decurrens* exhibited the most significant inhibitory activity against α-amylase and α-glucosidase enzymes with IC_{50} value of 1.8 mg/ml and 1.40mg/ml respectively. The presence of phytochemical metabolites contributes to both antioxidant activity and anti-diabetic activities. These results indicate that the extract of *T.decurrens* serve as a natural source of antioxidants and anti-diabetics compounds possessing the potential to address a wide array of serious health conditions.

1. INTRODUCTION

Seaweeds have garnered significant attention recently due to their high concentrations of physiologically active compounds. They can be utilised in various applications within the food, cosmetics, pharmaceutical, and environmental industries [1]. Seaweeds hold significant potential for medication research and nutritional supplements due to their abundance of bioactive components, including polysaccharides, polyphenols, pigments, and peptides, which exhibit anti-inflammatory, antiviral, antioxidant, and anticancer activities. Seaweeds are primarily classified into three types based on their pigment composition: Green Algae (Chlorophyta), Red Algae (Rhodophyta), and Brown Algae (Phaeophyceae). In literature, numerous chemicals like Agar, Carrageenan, Fucoidan, Laminaran, and Phlorotannins have been extracted from seaweeds, acknowledged for their distinctive properties and interactions with biological systems that may confer health advantages [4-5].

The species T. decurrens is notable and extensively spread among brown algae. The genus Turbinaria is classified within the family Sargassaceae and the class Phaeophyceae. T. decurrens is a common component of both stony and coral reefs, suggesting its significant role in the ecosystem [7]. Compounds extracted from T. decurrens have been observed to prevent aberrant cell proliferation, have antipyretic activities, reduce inflammation, enhance immunological function, and assist in hyperglycemia management [8]. Hyperglycemia is a defining characteristic of diabetes, a metabolic disorder necessitating ongoing medical management. This can arise from several factors, including tissue resistance to insulin, diminished insulin activity, or complete insulin shortage. Diabetes Mellitus (DM) is classified into Type 1 diabetes mellitus, often referred to as insulin-dependent diabetes mellitus (IDDM), which is characterised by the absence of insulin secretion due to damage to pancreatic β cells. "Furthermore, it accounts for roughly 10% of all diabetes cases. Conversely, Type 2 diabetes mellitus, also known as non-insulin dependent diabetes mellitus (NIDDM), impacts approximately 90% of individuals and is characterised by tissue insensitivity to insulin activity." Hyperglycemia is thought to substantially contribute to oxidative stress. It initiates the auto-oxidation of glucose, leading to the excessive generation of free radicals that induce cellular

damage, perhaps resulting in problems associated with diabetes mellitus [10] Antioxidants have a vital function in neutralising free radicals, hence avoiding oxidative damage induced by hyperglycemia. It mitigates oxidative stress by neutralising reactive oxygen species (ROS) and diminishing inflammation; it furthermore safeguards cellular structures [11]. Numerous synthetic antioxidant compounds exhibit carcinogenic properties, impair endocrine function, induce neurotoxicity, and depress the immune system [12]. Due to growing public concern about synthetic antioxidants, there is an increased interest in natural antioxidants sourced from plants, algae, and whole foods. T. decurrens has been extensively studied primarily for its phenolic and flavonoid components, and secondarily for the role of polysaccharides, both of which demonstrate free radical scavenging capabilities [14].

Seaweeds are acknowledged for their anti-diabetic properties, mainly attributed to their capacity to modulate glucose metabolism and impede starch-hydrolyzing enzymes, particularly α-amylase and α-glucosidase [15]. These two enzymes collaborate in the digestion and absorption of carbohydrates, consequently affecting postprandial glucose levels [16]. α-Amylase inhibitors impede the degradation of starches into simpler sugars, postponing glucose absorption, while α-glucosidase inhibitors obstruct the conversion of oligosaccharides into glucose, hence retarding glucose absorption in the small intestine [17]. Presently, the enzyme inhibitors Acarbose, Miglitol, and Voglibose are employed in the treatment of postprandial hyperglycemia (PPHG). These inhibitors operate by competitively blocking α-glucosidase enzymes in the small intestine, thereby decelerating the degradation of complex carbs into glucose [18]. Although effective in managing PPHG, these inhibitors are not favoured for long-term treatment due to gastrointestinal side effects and high costs [19]. In light of these concerns, seaweed-derived α-amylase and α-glucosidase inhibitors are attracting interest as viable natural, cost-effective, and gut-friendly options. Herbal medications have exhibited less negative effects and have been utilised for millennia to treat diabetes globally. Consequently, much attention has been directed on the screening of medicinal plants for α-amylase and α-glucosidase inhibitors. The current study sought to assess the morphology and genetic identity of T. decurrens, in addition to analysing its antioxidant and anti-diabetic activities.

2. MATERIALS AND METHODS

2.1 Sample location & collection:

T.decurrens sample was collected from Gulf of Mannar coast Rameshwaram (Mandapam), Tamil Nadu, India (DMS Lat: 11° 7' 37.6428" N DMS Long: 78° 39' 24.8076" E) by hand pick method. It was washed several times with distilled water to remove sand debris and other associated organisms such as planktons and microorganisms. Before being sent to the lab, the seaweed sample was shade dried for eight days.

Figure-1 Collection site of seaweed *T.decurrens (Map of Gulf of Mannar)*

2.2 Sample identification

2.2.1 Taxonomical and Morphological identification:

Using botanical methods, physical characteristics of the seaweed sample was initially identified [21]. The specimen was affixed to a herbarium sheet and a detailed label was attached. Live and herbarium sample of seaweed was sent to Herbasia Biotech., Amritsar Punjab, India for identification of morphological and taxonomical features.

