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ANTI DIABETIC AND CYTOTOXIC POTENTIAL USING METHANOLIC CRUDE EXTRACT PREPARATION FROM SARGASSUM WIGHTII SEAWEED

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Abstract:

Introduction:

Our study explores the potential of using *Sargassum wightii* seaweed as a source for alternative fuels due to its rich composition of bioactive compounds. The seaweed, belonging to the brown algae category, is widely distributed in tropical and temperate environments. Extracts from these seaweeds can be processed into a third-generation biofuel, surpassing land biomass techniques in efficiency.

Aim:

The primary objective is to evaluate the anti-diabetic and cytotoxic potential of methanolic crude extract derived from *Sargassum wightii* seaweed. Additionally, the research aims to understand the bioactive composition of the seaweed and its suitability for biofuel production.

Materials and methods:

Sargassum wightii samples were collected and washed thoroughly , the seaweed was shade-dried, powdered, and subjected to methanolic extraction. The crude extract underwent concentration and

was stored for subsequent analysis. Preliminary phytochemical screening tests were conducted to identify bioactive compounds. In vitro tests, including α-amylase and α-glucosidase inhibition assays, assessed the anti-diabetic potential. Cytotoxicity was evaluated using MTT assays on breast cancer cells. A GC-MS analysis was performed to identify the chemical composition of the seaweed extract.

Results:

The study revealed significant anti-diabetic activity, with the extract inhibiting α-amylase and α-glucosidase enzymes. Cytotoxicity assays demonstrated a dose-dependent reduction in breast cancer cell viability. GC-MS analysis identified various bioactive compounds in the seaweed extract, including fucoxanthin.

Conclusion:

Sargassum wightii exhibits promising anti-diabetic and cytotoxic properties, validating its potential as a natural source for pharmaceutical applications. The presence of bioactive compounds underscores its significance in biofuel production. Further investigations are warranted to isolate specific active compounds and advance understanding for potential clinical applications in diabetes management and cancer treatment.

Keywords:*Sargassum wightii,*alternative fuels, anti-diabetic, cytotoxic, bioactive compounds, biofuel, seaweed extract, pharmaceutical applications, diabetes management, cancer treatment.

Introduction:

Algae is one of many resources found in the marine environment that can be used to make alternative fuels. Red, brown, or green algae are the three main categories of algae. The nutritional makeup is shown by the colours of the algae. *Sargassum wightii*, a species of seaweed that is prominent among the algae and is also a member of the Phaeophyceae family, is extensively distributed in tropical and temperate environments.(1)

The seaweeds are commonly found in rocky/coral of shallow sea water or backwaters. The oil derived from algae may also be referred to as third-generation biofuel, or algae, as its main source is seaweed. Using various advanced technologies, the biofuel from these seaweeds is improved to about 30 times than the feedstocks produced from land biomass technique.(1)

A large range of bioactive substances, including proteins, vitamins, soluble dietary fibres, polyunsaturated fatty acids, minerals, and antioxidants, are present in edible seaweeds.(1)

The marine seaweeds contain between 1500 and 2000 classes of marine species of brown algae, the bulk of which are being extensively researched for use as medicines, nutraceuticals, etc. Additionally, the presence of the most important biologically active secondary metabolites, including fucoidans, phycocolloids, phlorotannins, alginic acids, fucosterols, and fucoxanthin, is

a characteristic of brown algae. These compounds are in charge of the notable biological properties, including anti-inflammatory, cytotoxic, antioxidant, and antidiabetic properties. Brown algae have been identified as a potential source of phlorotannins, which may be used to treat a variety of human ailments, such as type 2 diabetes.

(1,2)

The antidiabetic potential of methanolic extracts of *Sargassum wightii* was investigated by Emilin Renitta et al.(3).Based on the findings, they indicated that Sargassum wightii could be used as a potential antidiabetic agent because it showed anti-hyperglycemic activity. (4)

In a previous study from a laboratory, fucoxanthin purified from brown algae, *Sargassum wightii* Greville has found to exhibit antioxidant activity and inhibition of angiotensin-I-converting enzyme (ACE) in vitro.

