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ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF POLYPHENOL EXTRACTED FROM THE BROWN SEAWEED *SARGASSUM VULGARE*

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ABSTRACT
Marine algae are an important source of bioactive metabolites in drug development and nutraceuticals. Due to changes in lifestyle brought on by increasing urbanization, diabetes mellitus, a metabolic illness, is currently the third largest cause of mortality globally. Seeking an efficient natural-based antidiabetic medication is crucial to battling diabetes and associated consequences because the existing antidiabetic medicines have unfavorable side effects. Brown algae are among the marine seaweeds that have a large number of naturally occurring bioactive chemicals that may be used as active ingredients in pharmaceuticals and nutraceuticals. Brown algae-derived polyphenols also have the potential to lessen the problems associated with diabetes. The antidiabetic activity will be investigated using the alpha-amylase enzyme inhibition test. The antioxidant properties were determined by total antioxidant assays, Hydrogen peroxide assay and DPPH scavenging. Hence, the present study focuses on the antidiabetic and antioxidant activity of secondary bioactive compounds present in marine brown algae.

Keywords: Antioxidant, antidiabetic, Polyphenol, DPPH.

INTRODUCTION
The marine environment is rich in bioactive secondary metabolites, many of which have structural characteristics that are not seen in terrestrial natural products (Cantillo-Ciau et al., 2010). Moreover, seaweeds are in charge of preserving marine biodiversity because they are necessary for the survival of a number of other organisms. For the last few decades, coastal residents have had job opportunities since the harvesting of marine algae is dependent on the use of industrial needs (Dere et al., 2003). Seaweeds are rich in bioactive compounds such polyphenols, carotenoids, and polysaccharides. These bioactive substances provide consumers health benefits, making them suitable for usage in functional foods, pharmaceuticals, and cosmetics (Shibata et al., 2002). in vitro research has demonstrated the antibacterial, antidiabetic, and antioxidant qualities of the polyphenol chemicals found in catechins and glycosides in methanol extracts of seaweeds.
One important indicator of a food's potential as an antioxidant is its phenolic content (Parr Adrian and Bolwell, 2000). Phenolics are one of the biggest and most prevalent groups of photochemical, have attracted a lot of attention because of their pharmacological action and several health-promoting advantages.
A few studies have looked at the phenolic potentials of sea algae, namely brown algae (Pheophyceae),

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which are known to contain phlorotannins. Recently, researchers have focused their attention, particularly on brown algae that have high polyphenol content. Products of algae's secondary metabolism, polyphenolic compounds are categorized into a number of classes based on the kind of bond that connects the hydroxyl group to the aromatic hydrocarbon group.
The current treatments for diabetes mellitus, such as the use of insulin and oral antidiabetic medications, are either ineffective or have unfavorable side effects. (Marín-Peñalver et al., 2016). Consequently, it's critical to never stop looking for a medication that works for people with diabetes mellitus in order to help them get better and eventually become well. Neither the scientific research nor any of the medications now utilized to treat diabetes mellitus have shown any promise as effective treatments. Consequently, there is a growing need to find novel plant-based chemicals that have little to no negative effects on patients.

Plant-based resources have been demonstrated to be an effective application and to have less chemical dangers. Plant extracts are known to include phytochemical substances that have a secondary metabolite that may be utilized to treat a variety of illnesses, including diabetes mellitus. (Wang, 2013). Together with therapeutic plants, marine algae are a rich source of naturally occurring bioactive chemicals that may be used as active components in the treatment of diabetes mellitus. (Unnikrishnan and Jayasri, 2018). Therefore, it is crucial to identify the chemical components and isolate the active chemicals from underutilized marine algae in order to manufacture herbal drugs with the fewest possible adverse effects and a large financial return.

Phlorotannins, an essential secondary metabolite that has been shown to have antidiabetic properties, are found in abundance in the majority of brown algae. (Gupta and Abu-Ghannam, 2011). Thus, the antidiabetic properties of brown algal polyphenols and their potential applications in the pharmaceutical sector are the main topics of this work.

