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# ANTIDIABETIC AND ANTIOXIDANT POTENTIALS OF *FUCUS SPIRALIS* METHANOLIC EXTRACTS

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## ABSTRACT
Diabetes Mellitus (DM) is an endocrinological disorder and not a single disorder which is a group of metabolic or heterogeneous affliction resulting from an irregularity in insulin secretions and insulin actions or both. Compounds and extracts derived from the Mother Nature's diversity have found uses in medicine i.e., allopathic, homeopathy and Ayurveda, agriculture, beauty products, and health products in ancient and modern culture around the world. Therefore, the visionary to access natural products, understanding their usefulness and derivation applications in diabetes management has been a major driving force in the field of natural product research. Hence, the present study aimed to evaluate the medicinal quality of *Fucus spiralis* by screening of antidiabetic and antioxidant properties. Screening of antidiabetic potentials by the studies such as effect of extract on glucose adsorption capacity, percentage of glucose uptake in yeast cells, and α-amylase inhibitory activities demonstrated that the increasing extract concentrations exhibited significant (P>0.0001) hypoglycemic activities and higher activities found at 100 mg/ml concentration. The EC50 value of *Fucus spiralis* methanolic extracts for glucose adsorption capacity, percentage of glucose uptake in yeast cells, and α-amylase inhibitory activities were reported as 62.578, 35.84, and 45.95 mg respectively. The results of DPPH, FRAP, and NO scavenging activities revealed that 100 mg/ml extract concentration exhibited the highest percentage of inhibition such as 83.5±0.45%, 37.51±0.3, and 58.66±0.6% respectively. While, the EC50 values for DPPH, FRAP, and NO scavenging activities were reported as 47.46, 132.47, and 68.54 mg/ml. These results concluded that *Fucus spiralis* methanolic extracts can be useful as alternate for clinical applicability in treatment of diabetes.

***Keywords:*** *Fucus spiralis, Methanolic extract, Diabetes, Antioxidants, Glucose, DPPH, FRAP*

## INTRODUCTION
Diabetes Mellitus is an endocrinological metabolic disorder resulting from an irregularity in insulin secretions and insulin actions or both. Absence or reduced insulin in turn leads to persistent abnormally high blood sugar and glucose intolerance (Jahan *et al.,* 2015). Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both (Votey and Peters, 2004). Diabetes mellitus (DM) is rapidly becoming one of the most common non-communicable diseases globally (Shaw *et al.,* 2010). In 2013, there were 382 million people with diabetes and this number is expected to rise to 592 million by 2035. Population growth, aging of population and urbanization with associated lifestyle change is likely to lead to a 55% increase in worldwide numbers with diabetes by 2035. According to WHO, (1994), this problem has been aggravated by rapid cultural and social dynamics, ageing populations, increasing urbanization, dietary changes, reduced physical activity and other unhealthy lifestyle and behavioural patterns.
Compounds and extracts derived from the Mother Nature's diversity have found uses in medicine i.e., allopathic, homeopathy and Ayurveda, agriculture, beauty products, and health products in ancient and modern culture around the world. Therefore, the visionary to access natural products, understanding their

usefulness and derivation applications in diabetes management has been a major driving force in the field of natural product research. In addition, there is a massive sea of secondary metabolites typically confined to a particular group of organisms, or a particular species. Seaweeds are a group of macroscopic marine algae that form the biomass in the intertidal zone (Wong and Cheung, 2000). They are the eukaryotic o multicellular and macrothallic organisms that live in salty water and growing almost exclusively at narrow interface where land and sea meet. They recognized as a potential source of bioactive natural products. Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine. In addition to their health promoting effects some marine derived compounds have exhibited potential for use as food ingredients due to their bioactive properties (Rajauria *et al.*, 2013). Seaweeds are also used as source of cosmetics, biodiesel, gelling purposes, and for the extraction of industrial gums and chemicals. They have the potential to be used as a source of long and short-chain chemicals with medicinal and industrial uses. Seaweeds are the raw material for industrial production of agar, algin and carrageenan but they continue to be widely consumed as food in Asia countries (Mishra *et al.*, 1993). Hence, the present study focused on the antidiabetic properties of *Fucus spiralis* methanolic extracts. The brown alga *Fucus spiralis* is one of the common algae of the Indian coast and occurs predominantly in the lower littoral zone of the Atlantic coasts of Europe and North America, and is commonly found in the Portuguese coast. It also inhabits occasionally in the intertidal rock pools as submerged population. It has the common names of spiral wrack and flat wrack. *Fucus spiralis* is olive brown in colour and similar to *Fucus vesiculosus* and *Fucus serratus*. The intertidal algae often get exposed to the atmosphere periodically during low tide regimes and experience an oxidative stress on regular basis with the turning tides. They distribute worldwide in India especially they distributed along the coasts of Dwarka, Bombay, Karwar, Goa, Quilon, Vizhinjam, Muttam, Idinthakarai, Manapad, Tiruchendur, Tuticorin, Mandapam, Mahabalipuram, Madras, Visakhapatnam and Andaman-Nicobar. The present study aimed to evaluate the medicinal quality of *Fucus spiralis* by screening of antidiabetic and antioxidant properties.

