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Fucoidan and Fucoidanase from Brown Seaweeds and Applications

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A B S T R A C T

Fucoidan is a biologically active polysaccharide found in brown seaweeds. The present study was carried out to isolate and investigate the biological activities of fucoidan and the activity of fucoidanase (EC: 3.2.1.44) from *Padina pavonica*, *Sargassum latifolium and Colpomenia sinuosa*. *C. sinuosa* expressed the highest fucoidan content and the highest fucoidanase activity. The isolated fucoidan from *C. sinuosa* expressed appreciable anticoagulant and antibacterial activities against *Bacillus sp, Pseudomonas aeruginosa, Echerichia coli, Staphyllococcus aureus and Proteus vulgaris*. It also showed antioxidant activity using DPPH [DPPH (1,1-diphenyl-2-picrylhydrazyl)] and ABTS [2, 2-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid)] methods. Fucoidan from *C. sinuos* inhibited both α-glucosidase (EC: 3.2.1.20) and α-amylase (EC: 3.2.1.1). The inhibition of fucoidan for α-glucosidase activity was stronger than that of α-amylase activity. The results suggest that fucoidan from *C. sinuosa* can be used as antibacterial, antioxidant and antidiabetic

Introduction

The anticoagulants are a group of pharmaceutical compounds used for thrombotic disorders. Heparin is a sulfated polysaccharide and it is the first compound used for anticoagulation (Shanthi *et al.,* 2014).

Seaweeds contain different bioactive compounds, they are valuable resource for nutraceutical and pharmaceutical products (Shanthi *et al.,* 2014). Fucoidan is a sulfated polysaccharide found mainly in brown algae of Phaeophyceae (Pomin and Mourão 2008; Berteau and Mulloy, 2003).

Fucoidans are located in the cell-wall matrix of brown algae. This matrix contains large content of sulfate and L-fucose with minor amounts of other sugars including glucose, xylose, mannose, galactose, rhamnose and uronic acids (Berteau and Mulloy, 2003). Several studies have shown that fucoidan has various biological activities such as anticoagulant and antithrombotic activities. In addition fucoidan expressed antiproliferative, antiadhesive and anti-inflammation effect on cells. Furthermore, it was found that fucoidan protects can cells from viral infection (Khatuntseva *et al.,* 2000).

Athukorala *et al.,* (2007) reported structural similarities between heparin and fucoidans. However, fucoidan from seaweeds is safer than heparin and thus it is safe alternative drug which gained much attention in the

pharmaceutical industry with low or less side effects (Athukorala *et al.,* 2007, Yamazaki *et al.,* 2016).

Therefore, several studies have been made on characterization of flucoidan, from brown seaweeds, as the second largest amount next to alginate in the cell wall matrix (Eldeen *et al.,* 2009).

Thus, the present work aimed to investigate the biological activities of fucoidan from *Colpomenia sinuosa*. These included the antioxidant, anticoagulant and inhibition of the enzymes activities involved in starch digestion such as alpha glucosidase and alpha amylase. In addition, it aimed to measure the activities of fucoidanase from the same seaweed.

Materials and Methods

Algal samples

Algal samples were collected by hand from submerged rocks on the coast of Abu Qir Bay, Alexandria, Egypt. The samples were then transported in plastic bags to the laboratory and these bags contain sea water to prevent evaporation.

The samples were rinsed thoroughly with fresh water for elimination of the foreign materials such as shells, sand, epiphytes and their hold-fasts. Some of the collected samples were preserved for identification. The seaweed was identified following Aleem (1993).

The dried algal mass was powdered and then sieved through a mesh of size 1 mm and kept in plastic containers at -20 ºC in plastic containers.

Extraction of fucoidan

The extraction process was carried out according to Berteau and Mulloy (2003).

Anticoagulant activity of fucoidan

The prothrombin time was estimated in the presence of 10 g of fucoidan. Aqueous fucoidan solutions were added to control plasma and the mixture was incubated for 1 min at 37 ºC.

Time of clot formation was recorded after addition of a thromboplastin calcium mixture which is preheated for 30 min at 37 °C.

The thrombin time was estimated in the presence of 2–20 g of fucoidan in a sample (in dependence of fucoidan activity). Aqueous fucoidan solutions were added to control plasma. The mixture was incubated for at 37 °C 2 min. Time of clot formation was recorded after addition of thrombin solution.

Antibacterial activity of fucoidan

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts (Valgas *et al.,* 2007). The microorganisms tested were *Bacillus sp*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris*.