Materials and Methods

Collection of brown algae

The seaweed, Sargassum vulgare (Phaeophyceae) was collected from the rocks in coastal areas at Colachel beach in kanyakumari. First, morphological traits were used to separate specimens. The moment a sample was collected, it was washed in seawater several times to remove impurities, sand particles, and epiphytes, and brought to the research laboratory in taped up polythene bags. The seaweed was washed thoroughly in tap water several times, wearied and spread on blotting paper to remove excess water, and shade dried for 4 to 8 hours.

Extraction of crude polyphenol from Sargassum vulgare

Polyphenol were extracted from the brown seaweeds Sargassum vulgare following the method of Subash et al. (2010). Three liters of water were used to extract 100 g of dried seaweed powder over the course of 16 hours at 90–95°C.After being filtered using Whatman No. 3 filter paper and concentrated to one-fourth of its initial volume, the brown-colored thalli was allowed to cool and precipitate using three volumes of ethanol. Centrifugation was used to collect the precipitate, which was then dried using diethyl ether. For additional analysis, the crude polyphenol extract (10% yields) was utilized.

ANTIOXIDANT ACTIVITY

(i) Determination of total antioxidant capacity (TAC)

Total antioxidant activity of polyphenol from Sargassum vulgare. I was determined according to the method of Prieto et al. (1999). In short, 3.0 ml of the reagent solution (0.6 M sulfuric acid, 28 mM

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sodium phosphate, and 4 mM ammonium molybdate) was combined with 0.3 ml of the sample. The reaction mixture was submerged in a water bath and incubated for 90 minutes at 95ºC. After 15 minutes, the absorbance of each sample combination was measured at 695 nm. The standard was ascorbic acid.

(ii) Hydrogen peroxide scavenging assay

The free radical scavenging activity of the polyphenol from Sargassum vulgare was determined by hydrogen peroxide assay (Gulcin et al., 2004). A phosphate buffered saline (0.1M, pH 7.4) solution containing 10 mM hydrogen peroxide was made. A fast mix of 1 milliliter of the extract including samples ranging in concentration from 100 to 1000 micrograms was conducted with 2 milliliters of hydrogen peroxide solution. After 10 minutes of incubation at 37°C, the absorbance was measured at 230 nm in the UV spectrophotometer against a blank (without hydrogen peroxide).

The formula was used to determine the proportion of hydrogen peroxide scavenging.

Percentage scavenging (H_2O_2) = ((A_o-A_1) / A_o) x 100

A_o - Absorbance of control; A_1 - Absorbance of sample

(iii) DPPH radical scavenging activity

By using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), the free radical scavenging capacity of Sargassum extracts was determined. The scavenging activity for DPPH free radicals was measured according to the procedure described by (Braca et al., 2001). An ascorbic acid/plant extract aliquot ranging from 0.5 to 2.5 µl at different doses was combined with 3 ml of a 0.004% DPPH solution in methanol. After giving the combination a good shake, it was left to stabilize at room temperature for half an hour. The absorbance at 517 nm was used to measure the decolorization of DPPH. Instead of utilizing plant extract or ascorbic acid, 0.1 ml of the appropriate vehicle was used to provide a control. The extract/compound's % suppression of DPPH radicals was calculated by comparing the absorbance values of the experimental and control tubes

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)}}{\text{A518 (control}} \times 100$$

ANTIDIABETIC

Alpha-amylase enzyme inhibition assay

390 ml of 0.02M Phosphate buffer pH 7 was added to Positive control and different concentration of test samples with 10 µ L of Alfa Amylase Pre-incubated at 37^0 C for 10 mins. 10 ml of Starch Re-Incubated at 37^0 C was added for 1h. 0.1 ml 1% Iodine solution+ 5ml of distilled water was added to all the tubes containing test samples and OD was measured at 565 nm.

RESULTS

Free radical scavenging activity of polyphenol

(i) Total antioxidant capacity (TAC)

The total antioxidant activity of polyphenol from Sargassum vulgare along with the standard ascorbic acid is shown in Fig. 1. The activities of polyphenol were calculated based on inhibition percentage and was recorded as 76.2±0.14%.