2.2.2 Molecular identification (Genomic DNA isolation and PCR analysis)

A QIAGEN DNA isolation kit was used to extract genomic DNA from the seaweed sample. Next, 100µl of elution buffer (10 mM/L Tris-HCl, pH 8.5) was added to the isolated genomic DNA, and the DNA was measured. PCR was carried out with the use of a Taq DNA Polymerase Master Mix reaction mixture. 20 microlitres of distilled water, 1 microlitre each of forward and reverse primers (10 microMolar), and 1 microlitre of DNA template. "The presence of the amplified products

(~757 bps) that were used for DNA sequencing was confirmed by analysing the amplified PCR products of rbcL using 1.5% agarose gel electrophoresis." Using an ABI PRISM Genetic Analyser, the amplified product was sequenced [22-23]. The rbcL primer sequences that were utilised were listed below.

'rbcLa- F: (5'-ATGTCACCACAAACAGAGACTAAAGC-3')

rbcLa-R: (5′-GTAAAATCAAGTCCACCRCG-3')'

2.2.3 Phylogenetic analysis:

The rbcL gene sequences were examined by comparing them with the sequences available in GenBank utilizing the BLASTIN program. Following this, alignment was conducted using CLUSTAL W software. Phylogenetic trees were constructed using the Maximum Parsimony algorithm. All analyses were executed using the MEGA11 package [24-26].

2.3 Seaweed extract preparation:

The powdered sample of the seaweed was subjected to Soxhlet extraction using a Soxhlet apparatus (Borosil, Mumbai, India) with acetone, aqueous and methanol solvents. A total of 25gms of the powdered material were placed into filter paper thimbles, and positioned in the extractor chamber with corresponding solvent. Heating was applied to a round-bottom flask using a heating mantle, with temperatures set at 56°C for acetone, 85°C for the aqueous solvent and 65°C for methanol,. Each sample underwent a minimum of 10 reflux cycles to ensure high-quality seaweed extract. Following extraction, a rotary evaporator was used to condense the resultant extracts under reduced temperature and vacuum conditions.

2.4 Phytochemical qualitative and quantitative analysis:

The crude extract obtained from acetone, aquoeus and methanol underwent qualitative and quantitative phytochemical analysis. The qualitative phyto-components were examined primarily and then quantitatively using standard methods quoted by various researchers.

2.4.1 Qualitative analysis of phyto-components:

The qualitative analysis encompasses Alkaloids (Mayer's test) wherein a 3 ml sample is mixed with 3 ml of 1% HCl on a steam bath, and turbidity is seen following the addition of Mayer and Wagner's reagents, as well as Anthraquinones (Borntrager's test). One gramme of powdered seaweed was introduced into a test tube containing 20 millilitres of chloroform. The mixture was subsequently heated and filtered, followed by the addition of an equivalent volume of 10% ammonia to the filtrate, resulting in the observation of a vibrant pink hue in the reaction mixture. Coumarins (Sodium hydroxide (NaOH) test): To 2 ml of the test solution, sodium hydroxide was added, resulting in a yellow colour indicative of coumarins. Glycosides (Keller-Killiani test): To 2 ml of the sample solution, 2 ml of acetic acid was added, followed by careful addition of sulphuric acid, leading to a colour change from violet to blue-green. Flavonoids (Alkaline Reagent): To 2 ml of filtrate, a few drops of ferric chloride solution were added, resulting in the observation of a blue-green colour. "Phenols and Tannins (Ferric Chloride (FeCl3) test): Mix 5 ml of the tested solution with 1% FeCl3 in a test tube and observe the colour change. Saponins (Foam test): 5 ml of the sample was vigorously shaken with 5 ml of distilled water in a test tube, resulting in the formation of stable foam." Steroids (Chloroform test): 2 ml of chloroform, 2 ml of concentrated sulphuric acid, and 2 ml of the sample were combined in a test tube, resulting in the observation of a red colour in the lower chloroform layer. Terpenoids (Salkowski test): 2 ml of the sample was dissolved in 2 ml of chloroform and subsequently evaporated to dryness. Subsequently, 2 ml of concentrated sulphuric acid was introduced and heated for approximately 2 minutes. The emergence of a greyish hue signifies the presence of terpenoids [27-28].

2.4.2 Quantitative estimation of phyto-components:

The phytochemical components that appeared qualitatively in all three extracts were subjected to quantitative estimation that included total coumarin, total glycosides, total phenol, total tannins and total terpenoid content.

2.4.2.1 Total coumarin content:

10mg of each extract were dissolved in 1ml of methanol: acetone (1:1, v/v) in a micro-centrifuge tube [29]. The sample was subjected to vortexing and allowed to stand for 30minutes. After centrifugation the supernatant was decanted and the volume was adjusted to 2ml of methanol: acetone (1:1, v/v) followed by thorough vortexing. The sample was was read at 327nm against methanol: acetone (1:1, v/v) mixture as a blank. The content was determined using esculin standard.

2.4.2.2 Total glycoside content:

100 µL of extract was combined with 1 mL of freshly produced Baljet's reagent. After one hour, the mixture was diluted with 2 mL of distilled water, and the absorbance was measured at 495 nm using a Shimadzu UV/VIS spectrophotometer model UV 1800 [30]. Total glycosides from triplicate samples were quantified as mg of digoxin per g of dried extracts.