Antioxidant activity of fucoidan

DPPH scavenging activity determined by using the procedure of Brand-Williams *et al.,* (1995). ABTS radical scavenging activity was carried out according to Re *et al.,* (1999).

Preparation of enzyme extract

The fresh seaweed material (20mg) were macerated in 25 ml of pre-chilled 100 mM potassium phosphate buffer (pH 7.0) containing 1mM cysteine. The resulting homogenate was centrifuged at 300 xg and 40^{o}C for 15 min. The resulting homogenate represents the crude extract and was used for subsequent analysis.

Assay of fucoidanase

Sample of enzyme preparation (0.2 ml) was added to 2.5 ml of 50 mM sodium acetate buffer (pH 5.6) containing 0.2% of fucoidan. The mixture was incubated for 20 min and the produced reducing sugar was determined using Somogyi-Nelson method (Smogyi, 1952).

Antidiabetic activity of fucoidan

The inhibitory assay of α-amylase and the inhibitory assay of α-glucosidase was done by the method of Mogale *et al.,* (2011).

Results and Discussion

Fucoidan content in three seaweeds

In this experiment the fucoidan content was estimated in three seaweeds namely *Padina pavonica, Sargassum latifolium, Colpomenia sinuosa*. The results in Fig. 1 show that *Colpomenia sinuosa* expressed the highest yield (5.6 %). Higher content of fucoidan was found in leaves of *Macrocystis pyrifera*, *Laminaria digitata, Ascophyllum nodosum,* and *Fucus vesiculosus* (Skriptsova, 2014).

Fucoidanase activity in the three seaweeds

In this experiment fucoidanase was determined in extractions from *Colpomenia sinuosa*, *Sargassum latifolium* and *Padina pavonica*. The enzyme activity was expressed as U mg^{-1}protein. The results in Fig. 2 demonstrate that fucoidanase activity from *Colpomenia sinuosa* was the highest among the tested seaweeds followed by *Sargassum latifolium and Padina pavonica*.

Anticoagulant activity of fucoidan

In the anticoagulant test there was continuous increase in the time of clotting by increasing fucoidan content in the assay medium. It reached 45 second at 100 µg ml^{-1} fucoidan in the prothrombin test (Fig. 3) and 72 second at 100 µgml^{-1} fucoidan in the thrombin test (Fig. 4). The anticoagulant activity of fucoidan from *C. sinuosa* may be promising candidate for the development of an anticoagulant that can replace heparin, which has strong anticoagulant activity but shows potentially serious side effects including hemorrhage, thrombocytopenia and osteoporosis (Mourao and Pereira, 1999, Boisson-Vidal *et al.,* 2000, Berteau and Mulloy, 2003). Fucoidan from Korean *Undaria pinnatifida* sporophyll showed anticoagulant activity (Kim *et al.,* 2007). Also, the fucoidan showed antithrombotic/ antithrombin activity (Nishino *et al.,* 2000).

Antibacterial activity of fucoidan

The antibacterial activity of fucoidan from *Colpomenia sinuosa* against different strains of bacteria namely *Bacillus sp, Pseudomonas aeruginosa, Echerichia coli, Staphyllococcus aureus and Proteus vulgaris.* Fig. 5 show that the zone of inhibition ranged from 10-20 mm. Maximum inhibition zone of 20 mm was recorded against *Pseudomonas aeruginosa.* Antibacterial effect of fucoidan from *Sargassum wightii* was reported by Marudhupandi and Kumar (2013).

Antioxidant activity of fucoidan

The results in Fig. 6 indicate that fucoidan from *Colpomenia sinuosa* expressed appreciable antioxidant activity. The higher concentration 100 µg ml^{-1} showed the highest free radical scavenging activity. The results of antioxidant activity of ABTS are recorded in Fig 7. The results reveal that the antioxidant activity increased continuously up to 61 % at 100 µgml^{-1}. It has been found that fucoidan is unlike synthetic antioxidants since it is a natural antioxidant and has large potential for avoiding or delaying free radical mediated illness (Li *et al.,* 2008).

