RESEARCH PAPER

Screening of Antidiabetic Properties of Polysaccharides Extracted from *Sargassum* Species

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ABSTRACT

Diabetes is a metabolic disorder characterised by excessive glucose levels in plasma. Type I diabetes is characterised by autoimmune disintegration of pancreatic β-cells, while Type II diabetes is caused by abnormally elevated hepatic glucose production due to hyperglycemia (excess glucose in the blood). *Sargassum* is a marine brown algae with numerous species. The immense resources of marine algae can supply bioactive compounds that are abundant and useful in the health sector for their bioactivity. Sulfated polysaccharide is a water-soluble, negatively charged polymers found in *Sargassum species.* Polysaccharides extracted from *Sargassum species* were more potent inhibitors of α-glucosidase, with 83.47±0.3% and 89.70 ± 0.3% inhibition of α-amylase at a 100 µg/mL concentration. The ability of polysaccharides to inhibit α-amylase and α-glucosidase varies according to *Sargassum species.* Thus, *Sargassum species* is more suitable as a source of polysaccharides to reduce glucose by inhibiting α-amylase and α-glucosidase activity.

Keywords: Polysaccharide, α-amylase, α-glucosidase, Diabetes, *Sargassum* spp. FTIR
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INTRODUCTION

In carbohydrate metabolism, starch (a polysaccharide) is the primary source of blood glucose in the human system compared to other macromolecules (lipids and proteins). Glucose is the principal molecule involved in energy production in the human body; hence, it is linked to all types of diabetes mellitus. The amount of glucose in the bloodstream is influenced by processes: digestion (e.g., starch to glucose), hormonal regulation (secretion of insulin, glucagon), and glucose transport mechanisms via glucose transporter molecules. Diabetes mellitus (DM) is a long-term metabolic disorder characterised by high blood sugar levels (hyperglycemia) caused by insufficient insulin production and defective lipid, protein, and carbohydrate metabolism. In most developed countries, it is one of the leading causes of death, and there is strong evidence that it is epidemic in many newly industrialised and economically developing countries. Over the last two decades, the number of people diagnosed with diabetes has increased. Type2 diabetes is characterised by hyperglycemia, which causes extensive damage to organs and the neurological and cardiovascular systems. Diabetes is reported to be the seventh leading cause of mortality globally by 2030 (Moini, 2019), due to higher blood glucose levels. The fundamental source of blood glucose levels has been confirmed to be carbohydrate (starch) breakdown by catabolic enzymes in the alimentary canal, such as amylases, maltases, and glucosidases. As a result, slowing carbohydrate digestion or blocking glucose absorption is regarded as a promising technique for treating diabetes and its complications.

MATERIALS AND METHOD

COLLECTION OF *Sargassum Species*

Fresh *Sargassum species* were collected at low tide along the coast of Raigad district, Maharashtra, and then manually cleaned to remove sand, epiphytes, and debris. The samples were then rinsed in seawater to eliminate any debris, plankton, or germs. Algae with diverse morphologies were packed in fresh polythene bags, stored in an icebox, and transported to the laboratory. Furthermore, the material was thoroughly rinsed with tap water to remove the salt from the sample surfaces, and the water was drained from the *Sargassum species* and spread on blotting paper to remove any excess water. The dried samples were washed again with sterile distilled water to remove any leftover salt from the surface. The samples were shade-dried for 5 days, then oven-dried at 50°C for 1 hour, and finally crushed in an electric mixer for 2 hours. Finally, the powdered seaweed sample was collected and stored in the refrigerator at 4°C for future use.

Extraction of Polysaccharide

The method described by Wassie *et al*. (2021) with slight modifications 15 g of extracted seaweed powder was dissolved in 450 ml of 0.5% EDTA-Na (w/v) and heated at 70°C with agitation for 3 hours. The mixture was centrifuged at 5000 g for 20 minutes at 4°C. The supernatant was neutralised with 0.1 M NaOH and adjusted to pH 7. The extracts were centrifuged and treated with $CaCl_2$. Sulphated polysaccharides were precipitated by adding 80%

(v/v) absolute ethanol, then incubated at 4 °C overnight.

