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## SCREENING OF ANTI-DIABETIC PROPERTIES OF FUCOIDAN EXTRACTED FROM *PADINA TETRASTROMATICA* HAUCK IN MANAPAD COAST, TAMIL NADU, INDIA.

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### ABSTRACT

In the present study, the anti-diabetic activity of fucoidan extracted from *Padina tetrastromatica* Hauck. collected from Manapad coast, the south east coast of Tamil Nadu, India was evaluated. Anti-diabetic property of fucoidan was tested by the inhibitory effect of  $\alpha$ -amylase and  $\alpha$ -glucosidase. Different concentrations of fucoidan such as 25, 50, 75 and 100 $\mu$ g were added to the starch as substrate and the enzyme mixture. After 5min the enzymes activity were arrested and estimated using spectroscopically. The result showed that all the concentrations of the fucoidan were inhibited both  $\alpha$ -amylase and  $\alpha$ -glucosidase. When the concentration of fucoidan was increased from 25 to 100 $\mu$ g, the inhibitory effect of the enzyme also increased. From the present study, it was concluded that low concentration of fucoidan has minimum anti-diabetic effect and the high concentration of fucoidan has the maximum anti-diabetic effect. The enzyme inhibiting activity of fucoidan was quite variable, depending on the concentration of fucoidan on the targeted enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase.

**Keywords:** Anti-diabetic,  $\alpha$ -amylase,  $\alpha$ -glucosidase Fucoidan, Seaweeds, *Padina*.

### INTRODUCTION

Diabetes is a metabolic disorder characterized by high plasma glucose levels. Dietary control is suggested as a safe and complementary treatment of diabetes. A diet based on glycemic index is currently one of the most recommended nutritional treatments. It has been reported that dietary therapy can be used simultaneously with other medical treatments in order to obtain a synergistic effect [1]. However, this has the drawback of limiting the types and quantity of food consumed. Another possible solution is to decrease the rate of blood sugar absorption from the small intestine by slowing and interrupting the digestion of dietary starch, the major source of glucose [2]. This approach is considered more efficient than controlling insulin secretion, for economic reasons, convenience and avoidance of side effects [3]. The inhibition of enzymes that digest dietary starch into glucose,  $\alpha$ -amylase and  $\alpha$ -glucosidase has been studied as a way of controlling blood sugar level [4].  $\alpha$ -amylase from human saliva catalyses the hydrolysis of  $\alpha$ -(1,4)-glucosidic linkages and produces maltose and glucose from starch [5], while  $\alpha$ -glucosidase releases glucose from maltose [6]. By inhibiting these two enzymes, the absorption of glucose into the bloodstream can be delayed and thus, ameliorating Type-2 diabetes symptoms like hyperglycemia.

Attempts have been made to identify  $\alpha$ -amylase

and  $\alpha$ -glucosidase inhibitors that can be used as food or food additives. Although Seo *et al.* [7] and Kato *et al.* [8] demonstrated that some sugar-like phenolic compounds have  $\alpha$ -glucosidase inhibitory activity, most studies on  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors have focused on the utilization of proteins or phenolic compounds [9]. Although phenolic compounds have high inhibitory activity of  $\alpha$ -amylase and  $\alpha$ -glucosidase, phenolic compounds are unstable, sensitive to light and heat treatment limits their uses as nutraceuticals.

Recently, algae have been considered as a source of enzyme inhibitors. As several plant extracts, algae contain some polyphenolic compounds as bromophenols [10], phlorotannins [11] which are inhibitors of  $\alpha$ -glucosidase. Also, polysaccharides, isolated from algae, have become attractive in the biomedical area for numerous bioactivities [12-13]. In this context, fucoidan a polysaccharide found in brown algae and having several bioactivities appears promising. Fucoidan is abundant in brown seaweed such as *Padina* species [14]. It is a mucilaginous, hygroscopic and sulfated polysaccharide [15] and it protects seaweed from dehydration [16]. The biological effects attributed to fucoidan are: anti-coagulant [17], anti-HIV [18] and anti-tumor activities [19]. Brown seaweeds contain up to 10% of fucoidan and the quantity

varies depending on the region, species and season. The structural and chemical characteristics of fucoidan also vary among algal species [20]. The aim of the present study was to investigate the inhibition of starch digestive enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase by fucoidan extracted from *Padina tetrastromatica* Hauck in Manapad coast, the south east coast of Tamil Nadu, India.