## MATERIALS AND METHODS

### *Sample collection*

Healthy *Fucus spiralis* L. samples were collected from Tenneti Park (Long: 83º20' 59.94" E; Lat: 17º 44' 48.36" N) have been situated on the North East coast of Andhra Pradesh, adjoining the Bay of Bengal, Visakhapatnam, India. The collected samples were aseptically transferred to the zip pouches and transported to the laboratory. First sample washed under tap water and then with distilled water. The samples were stored in a refrigerator at 4°C for the further study.

### *Methanolic extract preparation*

Methanolic extract was prepared according to modified method of Aquil *et al.*, (2006). Healthy sample of *F. spiralis* was shade dried and the shade dried samples were powdered and the 10 gm of coarse powder of sample was macerated and soaked separately in (twice i.e., 2×100ml) methanol for 8-10 days at room temperature in dark conditions, with gentle mechanical stirring at every 18 h, using a sterile rod. The extract was then filtered through Whatman filter paper No.1 and the extracts were concentrated by using a distillation apparatus. The concentrated extract was packed in air tight brown glass bottles with proper label and kept in a refrigerator at 4ºC until used for the experiment.100 mg of extract was taken and dissolved in 1 ml of Dimethylsulphoxide (DMSO), which is used as stock solution with the concentration. From this stock solution, different concentrations such as 10, 25, 50, 75, and 100mg/ml were prepared using DMSO solution (Chaudhari *et al.*, 2013) for the evaluation of antidiabetic and antioxidant assays.

### *Evaluation of invitro antidiabetic potential*

### *Determination of extract glucose adsorption capacity*

Glucose adsorption capacity of *F. spiralis* methanolic extracts were determined by the methodology of Ou *et al.*, (2001). To achieve glucose adsorption capacity, 1ml of extract from each concentration was added to 25 ml of 100 mmol/dl concentrated glucose solution. The mixture was stirred well, incubated in a shaker water bath at 37 °C for 6 h, centrifuged at 4800 r/min for 20 min and the glucose content in the supernatant

was determined by using GOD-POD glucose estimation kit. The concentration of bound glucose was calculated using the following formula:

Glucose bound= G1- G6 /Weight of the sample ×Volume of solution

G1 is the glucose concentration of original solution.

G6 is the glucose concentration after 6 h.

***Effect of extracts on glucose uptake in yeast cells***

A series of five different concentrations of *F. spiralis* methanolic extracts were used to study the percentage of glucose uptake in yeast cells. To perform this assay, the commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of extracts (10-100mg/ml) were added to 1ml of glucose solution (100 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension followed by vertexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and amount of glucose was estimated in the supernatant by adding the 100µl of glucose oxidase solution to 500µl of supernatant solution and kept for half an hour at room temperature. Then the percentage increase in glucose uptake by the yeast cells was measured at 520nm using a UV visible spectrophotometer. Distilled water was used as a blank. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

***α-amylase inhibitory assay***

The α-amylase inhibitory assay for methanolic extracts of *F. spiralis* were evaluated according to a previously described method by Malik and Singh *et al.,* (1980) with slight modification. To perform α-amylase inhibitory assay, 0.5 ml of extract from respective concentrations were mixed with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated using 1 ml of dinitro salicylic acid colour reagent. At this time, the test tubes were placed in a water bath (100°C and 5 min) and cooled until room temperature was reached. The mixture was then diluted with 10 ml of deionized water, and absorbance was determined at 540 nm. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. The inhibition of α-amylase was calculated using the following equation.

$$\text{Inhibition \%} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

***Screening of antioxidant properties***

***DPPH radical scavenging activity***

DPPH scavenging activity of antioxidants were determined by Mensor *et al.,* (2001). To evaluate the DPPH radical scavenging activity, 20 µl of each extract, 0.5 ml of 0.1 mM ethanol solution of DPPH and 0.48 ml of buffer were added in a test tube. Buffer and DPPH without extract was used as blank. The mixture was shaken vigorously for 1min by vertexing and allowed to incubation at room temperature in the dark for 30 min. Thereafter, the absorbance for the sample was measured using spectrophotometer at 517 nm against blank. Results were expressed in % of scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