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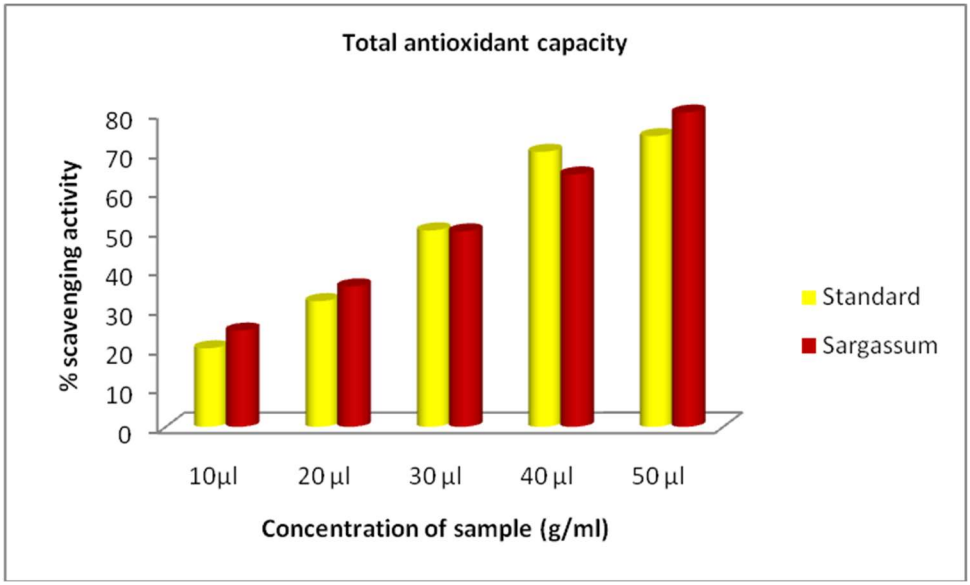

Fig. 1. Total Antioxidant Activity of polyphenol from Sargassum vulgare compared with standard ascorbic acid.

(ii) Hydrogen peroxide scavenging assay

The activity observed in hydrogen peroxide scavenging assay was directly proportional to the concentration of sample. The hydrogen peroxide inhibition activity for the polyphenol from Sargassum vulgare was 71.05±0.16% and it was compared with the standard gallic acid (Fig. 2.)

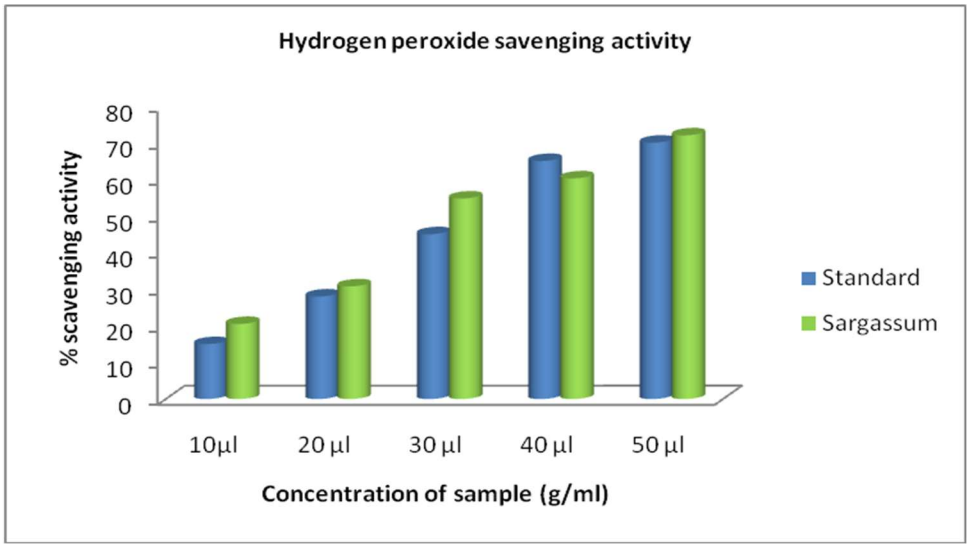

Fig. 2 .Hydrogen peroxide scavenging assay of polyphenol from Sargassum vulgare compared with standard gallic acid.