## MATERIALS AND METHODS

### Collection of Materials

The collection of *Padina tetrastromatica* Hauck (Figure 1) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking in Manapad coast, the south east coast of Tamil Nadu, India. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution. For drying, washed specimens were placed on blotting paper and spread out at room temperature in the shade. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use.

### Extraction of Fucoidan

Fucoidan was extracted by Rioux method [21]. Dried seaweed was mixed with 1% (w/v)  $\text{CaCl}_2$  solution (1:30 ratio) and then stirred for 4 hours at  $85^\circ\text{C}$  at  $455 \pm 5$  rpm using a stirrer RZR1 (Cafra Ltd. Canada). The supernatant was separated by centrifugation (16,887g, 20 min), and vacuum filtration on Whatman No. 4 filter. The filtered liquid was mixed with 2 volumes of 95% ethanol and 1 volume of 2% (w/v) NaCl and then stirred for 1 hour at room temperature for alcoholic precipitation of fucoidan. This solution was kept at  $-20^\circ\text{C}$  for 48 hours. The pellet containing fucoidan was recovered by centrifugation (16,887 g, 12 min). Then, it was resolubilized in 100 ml of fresh deionized water and dialyzed for 48 hr by using membrane of 15 KDa (Sigma, USA) to remove minor constituents and solvents. Fucoidan was recovered by freeze-drying and preserved at  $-20^\circ\text{C}$  in a sealed tube to keep away from humidity.

### Alpha-amylase inhibition assay

The method of Conforti *et al.* [22] was modified to determine the inhibitory effects of the fucoidan extract on  $\alpha$ -amylase (from human salivary, EC 3.2.1.1). 1% starch solution was prepared by stirring 1g of corn starch in 100ml of 20 mM sodium phosphate buffer with 6.7mM sodium chloride, pH 6.9. The solution was heated at  $100^\circ\text{C}$  for 15 min and then cooled to room temperature. The volume was brought to 100ml with distilled water.  $\alpha$ -amylase solution was prepared as 1unit/ml. The different concentration of fucoidan solution (25, 50, 75 and 100 $\mu\text{g}$ ) was added in 1ml of a starch solution. The tubes were prepared in duplicate and split into two groups, which were classified as a test group (TG) and a control group

(CG). All tubes were incubated at  $20^\circ\text{C}$  for 10min and 1ml of  $\alpha$ -amylase solution was added. For amylase activation, the TG tubes were incubated at exactly  $20^\circ\text{C}$  for 5min. The colorimetric reagent (1ml) was added to all of the tubes of the TG, heated at  $100^\circ\text{C}$  for 15min and cooled in an ice bath. 9ml of distilled water was added to each tube and the absorbance at 540nm was measured. The same steps were realized for the CG, but the incubation at  $20^\circ\text{C}$  for 5min was omitted. As blank, 0.1ml of distilled water was added instead of the fucoidan solution. The optical density of the blank (OD B) refers to the difference between test and control group. The degree of enzyme inhibition was calculated using the following equation,

$$\text{Enzyme inhibition (\%)} = \frac{(\text{OD}_{\text{TG}} - \text{OD}_{\text{CG}}) - \text{OD}_{\text{B}}}{\text{OD}_{\text{B}}} \times 100(\%)$$

### Alpha-glucosidase inhibition assay

For the determination of  $\alpha$ -glucosidase (EC 3.2.1.20) inhibitory activity of fucoidan, all solutions were prepared according to the method of Halvorson and Ellias [23]. 5ml of 67mM potassium phosphate (pH 6.8 at  $37^\circ\text{C}$ ) and 0.2ml of 3 mM glutathione solution were added into total 10 test tubes. The test tubes were split into two groups, the test group (TG) and control group (CG). 0.2ml of 1unit/ml of  $\alpha$ -glucosidase was added only into TG tubes and 0.2ml of distilled water was added into CG tubes instead of  $\alpha$ -glucosidase. 25, 50, 75 and 100 $\mu\text{g}$  of fucoidan were added in both TG and CG tubes in parallel. As blank, 0.1ml of distilled water was added instead of the fucoidan solution. The optical density of blank (OD B) refers to the difference between test and control group. All tubes were kept at  $37^\circ\text{C}$  for 20min. 0.5ml of 10 mM p-Nitrophenyl alpha-D-glucopyranoside was added into all tubes and then incubated at  $37^\circ\text{C}$  for 20min. 1ml of the previous mixture solution was transferred into new test tube containing 4ml of sodium carbonate (0.1 M) and the solution was mixed. The absorbance was measured at 400nm and the degree of enzyme inhibition was calculated using the following equation,

$$\text{Enzyme inhibition (\%)} = \frac{(\text{OD}_{\text{TG}} - \text{OD}_{\text{CG}}) - \text{OD}_{\text{B}}}{\text{OD}_{\text{B}}} \times 100(\%)$$