***Ferric reducing antioxidant power (FRAP) assay***

The ability to reduce ferric ions was measured using the method described by Benzie and Strain, (1996). The FRAP reagent was prepared by mixing 300 mμ sodium acetate buffer (pH 3.6), 10.0 mM (tripyridyl triazine) TPTZ solution and 20.0 mM FeCl3.6H2O solution in a ratio of 10:1:1 in volume. To evaluate the FRAP activity, 0.5ml of each methanolic extract, 3 ml of FRAP reagent was added and the reaction mixture was incubated at 37 °C for 30 min. The increase in absorbance at 593 nm was measured. Control tube was prepared with DMSO instead of extract. Results were expressed in % of ferric reducing activity.

$$\% \text{ Ferric reducing antioxidant power} = \frac{\text{Ab Control} - \text{Ab Test} \times 100}{\text{Ab Control}}$$

***NO radical scavenging activity***

The nitric oxide scavenging activity was assessed using the method of Balakrishnan *et al.*, (2011) with sodium nitroprusside. For this experiment, 0.5 mL of Fucus spiralis extracts were taken in individual test tubes. Subsequently, 1 mL of 10 mM sodium nitroprusside in phosphate-buffered saline was added, and the tubes were incubated at 25°C for 180 minutes. Following incubation, an equal volume of freshly made Griess reagent was added to all the tubes. The Griess reagent was produced by dissolving 1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride (NEDD) in 2.5% phosphoric acid. The absorbance of the produced chromophore after the diazotization of nitrate ions with sulfanilamide and subsequent binding to NEDD was quantified at 546 nm. Control was concurrently maintained in the absence of the methanolic extract.

***Statistical analysis***

The results were expressed as mean ± standard deviation of three individual replicates and the data was assessed by one-way analysis of variance (ANOVA) and subjected to regression-correlation analysis by using SPSS 10.0 software. The 'P' value less than 0.05 was considered as significant difference.

## RESULTS AND DISCUSSION

***Glucose adsorption capacity***

Glucose Adsorption Capacity of *F. spiralis* methanolic extracts was increased with the increasing extract. It was observed that the *Fucus spiralis* methanolic extracts (83.5 ± 0.45%) showed highest Glucose Adsorption Capacity at 100mg/ml extract concentration. At 10mg/ml extract concentration, the Glucose Adsorption Capacity of *F. spiralis* methanolic extract was reported as 32.56±0.41%. The Glucose Adsorption Capacity of *Fucus spiralis* methanolic extracts with increasing concentrations such as 10, 25, 50, 75, and 100mg/ml were found as 32.56±0.41, 34.74±0.05, 40.69±0.06, 72.5±0.2 and 83.5±0.45% correspondingly. The EC50 value of *Fucus spiralis* methanolic extracts for glucose adsorption capacity was reported as 62.578mg. The results have been shown in Table 1. These results indicated that the Glucose Adsorption Capacity was depended on the extract concentration. Increased extract concentration significantly (<.0001) increases the Glucose Adsorption potential. The glucose adsorption capacity shows strong positive correlation (r = 0.9422) with increasing extract concentration.

The results of the present study indicated that the all-tested extracts possessed a significant glucose adsorption capacity. Moreover, the glucose adsorption capacity of the samples was found to have a directly proportional relationship with the molar concentration of extract. Marine organisms represent excellent source for bioactive compounds (Bickmeyer *et al.,* 2005). Similar observation was reported by Bhutkar and Bhise, (2013) that the adsorption capacity of the extracts of *Albizzia lebbeck* and *Mucuna pruriens* was found to be directly proportional to the molar concentration of glucose. In vivo and in vitro studies of glucose adsorption have shown that the delay in glucose absorption in the gastrointestinal tract is determined mainly by the viscosity of soluble polysaccharides (Chau *et al.,* 2003). El Barky *et al.,* (2017) has been reported that the saponins content in the sea cucumber acts as an antidiabetic agent. Saponins from marine animals have been reported to have a hypoglycemic activity.

**Table 1. Glucose adsorption capacity of *Fucus spiralis* methanolic extracts.**

| S/N | Extract conc. (mg/ml) | Mean (%) | Std. Deviation | Std. Error | Variance |
|---|---|---|---|---|---|
| 1 | 10 | 32.56 | 0.41 | 0.24 | 0.17 |
| 2 | 25 | 34.74 | 0.05 | 0.03 | 0.003 |
| 3 | 50 | 40.69 | 0.06 | 0.03 | 0.003 |
| 4 | 75 | 72.5 | 0.2 | 0.11 | 0.04 |
| 5 | 100 | 83.5 | 0.45 | 0.26 | 0.21 |

**Table 2. One way ANOVA analysis glucose adsorption capacity of *Fucus spiralis* methanolic extracts.**

| Source | SS | df | Ms | F | P |
|---|---|---|---|---|---|
| Treatment (between groups) | 6638.3504 | 4 | 1659.5876 | 19291.55 | <.0001 |
| Within groups | 0.8603 | 10 | 0.08 | - | - |
| Total | 6639.2107 | 14 | - | - | - |

