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Extraction and purification of flaxseed proteins and studying their antibacterial activities

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Abstract: Flaxseed or linseed (*Linum usitatissimum* L.) is an annual herb belongs to the *Linaceae* family. It is cultivated worldwide and has been used for its oil seed and fiber since ancient times in Egypt, Rome and Greece. Nowadays it is considered as a medicinal plant in Asia, Europe and North America. Therapeutic effects of flaxseed fixed oil have been reported in several publications, but there are only few reports on biological activities of the flaxseed proteins. In the present study antimicrobial activities of the flaxseed proteins have been investigated. Hemogenized flaxseed flour was at first defatted and demucilaged, and its protein content was extracted in distilled water. After centrifugation, proteins of supernatant were isolated by adjusting pH. Gel filtration and ion-exchange chromatography on the protein extact gave fairly pure protein fractions, which antibacterial activities were examined against several microorganisms using the microbial micro-plate dilution method. The results showed that flaxseed proteins have an inhibitory activity on bacteria especially against *Entrococcus foecalis, Salmonella typhimurium* and *Escherichia coli.* Our experiment also revealed that mucilage composition as carbohydrates would not contribute in the inhibitory effect, but in fact, might cover and inhibit the antibacterial activity of flaxseed proteins. Moreover, it is possible that the carbohydrate components of flaxseed promote the microorganism growth.

Keywords: Linum Usitatissimum, Linseed, Flaxseed, Antimicrobial Activity, Protein Purification, Sephadex Gel Filtration, Ion Exchange, SDS-PAGE

1. Introduction

In the past two decades, a considerable increase of resistance in pathogenic bacteria against common antibiotics has emerged. Those bacteria such as *Escherichia coli, Salmonella sp, Kelebsiella sp,* although were susceptible to the usual antibiotic therapy sometime ago, have now made the main cause of challenging efforts in treating hospital infections (1). Rising bacterial resistance faster against synthetic antibiotics has turned attempts to search antibiotics from natural sources, again.

Among different categories of natural substances, proteinaceus materials isolated from plant sources have given promises in finding new natural compounds with good antimicrobial activities (refs 2-4). In this way many small proteins with some activity against microbial pathogens have been identified from plant seeds (5-7).

Flaxseed or linseed (*Linum usitatissimum* L.) cultivated worldwide has attracted people interest for its many benefits in human health (8, 9). Flaxseed contains lipid, fiber and protein as ingredients of its seed contents (9, 10). Another important component of flaxseed is mucilage which its composition presents a heterogeneous mixture of polysaccharides (10) The major product from flaxseed is oil which is rich in omega 3 fatty acids and helpful in preventing cardiovascular diseases and cancer (9,10). Since ancient time, the main usage of edible flaxseed has aimed on its oil, fiber and whole intact seed (9, 11). Traditionally, after extracting oil from flaxseed, the remaining meal with 35-40% protein content is used for livestock food (8, 12).

Accordingly, little attention has been paid on the other benefits of protein components of the seed.

There are only a few studies reported on the characteristics and activity of the protein contents of flaxseed. In one study, the seed proteins of flaxseed were extracted with water, buffer, urea and SDS, and analyzed by an array of electrophoretic techniques. Six major bands with MWs ranging from 55 to 41 KDa were detected. The majority of flaxseed proteins were reported to be legumin-like or albumin (13). Flaxseed proteins are rich in arginine and glutamine and so can be useful for preserving heart health and potentiating the immune system (14). In another attempt, studies on the isolated flaxseed proteins and related hydrolysate for inhibitory activity on angiotensin I-converting enzyme (ACEI activity), hydroxyl radical scavenging and bile acid binding behavior showed that intact proteins had only bile acid binding activity with no inhibitory effect on ACE or activity of OH scavenging. For the hydrolyzed proteins the reverse results were true (12). Some studies focused on the fungistatic activity of flaxseed protein against the growth of agronomically pathogens as well as that of the human pathogens (14, 15), which showed positive results. Since there was no report documented on the antibacterial activity of linseed proteins, as far as our library search shows, the present study were designed to investigate such information. The results may give another benefit from the other properties of flaxseed as well as a new hope to obtain antibacterial compounds from natural sources.