Fig.1 Fucoidan contents in three seaweeds

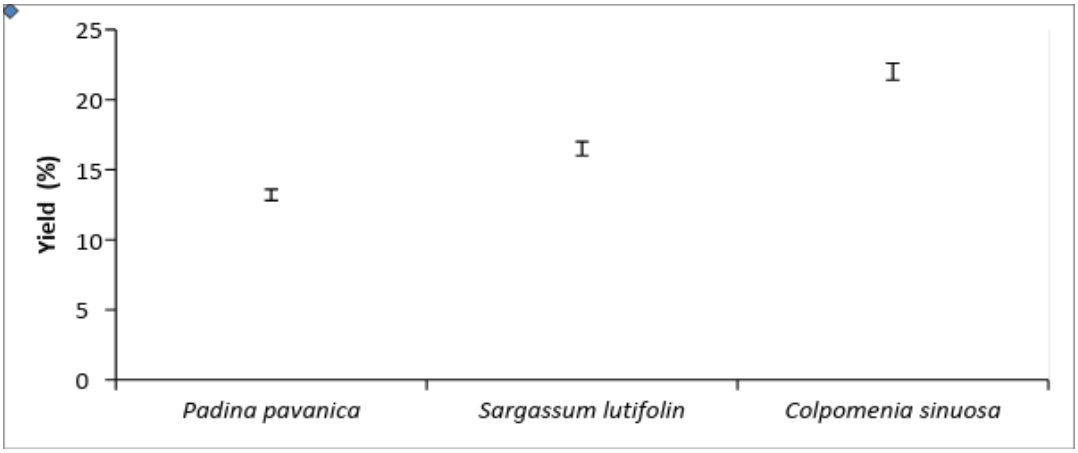


Fig.2 Fucoidanase activities in three seaweeds

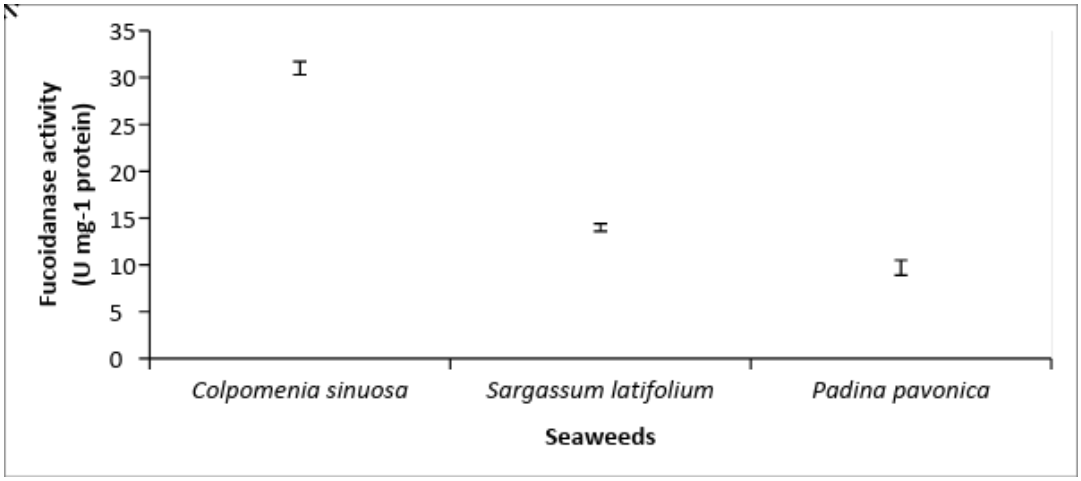


Fig.3 Effect of fucoidan from *Colpomenia sinuosa* on prothrombin time(s)