Figure 1. Dried *Sargassum*

Figure 2. *Sargassum* species extract

Characterisation of biochemical composition

Fourier transform infrared spectroscopy (FTIR)

FT-IR spectra (spectral range 4000–600 cm-1, resolution 2 cm-1), Procedure described by Sanjeewa *et al*.(2017). One milligram of dried sulphated polysaccharides was combined with KBr salts. The KBr pellets containing sulphated polysaccharides were prepared using the pressed disc method and measured with a SHIMADZU IR Affinity-1 spectrophotometer

Alpha-amylase inhibition activity

The α-amylase activity assay was used to determine the inhibitory potential of the extracted sulphated polysaccharides of *Sargassum Species* extracts and the control, acarbose, against α-amylase using the procedure described by Apostolidis *et al*.(2010). In brief, 2% (w/v) potato starch dissolved in 0.05 M sodium phosphate buffer (pH 7.0) was mixed with varying concentrations of extract ranging from 20 to 100µg/mL. To start the reaction, 0.1 mg/ml of α-amylase was added to a 400 µl final volume. After 20 minutes of incubation at 37 °C, the DNSA reagent was added to stop the enzymatic reaction, and then incubated at 100 °C for 8 minutes before chilling in a cold water bath. The absorbance was measured at 540 nm. Mean values were obtained from triplicate experiments. Using the following formula, the percentage inhibition of amylase activity was calculated.

$$Inhibition\ (\%) = \left(\frac{Abs\ Control - Abs\ Sample}{Abs\ Control}\right) \times 100$$

Alpha Glucosidase Inhibition Activity

Sargassum extracts were tested for the ability to inhibit α-glucosidase activity using a modified procedure from Devid et al.(2017). The substrate, 10 mM pNPG, was dissolved in 0.05 M sodium phosphate buffer (pH 7.0), and *Sargassum species* extract at different concentrations (20-100µg/mL) were added to the reaction. To activate the reaction, 0.1 U/ml α-glucosidase was added to a total volume of 400 µl. After 20 minutes of incubation at 37°C, the reaction was terminated by adding 400 µl of 2 M Na_2CO_3. The α-glucosidase activity was determined by measuring the absorbance at 405nm. The mean values were obtained from triplicate experiments. α-glucosidase inhibition activity was calculated by using the following equation

$$Inhibition\ (\%) = \left(\frac{Abs\ Control - Abs\ Sample}{Abs\ Control}\right) \times 100$$

RESULTS AND DISCUSSION:

Polysaccharide extracted from *Sargassum species* exhibited absorption bands at approximately 900 and 1740 cm-1, indicating the presence of polysaccharides (Sanjeewa *et al*., 2017).

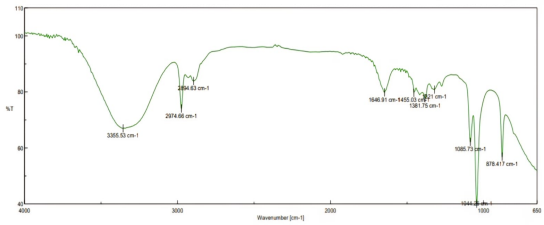

Figure 3. *Sargassum species* extract FTIR

Table 1. FT-IR absorption frequencies (cm-1) and functional groups of *Sargassum species*

Composition	Absorption frequencies	Functional group
Polysaccharides	3423.87-1633	OH, C=O, and COOH; hydroxyl, carbonyl, carboxyl (alginic acid)
	1730	C=O; carboxylic acid ester
	1675 and 1600	COO- stretch; carboxylate anion
	1320	C=C stretch