Fucoxanthin is carotenoid and is considered one of the most abundant pigments present in brown algae(5)

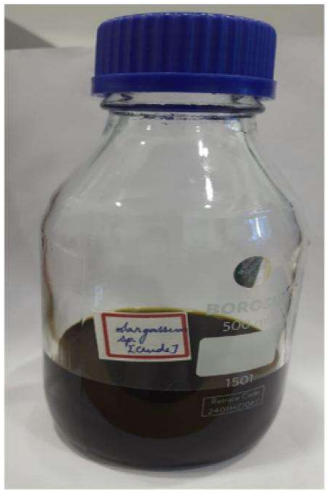
In a study conducted by V Raji et al, Fucoxanthin isolated from *Sargassum wightii* was examined against diabetes with hypertension mice in order to better understand the preventive effect of fucoxanthin against diabetes with hypertension. Fucoxanthin isolated from *Sargassum wightii* , 10 mg/kg body weight (bw), was given orally to diabetic with hypertension rats for 28 days. The blood pressure was checked after the procedure. The animals were killed after having their blood pressure checked, and tissues were taken for biochemical (liver, kidney, and heart biomarkers; activity of carbohydrate metabolic enzymes; glycogen content; monosaccharide components of glycoprotein; and antioxidant) and histological analysis. (6)

A metabolic illness with numerous etiologies known as diabetes mellitus is defined by persistent hyperglycemia and changes in the metabolism of carbohydrates, fats, and proteins as a result of a problem with insulin secretion, action, or both.(7) For the expanding population of the world, diabetes is one of the main causes of disease and mortality. By 2030, the International Diabetes Federation forecasts a global increase from 8.3% to 9.9%. Major abnormalities in fatty acid metabolism are linked to diabetes. Hypertriglyceridemia, high-density lipoprotein cholesterol (HDL-c), and normal plasma levels of low-density lipoprotein cholesterol (LDL-c) make up the most typical lipid pattern in type 2 diabetes. Due to its rising incidence and the accompanying incapacitating effects, it is one of the main risks to human health. (8)

Materials and methods:

Rhizhophora apiculata

Powdered sample



Crude extract of mangrove

Extract preparation

Filtration of crude extract

Figure 1:Preparation of the seaweed extract

Collection and identification of the seaweed:

The collected seaweed was washed with fresh water to remove epiphytes. Then the sample was brought to the laboratory in polythene bags and washed thoroughly with fresh water to remove salt and other extraneous material. The washed sample was shade dried to prevent degradation of bioactive components and powdered using an electric mixer.(9)

Methanolic crude extract preparation:

For extraction, weigh a precise volume of the seaweed powder.For several days, macerate the powdered seaweed material in methanol at room temperature. Stir the mixture occasionally.To get a crude extract, filter the methanolic extract with filter paper to eliminate solid particles.Vacuum concentrate or use a rotary evaporator to concentrate the crude extract.To create the final methanolic crude extract, re-dissolve the concentrated extract in methanol. To keep it safe from light and heat, store it at -20°C in amber-coloured vials.(10)

Results and discussion:

α-Amylase Inhibition activity					**α-glucosidase Inhibition activity**				
α-A	Sample	St.Er	Control	St.Er	α-G	Sample	St.Er	Control	St.Er
25	10	2.8	28	1.1	25	5	2.5	35	1.1
50	16	2.2	38	0.8	50	13	2.1	41	0.8
75	26	2.5	54	1.2	75	24	2.8	58	1.2
100	42	2.2	63	0.9	100	40	2.2	68	0.9