2.4.2.3 Total phenolic content:

Each 200µL extract was placed into screw cap test tubes followed by the addition of 1.0 ml of Folin-Ciocalteau and 1.0ml

of sodium carbonate. The tubes were subjected to vortex mixing and incubated for a duration of 2 hours. The absorbance was measured at 726nm using a spectrophotometer [31]. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/gms dry material.

2.4.2.4 Total tannin content:

500 µl of extracts was placed in a separate test tube and treated with 100 mg of polyvinyl polypyrrolidone, accompanied by 500 µl of distilled water. Following a 4-hour incubation at 4°C, the sample was centrifuged at 5000 rpm for 5 minutes, and 20 µl of the supernatant was extracted. The phenolic content of the supernatant was evaluated at a wavelength of 725 nm, quantified as the concentration of free phenolics on a dry matter basis in units of gallic acid equivalent (GAE) [32]. The tannin content was quantified using the prescribed calculations.

Tannins = Total phenol – Free phenol

2.4.2.5 Total terpenoids:

A known amount of sample (20 mg) and 1.5 ml of chloroform were put into a micro-centrifuge tube. Then 200 l of methanol was added. After the mixture was made, it was stirred and left to sit for three minutes. Each 2ml micro-centrifuge tube was filled with 100µl of pure H2SO4. The tubes were then left at room temperature (about 30°C) for two hours while they were kept dark. Care was taken to carefully pour off the reaction mixture's dregs. After that, 1.5 ml of 95% (v/v) methanol was added, and the mixture was vortexed well until all of the precipitate was gone [33]. At 538nm, the absorbance of the test was recorded compared to a blank. A standard curve was made from the blank-corrected absorption at 538nm of the Linalool standard to figure out the total terpenoids.

2.5 Antioxidant activity

2.5.1 ABTS assay: Free radical scavenging ability by the use of a stable ABTS radical cation 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid):

A 7mM solution of ABTS was created by dissolving it in water. The ABTS radical cation (ABTS*+) was produced by reacting the ABTS stock solution with potassium persulphate at a final concentration of 2.45 mM. The combination was permitted to rest in a dark setting at ambient temperature for 12 to 16 hours before utilisation. For the analysis, the ABTS*+ solution was diluted with 100% ethanol to attain an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30°C. After the introduction of diluted ABTS*+ solution to 50 µL of the test sample, the absorbance was recorded at 30°C precisely 6 minutes post-mixing (At). Each assay contained suitable solvent blanks. The percentage inhibition of absorbance at 734 nm was determined using the designated formula, relying on the difference in absorbance between A0 and At [34].

$PI = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$

2.5.2. DPPH assay: Free radical scavenging ability by the use of a stable DPPH radical (1, 1-diphenyl-2-picrilhydrazyl):

A 2ml aliquot of a 6 × 10^-5 M methanolic solution of DPPH was combined with 50µl of the sample solution at different concentrations (2µl extract + 48µl water, 4µl extract + 46µl water, 6µl extract + 44µl water, 8µl extract + 42µl water, 10µl extract + 40µl water). The absorbance at 515 nm was quantified with a spectrophotometer during a duration of 16 minutes at ambient temperature [35]. The scavenging effect was graphed over time, and the percentage of DPPH radical scavenging capacity of the sample was ascertained from the absorbance value measured at the conclusion of 16-minute intervals. The % inhibition of DPPH radical by the samples was calculated using the following formula.

Percent Inhibition (%) = $[(A_{C(0)} - A_{A(t)} / A_{C(0)})] \times 100$

2.6 Anti-diabetic assay

2.6.1 In vitro α-amylase inhibition:

At the outset Two hundred fifty microlitres of sample were transferred to a tube, followed by the addition of two hundred fifty microlitres of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase at a concentration of 0.5 mg/ml. The mixture was pre-incubated at 25°C for 10 minutes. Thereafter, at designated intervals, 250µl of 1% starch solution in the identical buffer was added, and the incubation persisted at 25°C for an extra 10 minutes. The reaction was concluded by the addition of 500µl of dinitrosalicylic acid (DNSA) reagent. The tubes were subsequently incubated in boiling water for 5 minutes and permitted to cool to room temperature. The reaction mixture was diluted with 5 ml of distilled water, and the absorbance was measured at 540 nm using a spectrophotometer [36]. The inhibitory activity of α-amylase was determined as a percentage inhibition (PI) utilising the subsequent formula:

$PI = [(Abs_{\text{Control}} - Abs_{sample} / \text{Abs}_{\text{Control}})] \times 100$

2.6.2 Yeast α-glucosidase inhibitory activity:

Yeast α-glucosidase was formulated in a 100 mM phosphate buffer at pH 7.0, containing 2 g/l bovine serum albumin and 0.2

g/l sodium azide (NaN3). A substrate solution was produced in the same buffer with 5 mM p-nitrophenyl-α-D-glucopyranoside. One thousand microlitres of the enzyme solution were mixed with one hundred microlitres of the test sample at concentrations varying from 100 to 500 microgrammes per millilitre. The absorbance was measured at 405 nm using a UV-1800 Shimadzu spectrophotometer. After a 5-minute incubation, 50µl of the substrate solution was introduced and incubated for a further 5 minutes. The alteration in absorbance from the initial measurement was documented [37]. The % inhibition was determined using a formula similar to that utilised in the α-amylase assay.