	1200-970	C-C, C-O pyranoid ring and C-O-C stretching of glycosidic
	1139.21-1036.60	Acidic polysaccharides
	1100	Mannuronic units
	1027 (1030-1025)	Guluronic units
	817 (825-815)	Mannuronic units
	1705-1595	C-O stretch
	1609 and 1420	COO- asymmetric and symmetric stretch of uronic acid
Sulphate groups	1280-1210	(S=O) Stretch of sulphate groups
	1250-1040	S=O stretching
	1030	S=O stretching
Uronic acid	670-600	C-S and C=S stretching
	1415-1395	C-OH stretch
	1090-1035	C-O vibration
	940-930	C-O-C stretch
Proteins	3600-3450	N-H stretch
	2347	N-H bond
	1660-1440	C=O, N-H, δCH2-CH3
Polyphenol	939	nitrogen grouping
	3300-3200	O-H and C-H stretch; phenol
	1384	OH phenolic groups
	1086	C-H stretch; aromatic nucleus

Alpha-amylase inhibition activity

The study observed that *Sargassum* species-extracted polysaccharide effectively inhibited α-amylase at a 100µg/mL concentration, showing 89.70 ± 0.3% inhibition, whereas standard polysaccharide exhibited 95.13± 0.3% inhibition.

Table 2. Alpha-amylase inhibition

Sr.No.	Concentration (µg)	% inhibition	
		Acarbose	*Sargassum* Spp.
1	20	23.51± 0.7	21.3±0.3
2	40	37.42± 0.6	32.57±0.5
3	60	63.94± 0.4	50.89±0.3
4	80	77.71± 0.5	72.82±0.4
5	100	95.13± 0.3	89.70±0.3

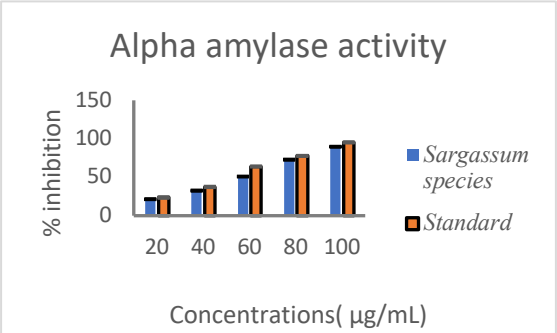

Figure 4. Alpha-amylase activity of *Sargassum species*

Alpha Glucosidase Inhibition Activity:

Table 3. Alpha-glucosidase inhibition

Sr.No.	Concentration (µg/mL)	% inhibition	
		Acarbose	*Sargassum Spp.*
1	20	31.22± 0.3	25.20 ±0.4
2	40	47.12± 0.3	35.84 ±0.5
3	60	64.26± 0.5	52.24 ±0.3
4	80	75.5± 0.3	74.32 ±0.3
5	100	89.39± 0.3	83.47 ±0.3

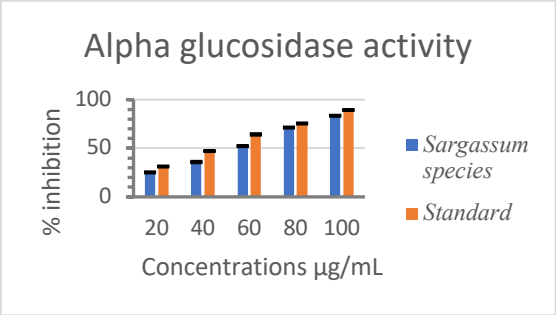

Figure 5. Alpha-glucosidase inhibition

Sargassum species extracts were studied for their ability to inhibit alpha-glucosidase enzyme at 100 µg/ml; standard polysaccharide exhibited 89.39 ± 0.3 % stronger alpha-glucosidase inhibitory activity, and *Sargassum species* extract exhibited 83.47± 0.3% inhibition.

CONCLUSION:

FT-IR analysis helps identify active functional groups in *Sargassum species.* This approach provides a speedy but precise analysis. Our data suggest that polysaccharides extracted from *Sargassum* species possess potential antidiabetic activity as they significantly lower blood glucose levels. The results suggest that marine algae possess significant antidiabetic activity and may be a good therapeutic agent for managing and treating diabetes mellitus. This opens up new opportunities for the seaweed industry globally. The bioactive compounds can be added as a food ingredient in food or beverage products to produce functional food that provides health benefits to humans.

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