Table 1: Inhibitory activity

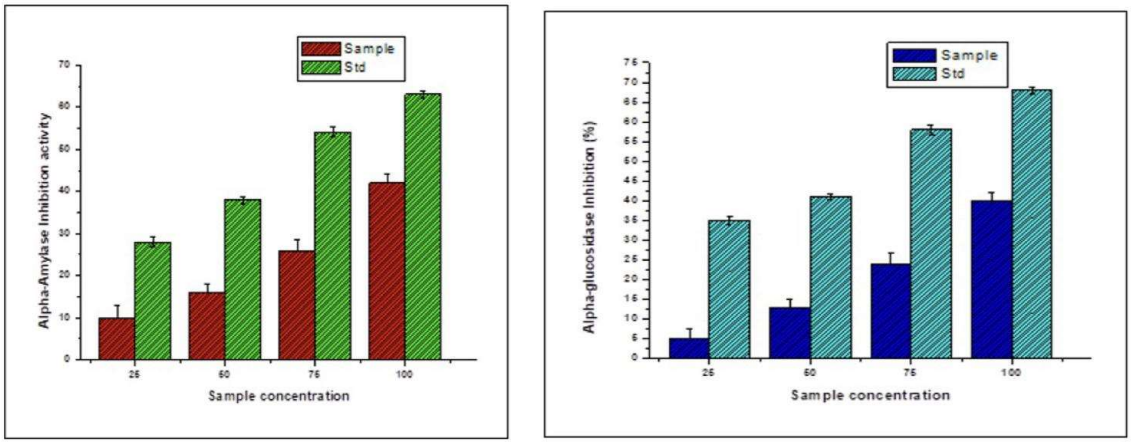


Figure 2 : Inhibitory assay - graph

Phytochemical Analysis:

Perform preliminary phytochemical screening tests to identify the presence of bioactive compounds, including alkaloids, flavonoids, phenolics, and saponins, in the extract(11)

Anti diabetic potential evaluation:

In vitro tests:

α-amylase inhibition assay:

To assess the ability of the extract to inhibit starch digestion.Algae samples were taken in test tubes at varying concentrations of 20, 40, 60, 80, and 100 µl. 250 µl of -amylase are added to each test tube, then they are combined. At room temperature, each test tube was incubated. Following this, 500 µl of starch was added and incubated at room temperature for 3 minutes. After being added, 500 µl of DNS was maintained in a water bath at 50°C for 5 minutes. (12)

α-glucosidase inhibition assay:

To evaluate its ability to inhibit glucose absorption in the intestine.A lower IC50 value suggests a stronger inhibitory effect and potential anti-diabetic activity.take various concentrations of the algal extract 5,13,24,40 µl and set up a microplate well.Incubate the microplate at 37°C for 10-15 minutes to pre-warm the solutions.Add the alpha-glucosidase enzyme to the control and test wells, and start a timer.The microplate must be incubated at 37 °C for a predetermined amount of time, often 30 to 2 hours. The enzyme will hydrolyze p-NPG during this incubation, resulting in the production of p-nitrophenol.Measure the absorbance of each well at 405 nm using a microplate reader.(13)

Sample concentration		
µg/ml	24Hrs	SE
25	74.5	2.4
50	62.5	1.8
75	53.4	2.6
100	32.5	2.8

Table 2 :Cytotoxicity activity- MTT assay against MCF cell line (breast cancer)