2.7 Statistical analysis

The results were expressed as mean ± SD (Standard deviation). Data analysis was conducted using one way analysis of variance (ANOVA). The level of significance was established at $p<0.05$.

3. RESULTS & DISCUSSION:

3.1 Authentication and Identification of collected seaweeds:

T.decurrens is identified as brown algae belong to the family of Sargassaceae and was characterized by an upright thallus with radially branched axes having blades of tough texture. The blades are of turbinate forms and looks like a pinecone [6]. The macroalgae was identified morphologically by a phycologist, and a certificate with reference number SHB-22/23 -04 was obtained from Amritsar, Punjab India.

Figure-1 Herbarium of *T.decurrens* macroalgae collected from Gulf of Mannar coast Rameshwaram (Mandapam) submitted to Sri Herb Asia Biotech Private Ltd Amritsar Punjab for taxonomical identification.

3.2 Phylogenetic analysis

3.2.1 Isolated genomic DNA: Genomic DNA was isolated from *T.decurrens* seaweed sample and approximately 25kb of genomic DNA was obtained from the seaweed samples. The DNA concentration results indicated that 73.7µg/sample have a purity of 1.87 (Table 1).

Table 1: DNA quantification

Sample code	Concentration (µg/ml)	Purity (A260/280)
sample	73.7	1.87

3.3 Amplification and Electrophoresis

gel image of DNA & its amplification showed the presence of DNA on agarose gel. The obtained genomic DNA was subjected to the amplification of the rbcL gene and it was amplified well and observed in a 1.5% agarose gel electrophoresis (Figure 2). The expected amplicon size was nearly 757 kb and also been recorded the same size in the obtained amplicon. The amplified product was purified using the Exo-sap method and run in an ABI Prism gene sequencer. About 757 base pairs were obtained in the sequence through forward primer.

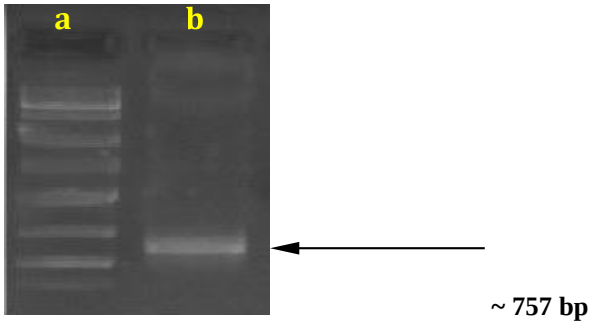


Figure 2: PCR amplification profile of selected seaweed sample (Conditions: 1.5% agarose gel electrophoresis)

(Lane a: 1kb DNA Ladder; b: Sample)

1 KB DNA Ladder (bp):10000, 8000, 6000, 5000, 4000, 3000, 2000, 1000, 500, 250)

3.4 Sequencing results for T.decurrens sample and Clustal W multiple sequence analysis

The sequences were analyzed using BLAST program at the NCBI website and showed sequence similarities with existing seaweed rbcL gene sequences up to 99.21% (Gen Bank Accession Number. DQ448830.1). Closed similar species rbcL gene sequences were obtained and used for CLUSTAL W alignment. The aligned sequences were analysed with Maximum Parsimony method and the resulting phylogenetic tree obtained from the sequences of selected seaweed is illustrated in Figure 3.

>Seaweed Sample TD

TGGCAATTTGGGCTCGAAAAGTGAGATGATTTTACATTTACATCGTGCTGGTAATT
CTACTTATGCACGTCAAAAAAATCATGGAATTAACTTTAGGGTTATTTGTAAATGG
ATGCGTATGTCCGGTGTAGATCATATTCATGCAGGTACAGTTGTGGGGAAACTAG
AAGGAGATCCTTTGATGGTTAGAGGATTTTACAATACGCTATTGTTAACTGAATTA
AAAATTAATCTGGCTGAAGGAATATTCTTCGATATCGATTGGGCATCGCTTAGAA
AATGTGTTCCTGTAGCATCAGGTGGTATTCATTGTGGTCAAATGCATCAACTTCTT
TATTATTTAGGTGATGATGTTGTTCTACAATTTGGAGGTGGTACAATTGGTCACCC
TGATGGTATACAAGCTGGTGCTACAGAAATCGTGTTGCTTTAGAAGCATGGTTCT
AGCTAGAAATGAGGGTCGTGAATATGTTGGTGAAGGGCCAGAAATTTTACGTACT
GCTGCAAGTACTTGTGGACCTTTAAAAGCAGCTTTAGATCTATGGAAAGATATTA
CTTTTGAATATACTTCAACAGACACACCTGATTTTGTTGAAGTAGCAACTGAAAGT
CCATAAAGTTATTCTGTTCTACAGTTTAATTTTTATTATAAAATCTAAAAGAATATA
TTAGTGAGCTTTAATCTTTTTAATACTTTACATTAAAATAAAAAACATAAAAGTTT
GGTAGTTAACTAAAAATAAAACTAAAATATTTA