***Effect of extracts on glucose uptake in yeast cells***

Percentage of glucose uptake in yeast cells were increased with the increasing concentration of extracts. It was observed that the *F. spiralis* methanolic extracts (82.83 ± 0.25) showed highest percentage of glucose uptake at 100mg/ml extract concentration. At 10mg/ml extract concentration the percentage of glucose uptake in yeast was reported as 14.36±0.21. Percentage of glucose uptake in yeast cells by different concentrations of *F. spiralis* methanolic extracts such as 10, 25, 50, 75 and 100mg/ml were reported as 14.36±0.21%, 57.66±0.25%, 69.43±0.35%, 78.53±0.31% and 82.83 ± 0.25%respectively. The results have been shown in Table 3. The EC50 value of percentage of glucose uptake by yeast cells with *F. spiralis* methanolic extracts was reported as 35.84 mg/mL. These results indicated that the percentage of glucose uptake in yeast was depended on the extract concentration. Increased extract concentration significantly increases the percentage of glucose uptake in all the tested extracts. The percentage of glucose uptake by yeast significantly (<.0005) increased with increasing extract concentration. The extracts show strong positive correlation (r = 0.86527) with increasing extract concentration.

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as a lucrative method for in vitro screening of hypoglycemic effect of various bioactive compounds. The in vitro assays of the present study indicated that methanolic extract possess good anti-diabetic activity. The rate of uptake of glucose into the yeast cells was increased with increased extract concentrations used in the study. In Yeast (*Saccharomyces cerevisiae*), glucose transport takes place through facilitated diffusion. Type 2 Diabetes is characterized by the deficiency of insulin, causing increased amount of glucose in blood. After the treatment of the yeast cells with these extracts, the glucose uptake was found to increase than standard drug metformin. The studies on the transport of non-metabolizable sugars, metabolizable glycosides have suggested that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers and takes place by facilitated diffusion process (Illiano and Cuatrecasas, 1971). It is reported that in yeast cells glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast is by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose (Teysink *et al.*, 1998). The data obtained clearly suggests that the *F. spiralis* methanolic extracts are capable of effectively enhancing glucose uptake which in turn suggests that it is capable of enhancing the effective glucose utilization, thereby controlling blood glucose level.

***α- amylase inhibitory assay***

The in-vitro α-amylase inhibitory studies demonstrated that extracts show significant anti diabetic activity. Percentage of *in-vitro* α-amylase inhibitory activity was increased with the increasing concentration of extracts in all the tested extracts. It was observed that the *F. spiralis* methanolic extracts (70.69 ± 0.63)

showed highest percentage of *in-vitro* α-amylase inhibitory activity at 100mg/ml extract concentration. At 10mg/ml extract concentration the percentage of in-vitro α-amylase inhibitory activity was reported as

**Table 3. Glucose uptake in yeast cells by *Fucus spiralis* methanolic extracts.**

| S/N | Extract Conc. (mg/ml) | Mean (%) | Std. Deviation | Std. Error | Variance |
|---|---|---|---|---|---|
| 1 | 10 | 14.36 | 0.21 | 0.12 | 0.04 |
| 2 | 25 | 57.66 | 0.25 | 0.14 | 0.06 |
| 3 | 50 | 69.43 | 0.35 | 0.2 | 0.12 |
| 4 | 75 | 78.53 | 0.31 | 0.18 | 0.09 |
| 5 | 100 | 82.83 | 0.25 | 0.15 | 0.06 |

**Table 4. One way ANOVA analysis of glucose uptake in yeast cells by *Fucus spiralis* methanolic extracts.**

| Source | SS | df | Ms | F | P |
|---|---|---|---|---|---|
| Treatment (between groups) | 9120.22 | 4 | 2280.055 | 29483.47 | <.0001 |
| Within groups | 0.7733 | 10 | 0.0773 | - | - |
| Total | 9120.9933 | 14 | - | - | - |

37.50±0.04. Percentage of *in-vitro* α-amylase inhibitory activity by different concentrations of *F. spiralis* methanolic extracts such as 10, 25, 50, 75 and 100mg/ml were reported as 37.50±0.04%, 42.38±0.02%, 50.81±0.06%, 59.67±0.04% and 70.69 ± 0.63% respectively. The results have been shown in Table 5. The EC50 value for the in-vitro α-amylase inhibitory activity with *F. spiralis* methanolic extracts was reported as 45.95mg/ml. These results indicated that the percentage of *in-vitro* α-amylase inhibitory activity was depended on the extract concentration. Increased extract concentration significantly increases the percentage of α-amylase inhibition in all the tested extracts. The percentage of α-amylase inhibitory activity significantly (<.0005) increased with increasing extract concentration. The extracts show strong positive correlation (r = 0.9963) with increasing extract concentration.