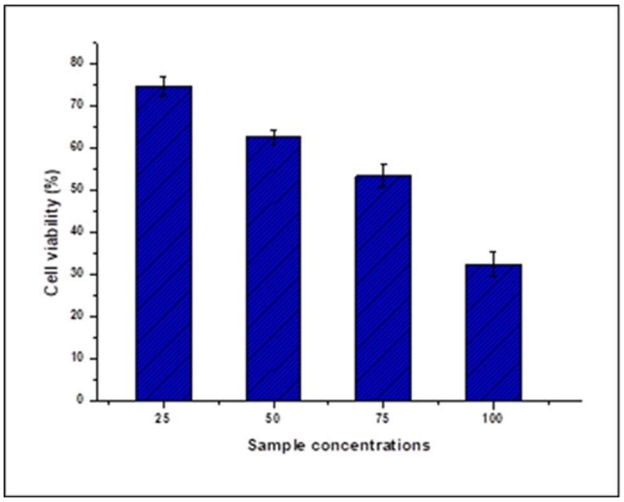


Figure 3: cell viability-graph

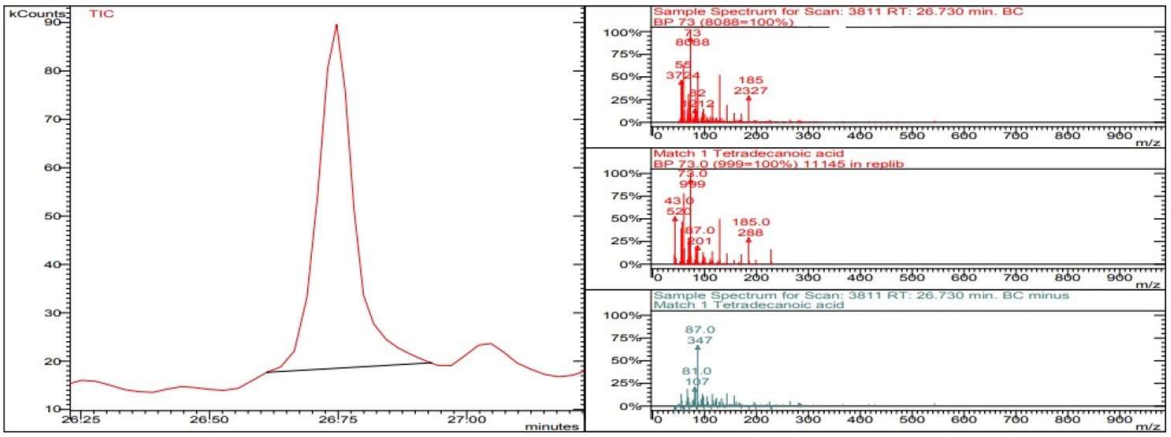


Figure 4 : GC-MS analysis

Cytotoxic Potential Evaluation:

Cytotoxicity assays using cell lines (e.g MTT assay)were performed to determine the effects of the extract on cell viability.Breast cancer cells are cultured in DMEM or medium supplemented with 10% FBS and 1% penicillin-streptomycin solution in a humidified incubator at 37°C with 5% CO2. Serial dilutions of *Sargassum wightii* seaweed methanolic crude extract are prepared in DMSO, with a final concentration of < 0.1%. Plates are placed in 96-well tissue culture plates, and cells are incubated for 24 hours. Different concentrations of seaweed extract are added in triplicate, and cells are incubated for a predetermined period. After incubation, prepare the MTT reagent and dissolve formazan crystals. Measure absorbance using a microplate reader at 570 nm.The cytotoxicity assessment revealed a dose-dependent reduction in cell viability of breast cancer cells treated with the extract. The half-maximal inhibitory concentration (IC50) was determined, indicating the concentration required to inhibit 50% of cell growth.(14)

In vivo tests (if applicable):

i. Administer the extract to diabetic animal models and monitor blood glucose levels.

ii. Assess insulin sensitivity and other relevant parameters.

Conclusion:

The methanolic crude extract derived from this seaweed exhibited significant anti-diabetic activity through the inhibition of alpha-glucosidase and alpha-amylase enzymes, as well as notable antioxidant properties. Additionally, it displayed cytotoxic potential against breast cancer cells. These findings underscore the promise of *Sargassum wightii* as a valuable natural resource for the development of novel anti-diabetic and anti-cancer agents. Further investigations, including the isolation and identification of specific active compounds, are warranted to advance our understanding of its mechanisms of action and potential clinical applications. *Sargassum wightii* holds great promise in the field of pharmaceutical research and may contribute to the development of innovative therapies for diabetes management and cancer treatment.(6)

Author contribution:

Mounithaa N - Contributed to data analysis,drafted manuscript,collected data and assisted in experimental analysis

Dr.Sivaperumal - Provided expertise in [specific area, e.g., molecular biology, statistics], contributed to study design and data analysis, and critically reviewed and revised the manuscript

Dr.Abirami Arthanari - Provided guidance on the research project, reviewed and approved study design and methodology, and participated in manuscript review and editing.

Ethical clearance number:

No ethical clearance is required because it is an in vitro study.

Conflict of interest:

Nil

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4.Sri sri dental specialities

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