Figure 3: Sequences of selected seaweed sample

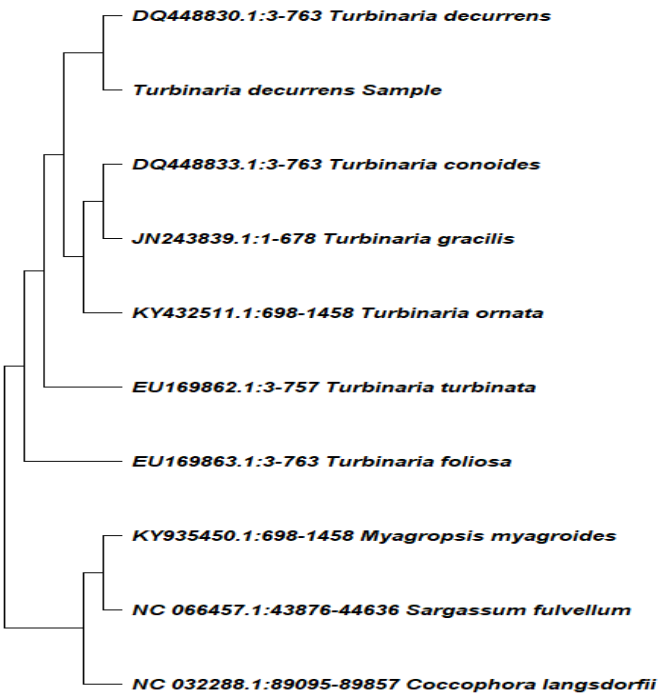


Figure 4: Maximum-Likelihood phylogenetic tree of family Sargassaceae (Seaweed *Turbinaria*)

3.5 Phytochemical qualitative and quantitative analysis:

3.5.1 Qualitative phytochemical analysis: In the phytochemical qualitative analysis of seaweed extracts (Table 3), it was observed that valuable secondary metabolites such as coumarins, glycosides, phenol, tannins and terpenoids were present in the crude extract derived from all the three solvents. The biological activity of the extract may be attributed to the presence of these bioactive substances. However, saponins were positive in the methanol extract only and alkaloids, anthraquinones, flavonoids and steroids were absent in all three extracts

Table 3: Results of phytochemical qualitative analysis

S.No.	Components	Method	Acetone extract	Aqueous extract	Methanol extract
1.	Alkaloids	Mayer's test	-ve	-ve	-ve
2.	Anthraquinones	Borntrager's test	-ve	-ve	-ve
3.	Coumarins	NaOH test	+ve	+ve	+ve
4.	Glycosides	Keller Killiani test	+ve	+ve	+ve
5.	Flavonoids	Alkaline Reagent	-ve	-ve	-ve
6.	Phenol	Ferric Chloride	+ve	+ve	+ve
7.	Saponins	Foam test	-ve	-ve	-ve
8.	Steroids	Chloroform test	-ve	-ve	-ve
9.	Tannins	Ferric Chloride	+ve	+ve	+ve
10.	Terpenoids	Salkowski	+ve	+ve	+ve

***(+ve-** Present) (**-ve-** Absent)*

3.5.2 Quantitative phytochemical analysis: The seaweed derived elements identified via qualitative analysis were subjected to additional quantification to ascertain their concentrations (Table 4). Total coumarin content was expressed as mg Esculin equivalent/EE. The quantity of coumarin content was highest from the *T.decurrens* methanolic extract (161.21±0.36mg esculin equivalent/EE) followed by acetone (124.9±0.95mg/EE) and aqueous extract (101.8±0.41 mg/EE). Total glycoside was expressed in mg digoxin equivalent/DE. The methanolic extract showed an increased amount (366.87±0.06mg/ dry extract) of total glycosides while acetone (66.81±0.18mg digoxin equivalent/ dry extract) and aqueous extract (29.92±0.15mg/ dry extract) showed lower quantities. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of dry extract (mg GAE/g dry extract). The total phenolic compound recovered from the *T.decurrens* methanolic extract were 242.26±0.61mg GAE/g dry extract, whereas the amount retrieved from the aqueous extract and acetone extract was 225.06±0.68 mg GAE/g dry extract and 206.71±0.67 mg GAE/g dry extract respectively. The total tannin content was expressed as gallic acid equivalents (GAE) in milligram per gram of dry extract (mg GAE/g dry extract). The quantity of tannin content in methanolic extract was 204.89±0.54mg GAE/g dry extract, acetone and aqueous extract has 194±0.59mg GAE/g dry extract and 181.54±0.26mg GAE/g dry extract respectively. Total terpenoid content was expressed as mg Linalool equivalent/g dry extract. The total content of terpenoid was higher in methanol extract (1393.78±0.11mg Linalool equivalent/g dry extract) followed by acetone extract (1294.08±0.33mg Linalool equivalent/g dry extract) and aqueous extract has relatively lesser quantities of terpenoid (181.7±0.69mg Linalool equivalent/g dry extract).

Table 4: Results of Quantitative phytochemical analysis

S.NO.	ASSAY	UNIT	RESULTS		
			Acetone Extract	**Aqueous Extract**	**Methanol Extract**
1	Total Coumarin Content	mg Esculin equivalent/EE	124.9±0.95	101.8±0.41	161.21±0.36
2	Total Glycosides Content	mg digoxin equivalent DE	66.81±0.18	29.92±0.15	366.87±0.06

3	Total Phenol Content	mg Gallic acid equivalent/g DE	225.06±0.68	206.71±0.67	242.26±0.61
4	Total Tannin Content	mg Gallic acid equivalent/g DE	194±0.59	181.54±0.26	204.89±0.54
5	Total Terpenoid Content	mg Linalool equivalent/g DE	1294.08±0.33	181.7±0.69	1393.78±0.11