2. Materials and Methods

2.1. Materials

All chemicals and culture media were from the Merck and Sigma Co, Germany. The seeds were collected from the dried fruit of Flaxseed plant cultivated in the botanic garden belonging to the School of Pharmacy, Shahid Beheshti Univ. Med. Sci., Tehran, Iran. Column beds were obtained from Pharmacia Biotech., Uppsala Sweden. UV spectrometer Model 1501 was from Shimadzu manufacturer, Japan. The slab gel cast marked AE-6210 was provided from Atto Corporation, Japan. Molecular weight protein marker, SDS7 (14000-66000 Da) was purchased from Sigma-Aldrich Inc., UK. The 96-well micro-plate ELISA reader, spectra model was obtained from Tecan, Switzerland. Bacteria with various species employed were from the stock cultures of the Microbiological Lab., School of Pharmacy, Shahid Beheshti Univ. Med. Sci., Tehran, Iran.

2.2. Methods

2.2.1. Mucilage Removing

Flaxseed contains a considerable amount of mucilage in its seed coat which interferes with the process of protein extraction from flaxseed. Removing mucilage was performed according to Marambe et al (12), with some modification. In order to remove mucilage, the seeds (50 g) were washed with distilled water and treated with 0.5 M NaHCO3 (1:8 w/v, 40°C) with stirring for 1 hr. The seeds were then collected after filtration with several times washing with distilled water and dried by remaining at room temperature for 24h.

2.2.2. Oil Removing

The plant seed contains nearly 40% lipid (8). After removing mucilage, the dried seeds were ground by a general lab grinder and the resulting meal was stirred with n-hexane (1:5, w/v) for 6 h at 4°C, during which hexane was renewed every 2 h by paper filtration of the suspension, using Marambe et al procedure (12) with modification. Following final filtration, the treated meal was kept in dark to be air dried and then stored at 4° C.

2.2.3. Protein Extraction

After demucilaging and deoiling, flaxseed powder was brought into distilled water, and while stirring, the pH was adjusted to 8.5 with 0.1 M sodium hydroxide (8). The volume of suspension was then corrected with distilled water to have the powder to water ratio as 1:10(w/v). The suspension was stirred for 1h, followed by centrifugation at 4500 rpm for 30min to recover protein extract. The alkaline supernatant was separated and the pH was adjusted to 3.8 (by 0.1M HCl) to collect protein content as a precipitate by centrifugation (20 min, 4500 rpm) considering the corresponding approximate pI (16).The collected protein was once rinsed with distilled water to remove trace acid and stored in freezer (-70° C).

2.2.4. Column Chromatography

2.2.4.1. Gel Filtration

The protein pellet (500 mg) was dissolved in 10ml of 50mM Tris buffer (pH 7.5) and loaded on a Sephadex G-100 column (1.5× 70 cm), previously equilibrated with the same buffer. Using the same Tris buffer as eluent, 50 fractions were collected in 3 ml volumes at the flow rate of 30-40 ml/h. Monitoring by UV spectrometer at 280nm, two major absorbent peaks were detected and the corresponding fraction samples were combined (Fr A, Fr B). These fractions were individually treated with cold acetone (2:1, v/v acetone to buffer), and the precipitates were collected by centrifugation (4500 rpm for 20 min).and reserved in freezer (-70° C) for further experiments.

2.2.4.2. Ion-Exchange Chromatography

After preliminary tests on Fr A and Fr B for possessing any antibacterial activity, Fr B was chosen for further purification by ion-exchange chromatography. An anion-exchanger column DEAE Sephadex A50 (2×50 cm) was equilibrated by a two-fold of its volume with 50mM Tris buffer (pH 7.5). Fr B (100 mg, dissolved in Tris buffer) was loaded onto the column which was then washed with 300 ml Tris buffer to remove any unbound protein out of the column. Elution condition was constructed as a stepwise salt gradient using different NaCl concentrations

(0.25, 0.5, 0.75 and 1 M) in Tris buffer with equal volumes (each for 300 ml). Two fractions, B1 and B2, were collected according to the UV absorption data, monitoring at 280nm. The two sample fractions were again treated with cold acetone and the precipitates were stored at -70° C.