(iii) DPPH radical scavenging assay

DPPH radical scavenging assay of Sargassum vulgare extracts was evaluated by employing extracts at different concentrations of 10 µl to 50 µl to react with DPPH reagent. Sargassum vulgare extract at a concentration of 50 µl showed highest antioxidant percentage of 88.11±0.24 %. The percentage of antioxidant capacity of Sargassum vulgare extract was found to be higher than standard ascorbic acid

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which was depicted in fig.3.

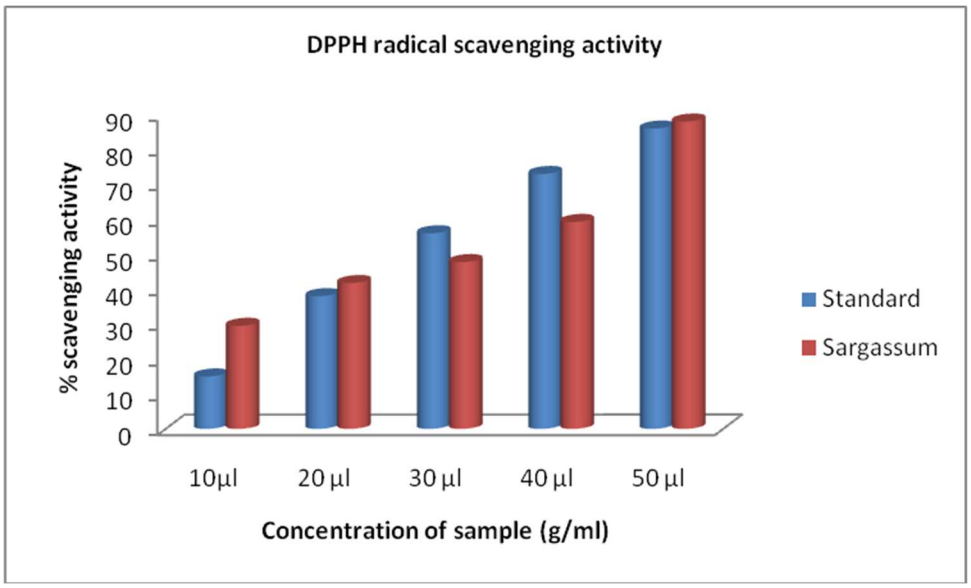

Fig .3. DPPH radical scavenging activity of Sargassum vulgare

ANTIDIABETIC ACTIVITY

Antidiabetic activity of Sargassum vulgare extracts was evaluated by employing extracts at different concentrations of 10 µl to 50 µl to react with DPPH reagent. Sargassum vulgare extract at a concentration of 50 µl showed highest antioxidant percentage of 72.20%. The percentage of antidiabetic capacity of Sargassum vulgare extract was found to be lower than standard acarbose which was depicted in Table 1 and fig.4.

Table 1. Antidiabetic activity of Sargassum vulgare

Concentration	**Standard (Acarbose)**	***Sargassum vulgare***
10 µl	32 %	29.00 %
20 µl	48 %	42.50 %
30 µl	55 %	50.30 %
40 µl	72 %	65.00 %
50 µl	87 %	72.20 %

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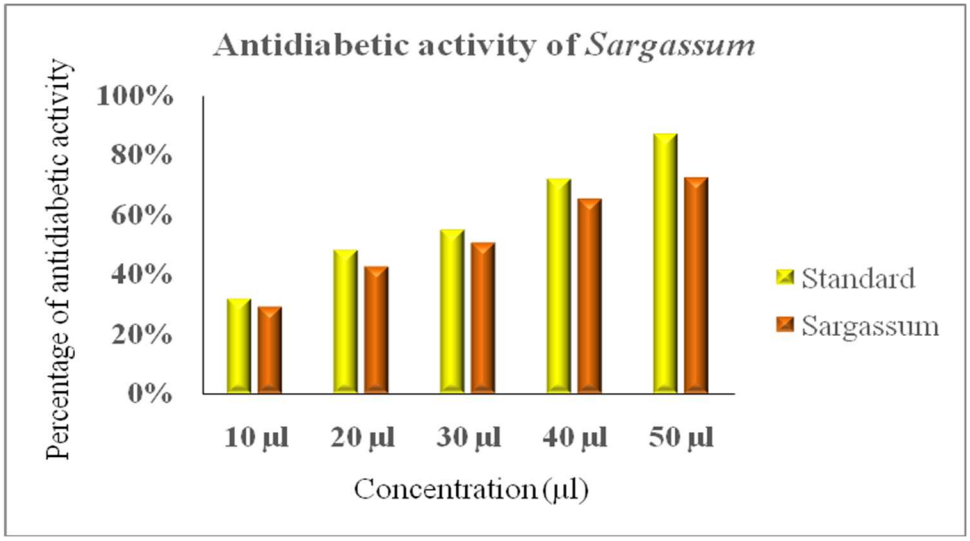