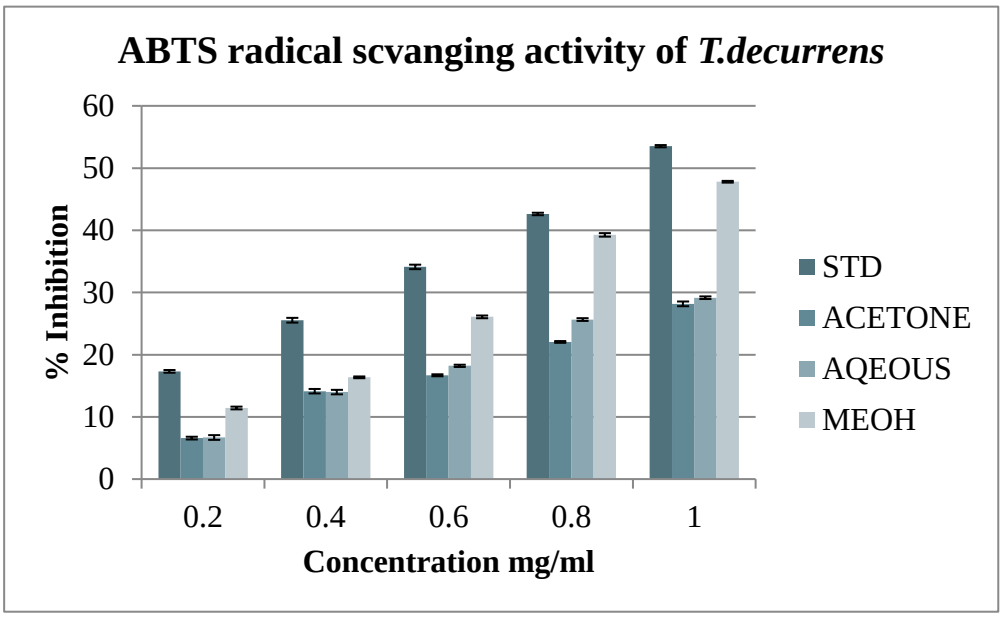
DE: Dry Extract

Results were expressed as Mean± Standard deviation (SD)

3.6 Antioxidant activity

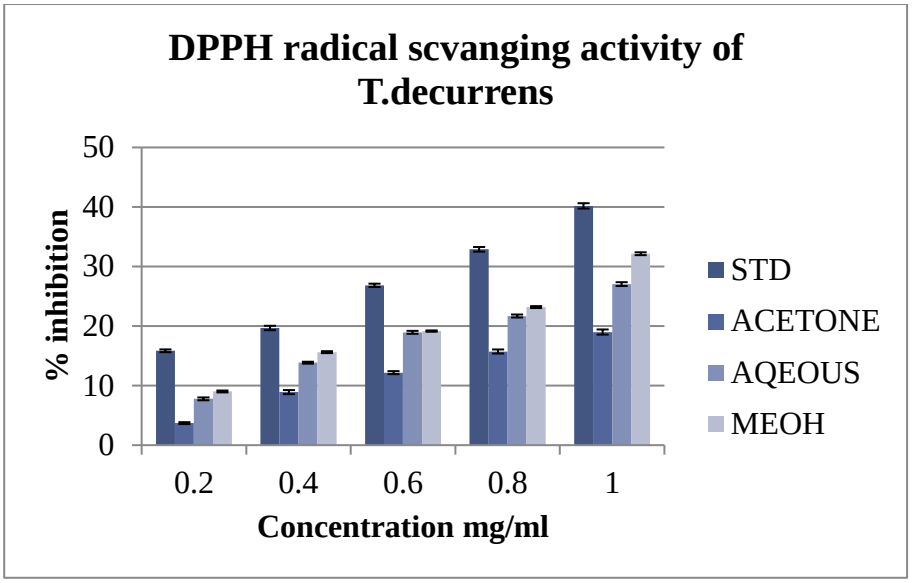
3.6.1 2,2azino bis (3-ethylbenzthiazoline)-6-sulfonic acid assay (ABTS assay)

The results showed (Graph 1) that among the three extract studied, methanolic extract of *T.decurrens* had the highest percentage 28.02±15.2% of scavenging activity with IC_{50} value 1.06mg/ml for all the concentrations (0.2-1.0mg/ml) followed by the aqueous extract (18.75±8.93%) with IC_{50} of 1.62mg/ml and, acetone extract (17.67±8.29%)with IC_{50} of 1.75mg/ml. These findings indicated that the methanolic extract of *T.decurrens* exhibited potent scavenging activity (p value<0.05) that can be compared with ascorbic acid. The different concentrations of ABTS used in the experiment likely influenced the amount of ABTS+ generated.



Graph 1: ABTS radical scavenging assay of standard (STD) ascorbic acid, acetone, aqueous, and methanol extract. Results expressed as Mean±Standard deviation (SD).

3.6.2 1, 1-diphenyl-2-picrilhydrazyl assay (DPPH assay): Antioxidant activity was evaluated using DPPH assay. For this, DPPH concentrations of 0.2, 0.4, 0.6, 0.8, and 1mg/ml were employed (Graph 2). The methanolic extract of *T.decurrens* resulted in an inhibition percentage (%) of 19.81±8.70% and IC_{50} value of 1.56mg/ml for DPPH radical. This was followed by the aqueous extract which exhibited slightly decreased radical scavenging effects 17.84±7.55% IC_{50} 1.75mg/ml while the acetone extract displayed an inhibition rate of 11.88±6.20% IC_{50} 2.54mg/ml .The DPPH assay of seaweeds varied significantly (p-value<0.05) among solvent extracts. Increase in the concentration of *T.decurrens* extract corresponds an increased capacity for DPPH scavenging. Ascorbic acid was utilized as a standard to compare the radical scavenging activity of DPPH assay compared to all three extracts.