2.2.5. Detecting Purification and Determining Quantity of Sample Proteins

Sodium dodecyl sulphate-polyacrylamide gel electophoresis (SDS-PAGE) was carried out on the crude protein extract as well as those sample fractions collected from the two column chromatography experiments using Laemmli and Favere' procedure (17). Molecular mass marker proteins were employed to estimate molecular weights of protein samples. The protein content of each fraction and that of the crude extract were measured according to the Bradford method using BSA as the standard protein (18).

2.2.6. Assay of Antibacterial Activity

Antibacterial activity of flaxseed proteins was examined against several species of gram negative and gram positive bacteria using the 96-well microplate –based broth dilution method (19). Primary suspensions of bacteria were made as 2×10^5 cfu/ml (20) in two–fold concentrated sterile Mueller-Hinton Broth (MHB).

2.2.6.1. Preparation of Protein Samples

Protein samples were prepared by dissolving precipitated protein in Tris buffer (pH 7.5, 0.05M) followed by centrifugation (5 min, 4500 rpm) and then filtration through a Millipore membrane filter (0.22 µm).

2.2.6.2. Preparing Microtitre Plate

Rows A to H and Columns 1 to 11 of a microtitre plate were marked off.

From the primary protein extract as well as from the protein fraction Fr B1, which was collected from the final chromatography, 5 different concentrations were made using the relevant mother samples (500µg/ml), i.e; 400, 300, 200, 150 and 100µg/ml in Tris buffer. One sample concentration was used for wells of each row A to F (100µl per well). Wells of each columns 1 to 10 were allocated for one of 10 different bacterial species, a suspension of which, prepared as mentioned earlier, was added to the wells of related column (100µl per well), so that every bacterial suspension was one-time diluted and brought into the normal concentration of MHB in each well. Sterile MHB, as a two-fold concentrated, was added to the wells of column 11(100µl per well). This column was considered as a sterility control for MHB and protein samples. Sterile normal saline was added to row G (100µl per well); this row was assumed as a growth control for bacterial suspensions. Row H was added Amikacin (100µg/ml, 100µl per well); this row was purposed as a bactericidal standard control. The microplates, constructed as above, was then covered with a plastic bag in order to be protected from dehydration, and incubated at 37° C for 24 h. The absorption of microbial growth was then measured at 570nm by an ELISA reader. The above experiment was applied for Fr. A, Fr. B and Fr. B2 with just one sample concentration.

2.2.6.3. Calculating IC %

Determining the percentage of inhibitory concentration of flaxseed protein samples was performed according to the following formula;

$$\frac{ODw-(ODa-ODb)}{ODw}=\text{IC }\%$$

Where *ODw* is bacterial growth absorption control in each well of row G, *ODa* is the absorption of bacterial suspension facing different concentrations of flaxseed protein samples in each column, and *ODb* is the absorption of flaxseed protein samples in column 11.

3. Results

Flaxseed contains lipid and fibre along with protein as its seed ingredients (8). To prevent interference with the process of protein extraction from flaxseed, removing mucilage from the seed, which was performed several times, ended with about 7-8 % reduction of dried seed weight. Since lipoid substances also interfere with the solubility of protein in aqueous medium during isolation, removing lipid from the ground powder with n-hexane was performed in cold temperature. After several times of treatment, the final powder, when used for the next step of protein extraction to remove insoluble waste materials, gave a fairly clear solution in distilled water.