In humans, the digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of polymeric substrates into shorter oligomers. Later on in the gut these are further hydrolysed by pancreatic α- amylases into maltose, maltotriose and small malto-oligosaccharides. α-amylase is responsible for hydrolysing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of α-amylase can lead to reduction in post prandial hyperglycemia in diabetic condition (Ferry-Roux *et al.,* 1998). The inhibition of α-amylase activity in the digestive tract of humans is considered to be effective tool to control diabetes. In addition, these effects may lead to diminished absorption of monosaccharides (Hara and Honda, 1990). In animals' alpha amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugars (Boivin *et al.,* 1988). This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood (Mohammed *et al.,* 2009). Hence diabetics tend to have low alpha amylase levels in order to keep their glucose levels under control. Plants also use alpha amylase inhibitors as a defence mechanism as a protection from insects. These inhibitors alter the digestive action of alpha amylases and proteinases in the gut of insects and inhibit their normal feeding behaviour. Therefore, alpha amylase inhibitors have potential roles in controlling blood sugar levels and crop protection (Bhosale and Hallale, 2011).

**Table 5. In-vitro α-amylase inhibitory activity by *Fucus spiralis* methanolic extracts.**

| S/N | Extract Conc. (mg/ml) | Mean (%) | Std. Deviation | Std. Error | Variance |
|---|---|---|---|---|---|
| 1 | 10 | 37.50 | 0.047 | 0.027 | 0.002 |
| 2 | 25 | 42.38 | 0.026 | 0.015 | 0.001 |
| 3 | 50 | 50.81 | 0.065 | 0.065 | 0.004 |
| 4 | 75 | 59.67 | 0.041 | 0.041 | 0.002 |
| 5 | 100 | 70.69 | 0.632 | 0.633 | 0.401 |

**Table 6. One way ANOVA analysis of in-vitro α-amylase inhibitory activity by *Fucus spiralis* methanolic extracts.**

| Source | SS | df | Ms | F | P |
|---|---|---|---|---|---|
| Treatment (between groups) | 2136.3818 | 4 | 534.0953 | 6516.54 | <.0001 |
| Within groups | 0.8196 | 10 | 0.082 | - | - |
| Total | 2137.2009 | 14 | - | - | - |

***DPPH radical scavenging activity***

The DPPH radical scavenging activity of methanolic extracts from Fucus spiralis exhibited a significant increase (<.0005) with increasing extract concentrations. Results are presented in Table 7. The results indicate that the methanolic extract of Fucus spiralis exhibited the highest DPPH radical scavenging activity at a concentration of 100 mg/ml, measuring 83.5±0.45%. The extracts exhibited DPPH radical scavenging activities of 32.56±0.41, 34.74±0.05, 40.69±0.06, 72.5±0.2, and 83.5±0.45% at concentrations of 10, 25, 50, 75, and 100 mg/ml, respectively. Moreover, the elevated concentrations of extract demonstrate a strong positive correlation of r = 0.9422 with DPPH scavenging activity. The $EC_{50}$ value for the DPPH radical scavenging activity of methanolic extracts was reported to be 47.46 mg/ml.

The DPPH radical exhibits a strong absorption at 517 nm in visible spectroscopy, attributed to its unpaired electron, resulting in a deep violet color. In the free radical scavenging reaction, DPPH is transformed into α,α-diphenyl-β-picrylhydrazine, accompanied by a color change as the electron pairs off in the presence of a free radical scavenger, resulting in a decrease in absorption due to decolorization. The decolorization is stoichiometric with respect to the number of electrons absorbed. The progressive reduction in color change reflects the scavenging capabilities of the sample (Ayoola *et al.*, 2008). Krishnaraju *et al.,* (2009) indicated that the effectiveness of antioxidants is frequently linked to their capacity to neutralize stable, highly reactive free radicals. The methanolic extracts of *F. spiralis* demonstrate a dose-dependent DPPH free radical scavenging ability in this study. The scavenging capacity of the extracts likely indicates the cumulative activities of diverse antioxidant-related bioactive secondary metabolites contained within them. Multiple studies have indicated that all bioactive compounds can decolorize DPPH solution through their hydrogen-donating capacity (Vadlapudi and Kaladhar, 2012). Priyanka *et al.,* (2013) reported that increased discoloration of the DPPH solution demonstrates greater antioxidant properties.