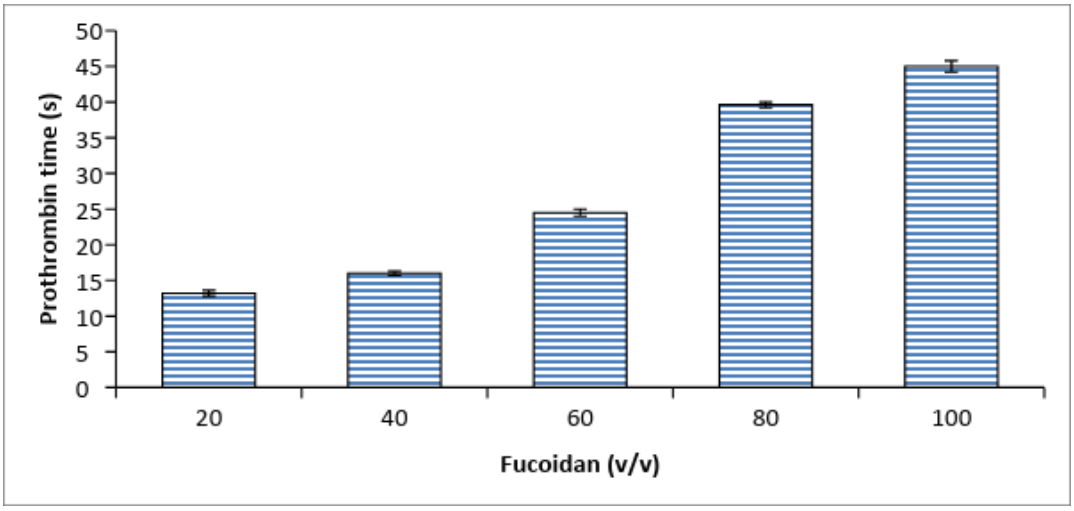


Fig.4 Effect of fucoidan from *Colpomenia sinuosa* on thrombin time(s)