The antibacterial activities of flaxseed proteins as a total extract and those of the column chromatography-fractionated samples were examined against several species of G- and G+ bacteria. Inhibitory effect of flaxseed total protein extract on the growth of 10 bacterial species was determined as IC %, shown in Table 1. As can be seen the effect is more pronounced on *Salmonella typhimurium* (40 %), *Entrococcus foecalis* (45 %), *Escherichia coli* (40 % and 45 %) and *Klebsiella pneumonia* (ATCC: 10031, 38 %). Also some antibacterial activity appears on *Staphylococcus epidermidis* (19 %) and *Klebsiella pneumonia* (ATCC: 10603, 15 %). It can be noted that for these bacteria, by the reduction of protein concentration, the inhibitory effect on bacterial growth inversely increased (e.g., 22 % to 40 % by *reducing protein concentration* from 250µg/ml to 50µg/ml for *Salmonella typhimurium*). The steep slope of inhibitory effect was also rising sharper on some bacteria (e.g., *Klebsiella pneumonia* (ATCC: 10031, from 8 % to 38 %, in Table 1). Gel filtration of the total protein extract by G-100 column gave two fractions, Fr A and Fr B as the profile of UV absorbance at 280 nm demonstrated (Fig 1).Both fractions were employed for antibacterial activity test. Although Fr A did not show any noticeable result, but Fr B revealed good antibacterial activities on several bacteria species, almost

the same microorganisms as those previously mentioned above for the total protein extract (Tables 2 and 3). Here, the inhibitory effect also relatively increased by the first step fractionation. Further fractionation of Fr B by an ion-exchange chromatography produced an UV absorption profile with two peaks (Fig 2). When the relevant eluted samples (Fr B1 and Fr B2) were collected from the column and used for antibacterial tests, only Fr B1 showed a good activity (Tables 4 and 5). Again by reducing protein concentration, Fr B1 demonstrated increasing antibacterial effect on the sensitive bacteria, with the similar pattern to that of previous experiment performed on the total protein extract; although Fr B1 showed a relatively higher level of activity than its ancestor did (Compare Table 4 with Table 1). Fr B1 also showed a slightly better antibacterial activity than Fr B (Table 4 compared with Table 3). Gel electrophoresis, SDS-PAGE, run on the flaxseed total protein extract as well as the relevant fractions showed the step by step isolation of the active ingredient obtained from the flaxseed protein extract, by the protocol used (Fig. 3). From this slab gel the molecular weight of Fr B1 was estimated to be 13500 Da.

The abbreviations used for genus and species names are as follows: *S. a*., *Staphylococcus aureus*; *S. e*., *Staphylococcus epidermidis*; *S. t*., *Salmonella typhimurium*; *E. c*., *Escherichia coli*; *E. f*., *Entrococcus foecalis*; *K. p*., *Kelebsiella pneumoniae*; *M. l*., *Micrococcus luteus*; *P. a*., *Pseudomonas aeruginosa*.

Table 1: *Inhibitory effect of flaxseed total protein extract on the growth of bacterial species shown as IC %.*

Protein Concentration (µg/ml)	**250**	**200**	**150**	**100**	**75**	**50**	**Amikacin (100 µg/ml)**
Staphylococcus. aureus (ATCC: 6538) (IC %)	% 3	% 8	% 4	% 9	% 3	% 3	%100
Staphylococcus. epidermidis (ATCC: 12228) (IC %)	%3	%9	%12	% 14	% 15	% 19	%100
Salmonella typhimurium (ATCC: 14028) (IC %)	% 22	% 22	% 26	% 27	% 30	% 40	%100
Escherichia coli (ATCC: 8739) (IC %)	% 8	% 21	% 34	% 39	% 41	% 46	%100
Escherichia coli (ATCC: 8439) (IC %)	% 6	% 15	% 19	% 23	% 31	% 40	%100
Entrococcus foecalis (ATCC: 29212) (IC %)	% 16	% 20	% 21	% 30	% 37	% 45	%100
Klebsiella pneumonia (ATCC: 10031) (IC %)	% 8	% 11	% 22	% 31	% 35	% 38	%100
Klebsiella pneumonia (ATCC: 10603) (IC %)	% 8	% 10	% 11	% 13	% 13	% 15	%100
Micrococcus luteus (ATCC: 9341) (IC %)	% 3	% 4	% 2	% 4	% 3	% 4	%100
Pseudomonas aeruginosa (ATCC: 9027) (IC %)	%0.9	% 1	% 1	% 1	%0.7	% 1	%100