## RESULTS AND DISCUSSION

Most studies looking for natural extracts contributing to diabetes prevention have been focusing on  $\alpha$ -glucosidase inhibition because the enzyme plays a role at the ending step of starch digestion by producing glucose from maltose. Although the inhibition of  $\alpha$ -amylase also results in the decrease of glucose release, its complete inhibition is not desired because it could provoke intestinal disorders [24]. The undigested starch could be utilized by the gut microflora for gas production. Therefore, partial inhibition of  $\alpha$ -amylase could contribute to modulate the rate of glucose release from starch.

Fucoidan is a natural polysaccharide made essentially of sulphated L-fucose residues. Also known as sulphated fucan, it was first extracted in 1913 from brown algae [25]. Fucoidan is present in the cell walls of brown algae especially *Padina tetrastromatica* Hauck. Though

many studies on identifying the structural properties of fucoidan have been carried out, the structure still remains uncertain due to the absence of strict regularity and the numerous components that make up fucoidan as a whole [26]. Figure 2 shows the general structure of fucoidan but the chemical composition and structure of fucoidan varies with species [27]. Most fucoidans have very complex chemical composition and only little regularity in the structural components is known present. Fucoidan largely contains sulphated L-fucose residues. Hence fucose is the primary sugar in fucoidan. Sulphate groups also represent a large component of fucoidan and the biological activities of fucoidan are strongly related to its sulphate content [28]. Besides fucose and sulphate, other monosaccharides (glucose, mannose, galactose, xylose, etc), uronic acids, and even protein are present in detectable amounts. All these compounds have increased the difficulty in structural elucidation of fucoidan [29].

#### Inhibition of $\alpha$ -amylase Activity

The  $\alpha$ -amylase activity effectiveness of different concentration of fucoidan extracted from *Padina tetrastromatica* Hauck were compared on the basis of the inhibition percentage. Among the different concentration of fucoidan used, 100 $\mu$ g of fucoidan extracted from *Padina tetrastromatica* Hauck inhibited 67.45% followed by 75 $\mu$ g fucoidan with 53.27% and 50 $\mu$ g with 36.96%. 25 $\mu$ g of fucoidan showed less inhibitory activity of  $\alpha$ -amylase with 21.43%. Acarbose, the positive control used in the study, inhibited the activity of  $\alpha$ -amylase at 75.5%. The present result showed that the percentage of  $\alpha$ -amylase inhibitory activity increased when the concentration of fucoidan increased from low concentration to higher concentration (Table 1 & Figure 3).

#### Inhibition of $\alpha$ -glucosidase Activity

In the present study fucoidan extracted from *Padina tetrastromatica* Hauck collected from Manapad, Tamil Nadu, India was found to possess favorable  $\alpha$ -glucosidase inhibitory effects on starch break down *in vitro*. The  $\alpha$ -glucosidase inhibitor activity effectiveness of different concentration of fucoidan extracted from *Padina tetrastromatica* Hauck were compared on the basis of the inhibition percentage. Among

the different concentration of fucoidan used, 100 $\mu$ g of fucoidan extracted from *Padina tetrastromatica* Hauck inhibited 58.59% followed by 75 $\mu$ g fucoidan with 43.85% and 50 $\mu$ g with 28.46%. 25 $\mu$ g of fucoidan showed less inhibitory activity of  $\alpha$ -glucosidase with 19.12%. Acarbose, the positive control used, inhibited the activity of  $\alpha$ -glucosidase at 81.2%. The present study showed that the percentage of  $\alpha$ -glucosidase inhibitory activity increased when the concentration of fucoidan increased from low concentration to higher concentration (Table 2 & Figure 4).