**Table 7. Inhibition percentage of DPPH radical scavenging activity with increasing concentration of *Fucus spiralis* methanolic extracts.**

| S/N | Extract Conc. (mg/ml) | Mean (%) | Std. Deviation | Std. Error | Variance |
|---|---|---|---|---|---|
| 1 | 10 | 32.56 | 0.41 | 0.24 | 0.17 |
| 2 | 25 | 34.74 | 0.05 | 0.03 | 0.003 |
| 3 | 50 | 40.69 | 0.06 | 0.03 | 0.003 |
| 4 | 75 | 72.5 | 0.2 | 0.11 | 0.04 |
| 5 | 100 | 83.5 | 0.45 | 0.26 | 0.21 |

**Table 8. One-way ANOVA analysis on inhibition percentage of DPPH radical scavenging activity with increasing concentration of *Fucus spiralis* methanolic extracts.**

| Source | SS | df | Ms | F | P |
|---|---|---|---|---|---|
| Treatment (between groups) | 6638.3504 | 4 | 1659.5876 | 19291.55 | <.0001 |
| Within groups | 0.8603 | 10 | 0.08 | - | - |
| Total | 6639.2107 | 14 | - | - | - |

***Ferric reducing antioxidant power (FRAP) assay***

The FRAP activity of methanolic extracts from Fucus spiralis exhibited a significant increase (<.0001) with higher extract concentrations. Table 9 presents the results. Based on these findings, the maximum FRAP activity was obtained at an extract concentration of 100 mg/ml (37.51 ± 0.3%). The methanolic extracts with increasing concentrations of 10, 25, 50, 75, and 100 mg/ml showed FRAP activities measured at

8.25±0.3%, 18.74±0.2%, 28.84±0.4%, 32.7±0.3%, and 37.51±0.3%, respectively. Furthermore, the elevated levels of extract concentration demonstrate a strong positive correlation of r = 0.9305 with FRAP activity. The $EC_{50}$ value for the FRAP activity of methanolic extract was reported to be 132.47 mg/ml.

The FRAP assay relies on the reduction of the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant under low pH conditions. Fe(II)-TPTZ exhibits a deep blue color and can be detected at a wavelength of 593 nm. The alteration in absorbance at 593 nm results from the conversion of colorless oxidized Fe+3 to blue-colored Fe+2, tripyridyltriazine, facilitated by the action of electron-donating antioxidants. The ferric reducing antioxidant power assay is commonly employed to assess the capacity of polyphenols to reduce ferric ions (Luximon *et al.*, 2005). Halvorsen et al., (2006) indicated that the majority of metabolites are redox-active compounds detectable by the FRAP assay. According to the current findings, Priyanka *et al.*, (2013) observed that increased antioxidant activity correlates with higher FRAP values. A higher FRAP value signifies increased antioxidant potential. The findings of this study indicate that methanolic extracts of Fucus spiralis exhibit significant FRAP activity.

**Table 9. Percentage of FRAP activity with increasing concentration of *Fucus spiralis* methanolic extracts.**

| S/N | Extract Conc. (mg/ml) | Mean (%) | Std. Deviation | Std. Error | Variance |
|---|---|---|---|---|---|
| 1 | 10 | 8.25 | 0.3 | 0.017 | 0.0009 |
| 2 | 25 | 18.74 | 0.2 | 0.012 | 0.0004 |
| 3 | 50 | 28.84 | 0.4 | 0.025 | 0.0019 |
| 4 | 75 | 32.7 | 0.3 | 0.021 | 0.0013 |
| 5 | 100 | 37.51 | 0.3 | 0.018 | 0.0012 |

**Table 10. One-way ANOVA analysis of FRAP activity with increasing concentration of *Fucus spiralis* methanolic extracts.**

| Source | SS | df | Ms | F | P |
|---|---|---|---|---|---|
| Treatment (between groups) | 1650.2652 | 4 | 412.5663 | 368362.72 | <.0001 |
| Within groups | 0.0112 | 10 | 0.0011 | - | - |
| Total | 1650.2764 | 14 | - | - | - |

***NO radical scavenging activity***

The nitric oxide scavenging activity from the methanolic extracts of *Fucus spiralis* exhibited significant (P= <0.0014) increase with increased extract concentrations. Highest and lowest NO scavenging activities were observed at 100 and 10mg/ml concentrations respectively. The NO scavenging activities from the methanolic extracts of *Fucus spiralis* were observed as 32.37±2.7, 38.48±0.7, 42.37±1.2, 53.78±1.3, and 58.66±0.6% corresponding to the 10, 25, 50, 75, and 100mg/ml extract concentrations. Furthermore, the $EC_{50}$ was measured as 68.54 mg/ml. The NO scavenging activity of the extract shows a strong positive correlation of r=0.9889 with respect to the increasing concentrations (Table 11).