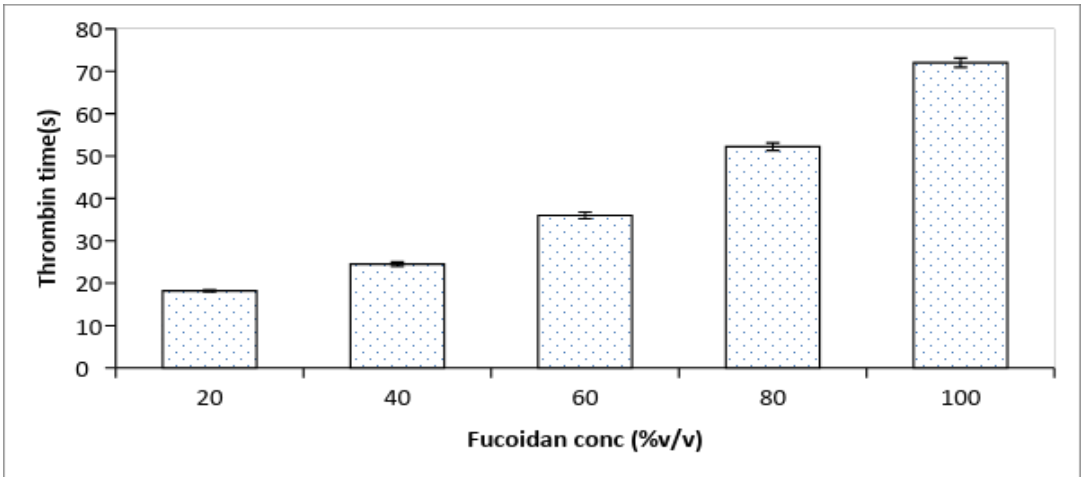


Fig.5 Antibacterial activity of the fucoidan from *Colpomenia sinuosa*

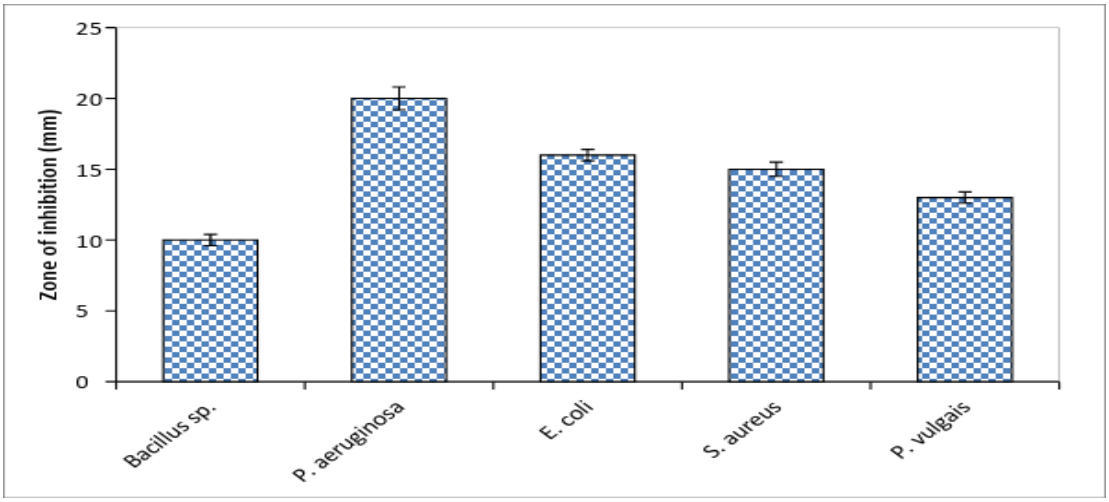


Fig.6 DPPH antioxidant activity of fucoidan from *Colpomenia sinuosa*

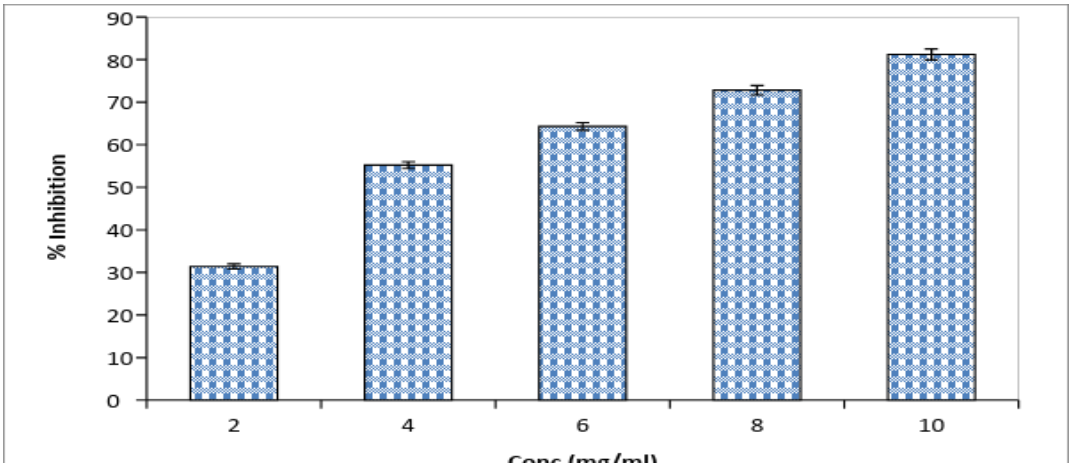


Fig.7 ATBS antioxidant activity of fucoidan from *Colpomenia sinuosa*

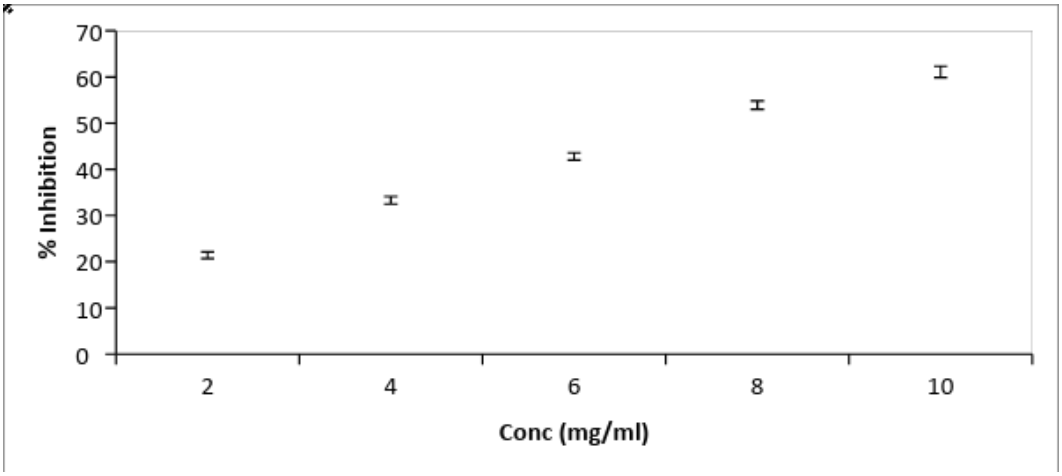


Fig.8 Reciprocals of S and V_o of α-amylase with and without fucoidan

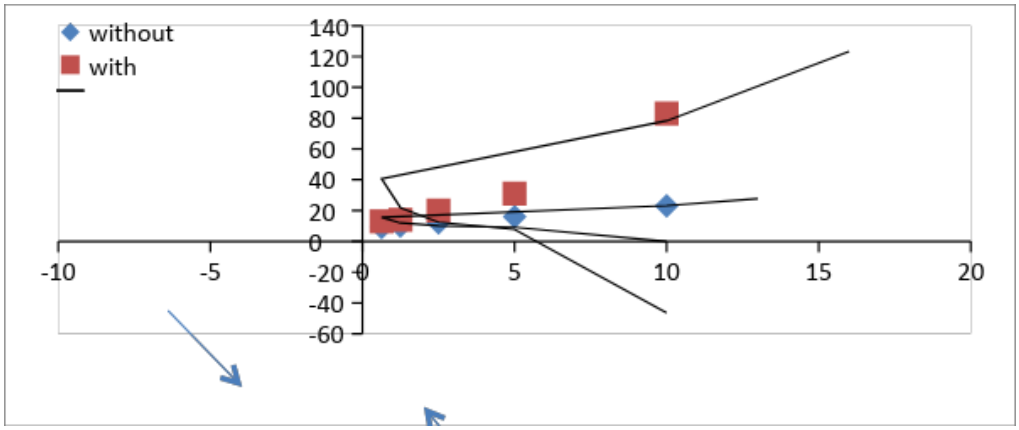
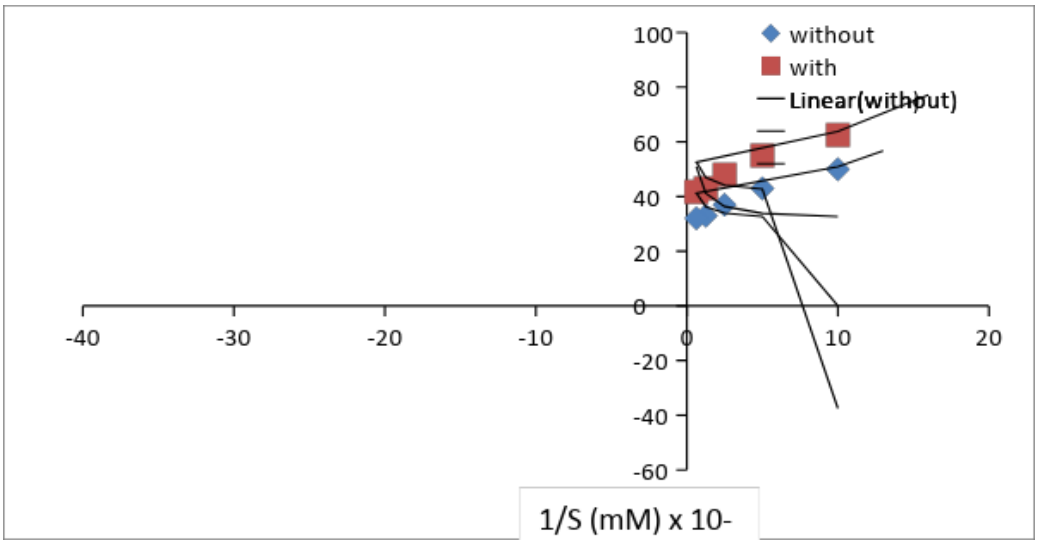


Fig.9 Reciprocals of S and V of α-glucosidase with and without fucoidan



Antidiabetic activity of fucoidan

α-amylase activity was determined in presence of 200 µg ml^{-1} fucoidan. Plotting 1/S against 1/V was carried out and the results are shown in Fig. 8 and illustrate that fucoidan inhibited α-amylase activity. V_{max} values were 24 and 30 with and without fucoidan. K_m values were 50 and 60 (% w/v) with and without fucoidan.

The influence of 200 µg ml^{-1} fucoidan on α-glucosidase activity was investigated and the plot of 1/S against 1/V (Fig. 9) show that V_{max} values were 0.12 and 3.3 units mg^{-1}protein with and without fucoidan whereas K_{max} values were 3.3 and 4.1 mM with and without fucoidan.