Fig.4. Antidiabetic activity of Sargassum vulgare

DISCUSSION

Many of the bioactive secondary metabolites found in the marine environment are structurally distinct from those found in terrestrial natural products. Seaweeds are rich in bioactive compounds such polyphenols, carotenoids, and polysaccharides. Functional foods, medications, and cosmetics may use these bioactive compounds because they provide consumers health advantages.

Seaweed methanol extracts include polyphenolic compounds called catechins, flavonoids, and glycosides that have been reported to have in vitro antioxidant and antibacterial action (Mahendran et al., 2022).

Polyphenols are widespread in nature, occurring in a great variety of marine organisms. Therefore, an attempt was made to study the presence of polyphenol from brown seaweed Sargassum vulgare. The yield of crude polyphenol extracted from 100 g of the seaweed powder was 12 g for Sargassum vulgare. The crude polyphenol extracted partially purified in DEAE cellulose 52 using 1 M sodium chloride and yield was 6.5 gm for Sargassum vulgare. Similarly, Rajamani Karthikeyan et al. (2010) reported the polyphenol from Gracilaria boergesenii was estimated at 5 g /100 ml of dry weight in polyphenol. In the present study, the total antioxidant capacity of polyphenol from Sargassum vulgare was found to be 76.2±0.14%. Meenakshi et al. (2009) reported that the total antioxidant activity of methanolic extract U. lactuca showed 0.91 ± 0.09 mg GAE/g. Mahendran et al. (2022) studied the TAC from S. tenerrimum was 88.11±0.25.

In the present study, the hydrogen peroxide scavenging activity of polyphenol of Sargassum vulgare was 71.05±0.16%. Many species of seaweed possess scavenging ability of hydrogen peroxide (Mahendran et al., 2022). The measurement of H_2O_2 scavenging activity is one of the useful methods of determining the ability of antioxidants to decrease the level of pro-oxidants such as H_2O_2 (Czochra and Widensk, 2002).

In this present study Sargassum vulgare was investigated. To assess the antioxidant capacity of Sargassum vulgare extract, the DPPH test was carried out. The percentage of DPPH scavenging activity in Sargassum vulgare extract is (88.11±0.24 %.). Similarly, the work done by Riwanti et al. (2024) showed that methanol extract of Sargassum vulgare has strong antioxidant activity consisting of phenolic compounds, flavonoids and carotenoids. .

Antidiabetic activity of Sargassum vulgare extract was analyzed. The percentage of antidiabetic activity in Sargassum vulgare extract is higher (72.20%). Similarly, the work done by Firdaus et al. (2010)

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showed that methanol extract of Sargassum echinocarpum lowered blood glucose level of diabetes mellitus rats to below 200 mg/dl at a dosage of 450 mg/kg.b.w.

Conclusion

Brown algae are a rich source of bioactive compounds which exhibit significant health-promoting properties. The majority of the investigated brown algae species have been shown to contain polyphenols, which have the most potential uses as antioxidants and antidiabetic agents. This study aimed to evaluate the possible antidiabetic and antioxidant properties of polyphenol extracts derived from Sargassum vulgare of brown algae. According to the study's findings, brown algal polyphenols have strong antioxidant and anti-diabetic properties. These extracts can be classified as nontoxic and our research suggests they may contribute to the development of new treatments.

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