Graph 2: DPPH radical scavenging assay of standard (STD) ascorbic acid, acetone, aqueous & methanol extract. Results were expressed as Mean±Standard deviation (SD).

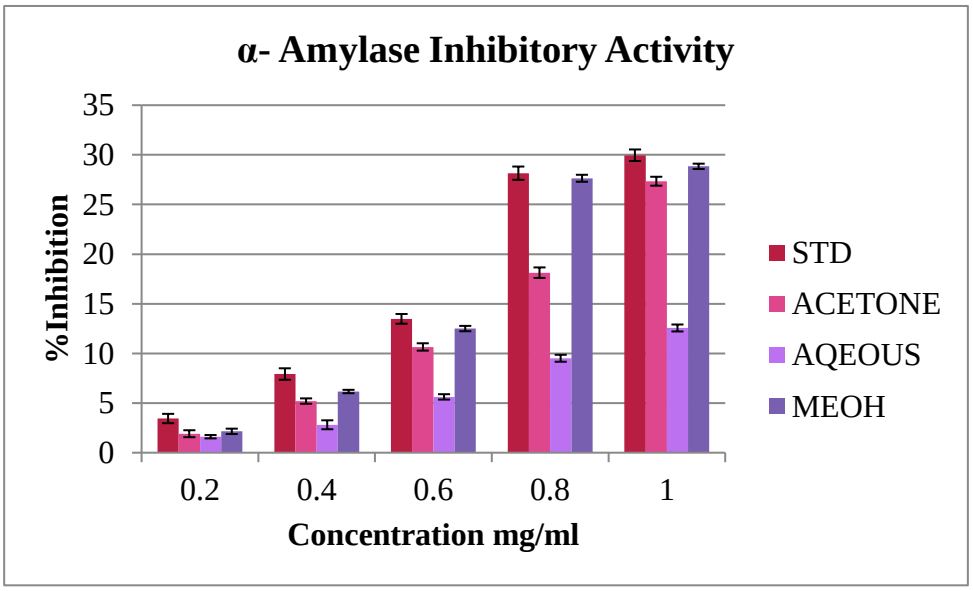
3.7 Inhibitory activity using acetone, aqueous and methanol extract of T.decurrens

3.7.1 α -amylase inhibitory assay:

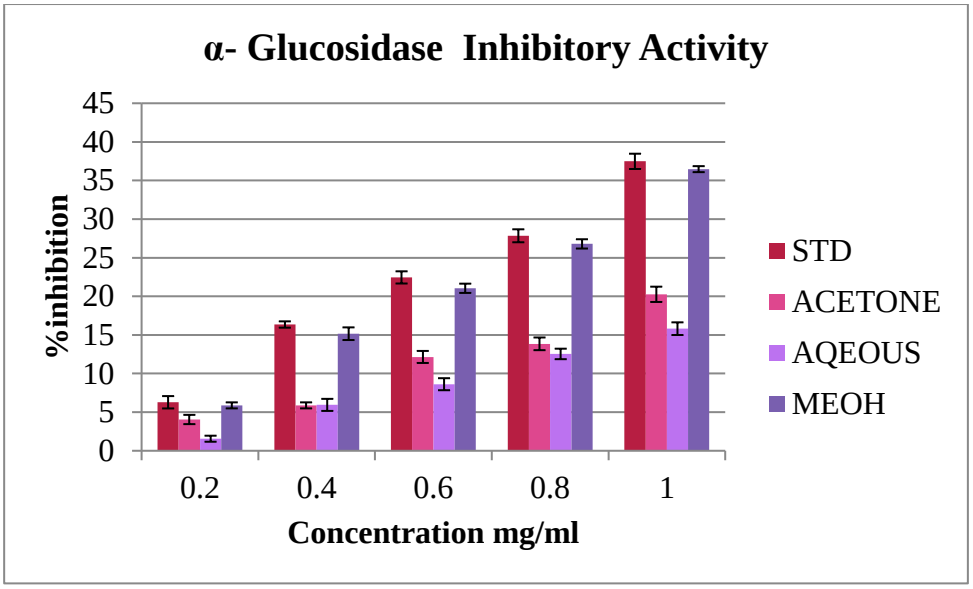
The inhibitory effect of seaweed extracts utilizing various solvents were evaluated for their impact on α-amylase enzyme. All extracts demonstrated significant inhibition of α-amylase activity which varied according to the solvent used. Organic solvents exhibited a more pronounced effect compared to aqueous solvents. Notably, methanol extract of *T.decurrens* displayed the highest inhibition capacity among the three extracts 17.52± 7.45% with an IC_{50} value of 1.8mg/ml followed by the acetone extract and aqueous extract which showed inhibition capacities of 13.43±9.98% & 5.30±4.45% with an IC_{50} value of 2.1 and 4.4 mg/ml respectively. Acarbose run as standard that showed the

3.7.2 α-glucosidase inhibitory activity:

The methanolic extract of *T.decurrens* demonstrated an inhibition rate of 20.94±11.90% and IC_{50} value 1.40mg/ml. in contrast, the acetone extract exhibited 11.61±6.85% inhibition with an IC_{50} 2.63mg/ml while the aqueous extract revealed an inhibitory activity 9.02±5.78% with an IC_{50} 3.26mg/ml.