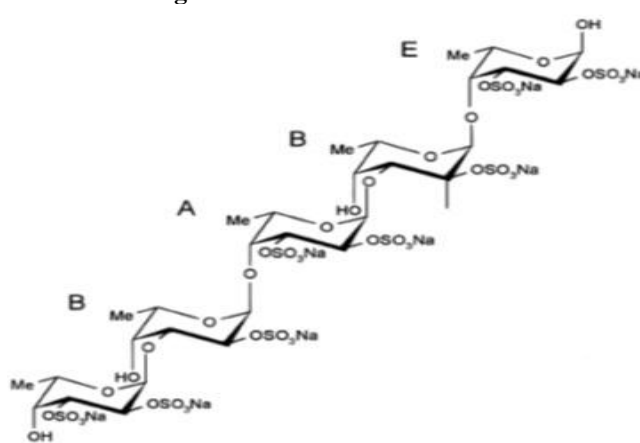
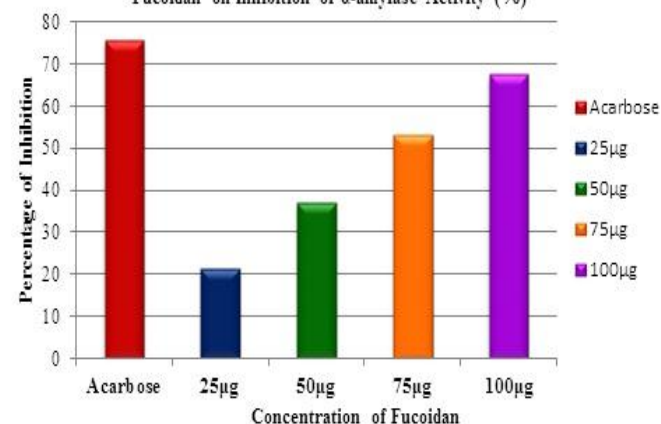
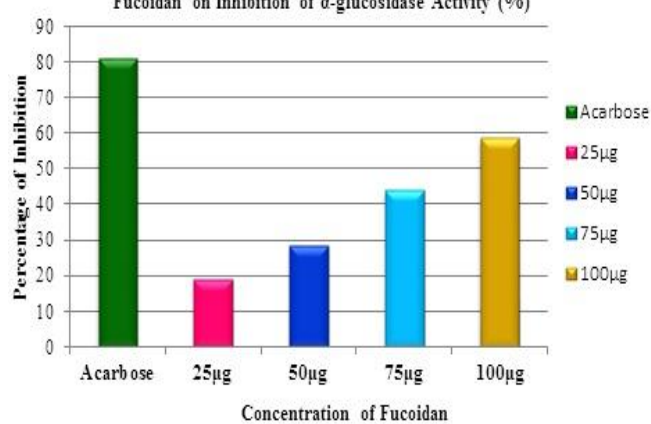
Mechanisms of  $\alpha$ -glucosidase inhibition differ among the various inhibitors reported. Some well-known inhibitors as acarbose mimic the enzyme substrate. Previously published articles on the mechanism of polyphenol compounds suggest that the principal factor acting on  $\alpha$ -glucosidase activity is hydrogen scavenging because  $\alpha$ -glucosidase provides hydrogen to catalyze the hydrolysis of the  $\alpha$ -(1,4)-glucosidic linkage [30]. The inhibitor acts by intercepting the hydrogen ion freed from the  $\alpha$ -glucosidase catalytic site. Most studies conducted with fucoidan showed that the sulfate content is closely related to its biological properties. Wang *et al.* [31] showed a relation between sulfate content and radical scavenging property using fucoidan from *Laminaria japonica*. So *et al.* [32] also reported free-radical scavenging ability of fucoidan from *Fucus vesiculosus*. Highly sulfated fucoidan (37% sulfate groups) showed better free radical scavenging activity than native fucoidan (28% sulfate groups). Increasing sulfate groups may enhance the scavenging activity of fucoidan and thus, promote its capacity to intercept free hydrogen. Numerous phenolic compounds such as flavonol [33], catechins and theaflavins [34] have an  $\alpha$ -glucosidase inhibitory activity. The intensity of the activity is related to the structure and conformation of polyphenols [35]. Fucoidan might inhibit  $\alpha$ -glucosidase by a mechanism similar to that of polyphenols that involves scavenging but more research will be needed in order to determine the mechanism of action. Structural features such as molecular weight and the amount of sulfate groups could seriously impact the enzyme activity. More work was realized in order to explain how those structural characteristics influence the enzyme activity.