The Nitric Oxide scavenging activity is based on the concept that sodium nitroprusside in an aqueous solution at physiological pH swiftly produces nitric oxide, which then interacts with oxygen to produce nitrite ions, detectable using the Griess reagent. Patel and Patel, (2011) proposed that nitric oxide scavengers suppress the production of nitrite ions by competing with oxygen. Parul et al., (2013) observed that the nitric oxide scavenging activity of the methanolic extracts of *Phyllanthus fraternus* was 60.807±0.005% at a concentration of 200 µg/ml. Saha *et al.*, (2004) observed that the nitric oxide scavenging activity of *Lasianthus oblongus* plant extract was 85.97% at a concentration of 250 µg/ml. Conversely, Ebrahimzadeh

*et al.,* (2010) revealed that the methanolic extracts of Hibiscus esculentus seeds exhibited 0.97±0.6% activity.

**Table 11. Percentage of NO scavenging activity with increasing concentration of *Fucus spiralis* methanolic extracts.**

| S/N | Extract Conc. (mg/ml) | Mean (%) | Std. Deviation | Std. Error | Variance |
|---|---|---|---|---|---|
| 1 | 10 | 32.37 | 2.7 | 1.6 | 7.4 |
| 2 | 25 | 38.48 | 0.7 | 0.4 | 0.5 |
| 3 | 50 | 42.37 | 1.2 | 0.7 | 1.4 |
| 4 | 75 | 53.78 | 1.3 | 0.8 | 1.6 |
| 5 | 100 | 58.66 | 0.6 | 0.4 | 0.4 |

**Table 12. One-way ANOVA analysis of NO scavenging activity with increasing concentration of *Fucus spiralis* methanolic extracts.**

| Source | SS | df | Ms | F | P |
|---|---|---|---|---|---|
| Treatment (between groups) | 1417.475 | 4 | 354.3687 | 156.4 | <.0014 |
| Within groups | 22.6574 | 10 | 2.2657 | - | |
| Total | 1440.1324 | 14 | - | -- | - |

## CONCLUSION

From this study, it could be suggested that *Fucus spiralis* is a promising source of bioactive compounds, which have the ability to modify the physiological function of cells and hence act as anti-diabetic drugs. The extract shows commendable antioxidant activity which also may be one of the contributing factors to its antidiabetic potential. By all these features it can be concluded that *Fucus spiralis* methanolic extracts can be useful as alternate for clinical applicability in treatment of diabetes. The development of such drugs with differential action will be very valuable in clinical treatment without the side effects.

## REFERENCES

**Aqil F, Ahmad I, Mehmood Z (2006).** Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish Journal of Biology,* **30**(3) 177-83.

**Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO (2008).** Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research,* **7**(3) 1019-24.

**Balakrishnan N, Balasubramaniam A, Nandi P, Dandotiya R, Begum S (2011).** Antibacterial and free radical scavenging activities of stem bark of *Psidium guajava* Linn. *International Journal of Drug Development and Research,* **3** 255-60.

**Benzie IF, Strain JJ (1996).** The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry,* **239**(1) 70-6.

**Bhosale UP, Hallale BV (2011).** Gamma radiation induced mutations in black gram (*Vigna mungo* (L.) Hepper). *Asian Journal of Plant Science and Research,* **2011** ⟨hal-03699676⟩.

**Bhutkar M, Bhise S (2013).** In vitro hypoglycemic effects of *Albizzia lebbeck* and *Mucuna pruriens*. *Asian Pacific Journal of Tropical Biomedicine*,**3**(11) 866-70.

**Bickmeyer U, Assmann M, Köck M, Schütt C (2005).** A secondary metabolite, 4, 5-dibromopyrrole-2-carboxylic acid, from marine sponges of the genus Agelas alters cellular calcium signals. *Environmental Toxicology and Pharmacology*,**19**(3) 423-7.

**Boivin M, Flourie B, Rizza RA, Go VL, DiMagno EP (1988).** Gastrointestinal and metabolic effects of amylase inhibition in diabetics. *Gastroenterology,* **94**(2) 387-94.