Graph 3: In vitro α-amylase inhibitory activity of standard (STD) Acarbose, acetone, aqueous and methanol extract. Results expressed as Mean±Standard deviation (SD).



Graph 4: *In Vitro* α-glucosidase inhibitory activity. (STD) Acarbose, acetone, aqueous and methanol extract. Results were expressed as Mean±Standard deviation (SD).

4. DISCUSSION

Marine algae have attracted considerable attention due to their essential roles in survival rather than their contribution to growth, development, or reproduction. These roles encompass offering protection against pathogens and environmental stresses [38] The brown algae *T.decurrens* collected from the Gulf of Mannar bears several bioactive compounds. Coumarin, Glycosides, Phenol, Tannin and Terpenoids were found in various extracts of *T.decurrens* showed that these can act mutually, encouraging their overall effects, especially in the areas of anti-diabetic and antioxidant activity. Similar findings were investigated in *Turbinaria decurrens, Ulva lactuca, Padina pavonica, Pterocladia capillacea, Sargassum muticum, Sargassum acinarium* by Ismail *et al.,* [39]. Quantitative analyzes of the phytochemical ingredients whose presence were detected qualitatively were carried out in parallel. Total coumarin content, total glycoside, Phenolic compounds, total tannin and terpenoids were estimated. Total phenolic content was measured in the methanol, acetone and aqueous extract and the results showed that the methanolic extract had a higher amount of phenols as compared to the acetone and aqueous extract. A research conducted by Ponnan *et al.,*[40] has revealed the same results in which a higher content was found in polar solvent ethyl acetate fraction of *Turbinaria conoides* (EtOAc). A higher phenolic content in brown seaweed *T.decurrens* ethyl acetate extract was also reported by Sami *et al.,* [14]. "The amount of phenolic content is influenced by the solvent's polarity during the extraction process." The higher phenolic content in brown seaweeds exhibits higher antioxidant activity which resulted from their ability to be a reducing agent. In order to determine how much activity a sample would require in order to inhibit DPPH radical stability by donating hydrogen atoms, antioxidant activity by the DPPH method was performed [41]. Antioxidant-active samples reduced DPPH to DPPH-H. The methanolic extract of *T.decurrens* showed higher percent inhibition as compared to the aqueous and acetone extract. Research conducted by Vijayraja and Jeyaprakash 2017, [42] revealed that increase in the concentration of *Turbinaria ornata* methanolic extract (TOME) enhances the scavenging ability. The antioxidant activity of *T.decurrens* was also evaluated by ABTS assay. The evaluation of antioxidant potential of seaweed cannot be adequately determined through the use of a single assay. Therefore, the study employed two different assays. The method evaluated the capability of various compounds to neutralize ABTS radicals, which are highly reactive species characterized by an unpaired electron. In our research, the findings were consistent with those obtained from DPPH assay. The methanolic extract showed a higher percent inhibition of ABTS radical scavenging as compared to aqueous and acetone extract. The study was support4ed by Gupta *et al.,* [43] claiming that presence of antioxidant compounds like fucoxanthin, polysaccharides and polyphenols in brown seaweeds are responsible for antioxidant activity. Another study also examined the antioxidant activity by ABTS assay explaining, the presence of polysaccharides *Turbinaria ornata* may act as antioxidant and potential anti-inflammatory agent [44]. Many plants have been studied and used throughout history for their potential to lower blood sugar levels or help manage diabetes. Alongside conventional medications, herbal and plant-based treatments are often seen as complementary options. In addition to this,many hypoglycemic drugs have been developed from herbal plants and seaweeds [45].

The hydrolytic enzymes involved in carbohydrate digestion, namely α-amylase and α-glucosidase, are crucial in postprandial hyperglycemia. Inhibiting these enzymes is vital for reducing postprandial blood glucose levels, rendering it a critical component of diabetes treatment and regulation [46]. A 2020 study demonstrated that the methanolic extract of T. decurrens displayed significant α-glucosidase inhibitory action, with an IC50 value of 0.011 mg/ml (11 µg/ml) [47]. Moreover, research

by Ismail et al. [39] indicated that the acetone extract of T. decurrens had the most pronounced inhibitory effects on the carbohydrate-hydrolyzing enzymes α-amylase and α-glucosidase, with IC50 values of 4.37 mg/ml and 2.85 mg/ml, respectively. Our investigation corroborates the findings of Ismail et al. [39], wherein all extracts exhibited inhibitory efficacy against both α-amylase and α-glucosidase, with no significant differences noted among the various solvents in comparison to the standard medication acarbose. The anti-hyperglycemic actions of the algal extract can be ascribed to bioactive chemicals that may interact with the active site of α-glucosidase, resulting in conformational alterations and inhibition of the enzyme's catalytic activity [47].

5. CONCLUSION

T.decurrens is characterized by a significant concentration of polyphenols, and demonstrated a wide array of antioxidant activities as well as the ability to inhibit α-amylase and α-glucosidase as reported in the literature . The antioxidant activity of all crude extracts was assessed, revealing that the methanolic extract exhibited the most substantial suppression of reactive oxygen species across all experiments. Furthermore, the abundance of phytoconstituents substantiates its significance in traditional applications for human diseases. The findings indicate that T. decurrens may play a substantial role in the advancement of natural phytochemical therapeutics for diabetes and disorders associated with oxidative stress. Future research should explore the precise mechanisms of action and possible therapeutic applications of antioxidants sourced from T. decurrens.

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