**Table 1. Effect of Standard and various concentration of Fucoidan on Inhibition of  $\alpha$ -amylase Activity (%)**

Groups	Percentage of Inhibition of $\alpha$ -amylase Activity
Acarbose	75.5%
25 $\mu$ g Fucoidan	21.43%
50 $\mu$ g Fucoidan	36.96%
75 $\mu$ g Fucoidan	53.27%
100 $\mu$ g Fucoidan	67.45%

**Table 2. Effect of Standard and various concentration of Fucoidan on Inhibition of  $\alpha$ -glucosidase Activity (%)**

Groups	Percentage of Inhibition of $\alpha$ -glucosidase Activity
Acarbose	81.2%
25 $\mu$ g Fucoidan	19.12%
50 $\mu$ g Fucoidan	28.46%
75 $\mu$ g Fucoidan	43.85%
100 $\mu$ g Fucoidan	58.59%

**Figure 1. Natural Habit of *Padina tetrastromatica* Hauck****Figure 2. Structure of Fucoidan****Figure 3. Effect of Standard and various concentration of Fucoidan on Inhibition of  $\alpha$ -amylase Activity (%)****Figure 4. Effect of Standard and various concentration of Fucoidan on Inhibition of  $\alpha$ -glucosidase Activity (%)**

## CONCLUSIONS

From the present study, it was concluded that all the concentrations of the fucoidan extracted from *Padina tetrastromatica* Hauck were inhibited both  $\alpha$ -amylase and  $\alpha$ -glucosidase. When the concentration of fucoidan was increased from 25 to 100 $\mu$ g, the inhibitory effect of the

enzyme also increased. Low concentration of fucoidan has minimum anti-diabetic effect and the high concentration of fucoidan has the maximum anti-diabetic effect. The enzyme inhibiting activity of fucoidan was quite variable, depending on the concentration of fucoidan on the targeted enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase.

## REFERENCES

1. Franz MJ. Protein controversies in diabetes. *Diabetes Spectrum*, 13, 2000, 132-141.
2. Ali H, Houghton PJ, Soumyanath A.  $\alpha$ -Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *Journal of Ethnopharmacology*, 107, 2006, 449-455.
3. Porte D. Clinical importance of insulin secretion and its interaction with insulin resistance in the treatment of type-2 diabetes mellitus and its complications. *Diabetes/Metabolism Research and Reviews*, 17(3), 2001, 181-188.
4. Geng P, Bai G. Two novel amino-oligosaccharides isolated from the culture of *Streptomyces Coelicoflavus* ZG0656 as potent inhibitors of  $\alpha$ -amylase. *Carbohydrate Research*, 343, 2008, 470-476.
5. Sogaard M, Abe J, Martin-Eauclaire MF, Svensson B.  $\alpha$ -Amylase: Structure and function. *Carbohydrate Polymers*, 21, 1993, 137-146.
6. Mohan S, Pinto BM. Zwitterionic glycosidase inhibitors: Salacinol and related analogues. *Carbohydrate Research*, 342, 2007, 1551-1580.
7. Seo WD, Kim JH, Kang JE, Ryu HW, Curtis-Long MJ, Lee HS, Yang MS, Park KH. Sulfonamide chalcone as a new class of  $\alpha$ -glucosidase inhibitors. *Bioorganic & Medicinal Chemistry Letters*, 15, 2005, 5514-5516.
8. Kato A, Kato N, Kano E, Adachi I, Ikeda K, Yu L, Okamoto T, Banba Y, Ouchi H, Takahata H, Asano N. Biological properties of D- and L-1-deoxyazasugars. *Journal of Medicinal Chemistry*, 48(6), 2005, 2036-2044.
9. He Q, Lv Y, Yao K. Effects of tea polyphenols on the activities of alpha-amylase, pepsin, trypsin and lipase. *Food Chemistry*, 101, 2006, 1178-1182.
10. Liu M, Hansen PE, Lin X. Bromophenols in marine algae and their bioactivities. *Marine Drugs*, 9, 2011, 1273-1292.
11. Nwosu F, Morris J, Lund VA, Stewart D, Ross HA, McDougall GJ. Anti-proliferative and potential anti-diabetic effects of phenolic rich extracts from edible marine algae. *Food Chemistry* 126, 2011, 1006-1012.

12. Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science & Technology*, 22, 2011, 315-326.
13. Holdt SL, Kraan S. Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, 23(3), 2011, 543-597.
14. John Peter Paul J, Yuvaraj P, Amster Regin Lawrence R. Screening of fucoidan extracted from *Padina distromatica* Hauck from Hare Island, Thoothukudi, Tamil Nadu, India. *International Journal of Pharmacy and Integrated Life Sciences*, 1(12), 2013, 67-75.
15. Black WAP. The seasonal variation in the combined L-fucose content of the common British Laminariaceae and fucaceae. *Journal of the Science of Food and Agriculture*, 5, 1954, 445-448.
16. Percival E, McDowell RH. Chemistry and enzymology of marine algal polysaccharides. *Academic Press*, London and New York, 1967, 6-28, 73-96 & 157-174.
17. Boisson VC, Chaubet F, Chevolut L, Sinquin C, Theveniaux J, Millet J, Sternberg C, Mulloy B, Fischer AM. Relationship between antithrombotic activities of fucans and their structure. *Drug Development Research*, 51(4), 2000, 216-224.
18. Schaeffer DJ, Krylov VS. Anti-HIV activity of extracts and compounds from algae and cyanobacteria. *Ecotoxicology and Environmental Safety*, 45, 2000, 208-227.
19. Koyanagi S, Tanigawa N, Nakagawa H, Soeda S, Shimeno H. Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities. *Biochemical Pharmacology*, 65, 2003, 173-179.
20. Mabeau S, Kloareg B, Joseleau JP. Fractionation and analysis of fucans from brown algae. *Phytochemistry*, 29(8), 1990, 2441-2445.
21. Rioux LE, Turgeon SL, Beaulieu M. Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers*, 69, 2007, 530-537.
22. Conforti F, Loizzo MR, Statti GA, Menichini F. Comparative radical scavenging and antidiabetic activities of methanolic extract and fractions from *Achillea ligustica* All. *Biological & Pharmaceutical Bulletin*, 28(9), 2005, 1971-1794.
23. Halvorson H, Ellias L. The purification and properties of an  $\alpha$ -glucosidase of *Saccharomyces italicus* Y1225. *Biochimica et Biophysica Acta*, 30, 1958, 28-40.
24. Cho M, Han JH, You S. Inhibitory effects of fucan sulfates on enzymatic hydrolyses of starch. *Food Science and Technology*, 44, 2011, 1164-1171.
25. Kylin K. Biochemie der Meeresalgen. *HoppeSeyler's Z Physiol Chem*, 83, 1913, 171-197.
26. Zvyagintseva T, Shevchenko NM, Chizhov AO, Krupnova TN, Sundukova EV, Isakov VV. Water soluble polysaccharides of some far-eastern brown seaweeds. Distribution, structure, and their dependence on the development conditions. *Journal of Experimental Marine Biology and Ecology*, 294, 2003, 1-13.
27. Hayashi K, Nakano T, Hashimoto M, Kanekiyo K, Hayashi T. Defensive effects of a fucoidan from brown alga *Undaria pinnatifida* against herpes simplex virus infection. *International Immunopharmacology*, 8, 2008, 109-116.
28. Yang C, Chung D, Shin S, Lee HY, Kim JC, Lee YJ. Effects of molecular weight and hydrolysis conditions on anticancer activity of fucoidans from sporophyll of *Undaria pinnatifida*. *International Journal of Biological Macromolecules*, 43, 2008, 433-437.
29. Li B, Lu F, Wei X, Zhao R. Fucoidan: Structure and Bioactivity. *Molecules*, 13, 2008, 1671-1695.
30. Melo EB, Silveira GA, Carvalho I.  $\alpha$ - and  $\beta$ -glucosidase inhibitor: chemical structure and biological activity. *Tetrahedron*, 62, 2006, 10277-10302.
31. Wang J, Zhang Q, Zhang Z, Li Z. Antioxidant activity of sulphated polysaccharide fractions extracted from *Laminaria japonica*. *International Journal of Biological Macromolecules*, 42, 2007, 127-132.
32. So MJ, Kim BK, Choi MJ, Park KY, Rhee SH, Cho EJ. Protective activity of fucoidan and alginic acid against free radical induced oxidative stress under *in vitro* and cellular system. *Journal of Food Science and Nutrition*, 12(4), 2007, 191-196.
33. Gamberucci A, Konta L, Colucci A, Giunti R, Magyar JÉ, Mandl J, Bánhegyi G, Benedetti A, Csala M. Green tea flavonols inhibit glucosidase II. *Biochemical Pharmacology*, 72, 2006, 640-646.
34. Bhandari MR, Jong AN, Hong G, Kawabata J. Alpha-glucosidase and alpha-amylase inhibitory activities of Nepalese medicinal herb *Bergenia ciliate* Haw. *Food Chemistry*, 106, 2008, 247-252.
35. Matsui T, Tanaka T, Tamura S, Toshima A, Tamaya K, Miyata Y, Tanaka K, Matsumoto K.  $\alpha$ -Glucosidase inhibitory profile of catechins and theaflavins. *Journal of Agricultural and Food Chemistry*, 55, 2007, 99-105.