**Chau CF, Huang YL, Lee MH (2003).** In vitro hypoglycemic effects of different insoluble fiber-rich fractions prepared from the peel of *Citrus sinensis* L. cv. Liucheng. *Journal of Agricultural and Food Chemistry,* **51**(22) 6623-6.
**Chaudhari MG, Joshi BB, Mistry KN (2013).** In vitro anti-diabetic and anti-inflammatory activity of stem bark of *Bauhinia purpurea*. *Bulletin of Pharmaceutical and Medical Sciences,***1**(2) 139-50.
**Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Pourmorad F (2010).** Nitric oxide radical scavenging potential of some Elburz medicinal plants. *African Journal of Biotechnology*, **9**(32) 5212-7.
**El Barky AR, Ali EM, Mohamed TM (2017).** Marine sea cucumber saponins and diabetes. *Austin Pancreatic Disorders,***1**(1) 1-7.
**Ferey-Roux G, Perrier J, Forest E, Marchis-Mouren G, Puigserver A, Santimone M (1998).** The human pancreatic α-amylase isoforms: isolation, structural studies and kinetics of inhibition by acarbose. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology,* **1388**(1)10-20.
**Halvorsen BL, Carlsen MH, Phillips KM, Bøhn SK, Holte K, Jacobs Jr DR, Blomhoff R (2006).** Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *The American Journal of Clinical Nutrition,* **84**(1) 95-135.
**Hara Y, Honda M (1990).** The inhibition of α-amylase by tea polyphenols. *Agricultural and Biological Chemistry,* **54**(8) 1939-45.
**Illiano G, Cuatrecasas P (1971).** Glucose transport in fat cell membranes. *Journal of Biological Chemistry,* **246**(8) 2472-9.
**Jahan S, Fariduddin M, Sultana N, Aktar Y, Hasan M (2015).** Predictors of post-partum persistence of glucose intolerance and its association with cardio-metabolic risk factors in gestational diabetes mellitus. *Journal of Diabetes and Metabolism,* **6** 609.
**Krishnaraju AV, Rao CV, Rao TV, Reddy KN, Trimurtulu G (2009).** In vitro and in vivo antioxidant activity of *Aphanamixis polystachya* bark. *American Journal of Infectious Diseases,* **5**(2) 60-7.
**Luximon-Ramma A, Bahorun T, Crozier A, Zbarsky V, Datla KP, Dexter DT, Aruoma OI (2005).** Characterization of the antioxidant functions of flavonoids and proanthocyanidins in Mauritian black teas. *Food Research International,* **38**(4) 357-67.
**Malik CP, Singh MB (1980).** Plant enzymology and histoenzymology, Kalyani Publishers, New Delhi, 278 p.
**Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TC, Coube CS, Leitão SG (2001).** Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research,* **15**(2) 127-30.
**Mishra VK, Temelli F, Ooraikul B, Shacklock PF, Craigie JS (1993).** Lipids of the red alga *Palmaria palmata*. Botanica Marina, **36** 169-174.
**Mohamed SA, Al-Malki AL, Kumosani TA (2009).** Partial purification and characterization of five α-amylases from a wheat local variety (Balady) during germination. *Australian Journal of Basic and Applied Sciences,* **3**(3) 1740-8.
**Ou S, Kwok KC, Li Y, Fu L (2001).** In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of Agricultural and Food Chemistry,* **49**(2) 1026-9.
**Parul R, Kundu SK, Saha P (2013).** In vitro nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. *The Pharma Innovation Journal,* **1**(12) 83-8.
**Patel Rajesh M, Patel Natvar J (2011).** In vitro antioxidant activity of coumarin compounds by DPPH, super oxide and nitric oxide free radical scavenging methods. *Journal of Advanced Pharmacy Education and Research,* **1**(1) 52-68.
**Priyanka C, Kadam DA, Kadam AS, Ghule YA, Aparadh VT (2013).** Free radical scavenging (DPPH) and ferric reducing ability (FRAP) of some gymnosperm species. *International Research Journal of Botany,* **3**(2) 34-6.

**Rajauria G, Jaiswal AK, Abu-Gannam N, Gupta S (2013).** Antimicrobial, antioxidant and free radical-scavenging capacity of brown seaweed *Himanthalia elongata* from western coast of Ireland. *Journal of Food Biochemistry*, **37**(3) 322-35.
**Saha K, Lajis NH, Israf DA, Hamzah AS, Khozirah S, Khamis S, Syahida A (2004).** Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. *Journal of Ethnopharmacology*, **92**(2-3) 263-7.
**Shaw JE, Sicree RA, Zimmet PZ (2010).** Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, 87(1) 4-14.
**Teusink B, Diderich JA, Westerhoff HV, van Dam K, Walsh MC (1998).** Intracellular glucose concentration in derepressed yeast cells consuming glucose is high enough to reduce the glucose transport rate by 50%. *Journal of Bacteriology*, **180**(3) 556-62.
**Vadlapudi V, Kaladhar DS (2012).** Antioxidant activities of marine algae: A review, In: A. Capasso (Ed.), *Medicinal Plants as Antioxidant Agents: Understanding Their Mechanism of Action and Therapeutic Efficacy*, Research Signpost, Kerala, India 2012, pp.189–203.
**Votey SR, Peters AL (2004).** Diabetes mellitus type 2. A review. Retrieved from http://www.emedicine.com/emerg/topic133.htm Accessed July, 2006.
**Wong KH, Cheung PC (2000).** Nutritional evaluation of some subtropical red and green seaweeds: Part I—proximate composition, amino acid profiles and some physico-chemical properties. *Food Chemistry*, **71**(4) 475-82.
**World Health Organization (1994).** Prevention of diabetes mellitus. Technical Report Series No. 844. Geneva: World Health Organization, 1994.