The inhibitory effect of both synthesized and natural therapies on starch digesting enzymes such as inhibiting the rate of carbohydrate absorption provides a safe way; with low risk of hypo-glycaemia and greater benefits on preventing diabetic complicates than conventional medications (Kang *et al.*, 2015).

The synthetic α-glucosidase inhibitors expressed gastrointestinal disturbances such as diarrhea and abdominal discomfort, which are diminished with reduced dose. A carbose is a common drug inhibiting the activity of α-glucosidase enzyme. Abdominal diarrhea is the major side effects of this drug (Kang *et al.*, 2015).

α-amylase activity was inhibited by fucoidan from *C. sinuosa*. Thus, the results reveal the possible use of fucoidan from *C. sinuosa* to reduce the digestion of carbohydrate. Fucoidan is characterized by low digestibility and large molecular weight, it less likely to be absorbed from gasterointestinal tract. Hence, one possible mechanism for its potential anti-diabetic effects could be inhibition of digestive enzymes (Cho *et al.*, 2011).

It has been reported that monosaccharide content, sulphate groups and the linear back bone of the polysaccharide may all contribute to the bioactivity of fucoidan (Li *et al.*, 2008; Skriptsova *et al.*, 2009). Thus, the present results introduce fucoidan from *C. sinuosa* as new candidate for antidiabetic agent.

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