Table 2*: Inhibitory effect of fraction A isolated from flaxseed protein extract on the growth of bacterial species, shown as IC %.*

Concentration.	***S.a.* (IC %)**	***S.e* (IC %)**	***S.t.* (IC %)**	***E.c.* (8439) (IC %)**	***E.c.* (8739) (IC %)**	***E.f.* (IC %)**	***K.p.* (10031) (IC %)**	***K.p.* (10603) (IC %)**	***M.l.* (IC %)**	***P.a.* (IC %)**
120(µ g/ml)	%13	%12	%16	%0.8	%0.8	%8	%14	%12	%9	%2

Table 3: *Inhibitory effect of fraction B isolated from flaxseed protein extract on bacterial species, shown as IC %.*

Concentration.	***S. a.* (IC %)**	***S. e.* (IC %)**	***S .t.* (IC %)**	***E.c.* (8439) (IC %)**	***E.c.* (8739) (IC %)**	***E.f.* (IC %)**	***K. p.* (10031) (IC %)**	***K. p.* (10603) (IC %)**	***M. l.* (IC %)**	***P. a.* (IC %)**
60 (µg/ml) amikacin	%14	%16	%53	%51	%52	%52	%42	%21	%4	%0.6
100 (µg/ml)	%100	%100	%100	%100	%100	%100	%100	%100	%100	%100

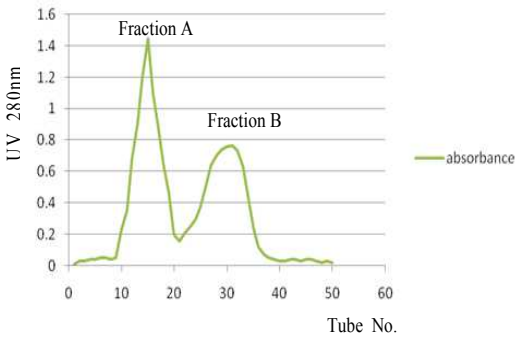

Fig 1*: UV profile for gel filtration (G-100) chromatography of the total protein extract of flaxseed.*

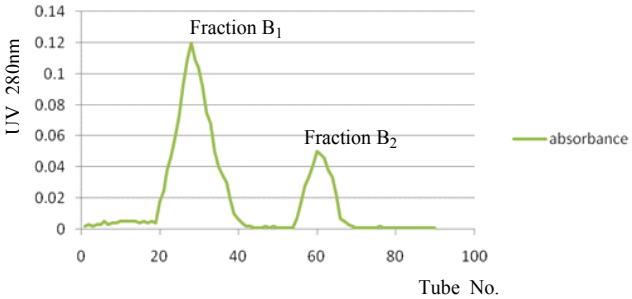

Fig 2*: UV profile for Ion-exchange chromatography of fraction B isolated from flaxseed protein extract.*

***Table 4**: Inhibitory effect of fraction B_1 isolated from flaxseed protein extract on the growth of bacterial species, shown as IC %.*

Concentration (µg/ml)	**250**	**200**	**150**	**100**	**75**	**50**	**amikacin (100)**
Staphylococcus aureus (IC %)	% 6	% 8	% 8	% 11	% 10	% 13	%100
Staphylococcus epidermidis (IC %)	% 9	% 11	% 13	% 14	% 14	% 16	%100
Salmonella typhimurium (IC %)	% 17	% 34	% 40	% 51	% 53	% 59	%100
Escherichia coli (8439) (IC %)	% 19	% 23	% 24	% 41	% 46	% 56	%100
Escherichia coli (8739) (IC %)	% 18	% 21	% 27	% 41	% 52	% 61	%100
Entrococcus foecalis (IC %)	% 20	% 26	% 34	% 44	% 54	% 61	%100
Klebsiella pneumonia (10031) (IC %)	% 20	% 21	% 22	% 24	% 36	% 46	%100
Klebsiella.pneumonia (100603) (IC %)	% 21	% 24	% 24	% 25	% 24	% 28	%100
Micrococcus. luteus (IC %)	% 2	% 2	% 4	% 5	% 3	% 4	%100
Pseudomonas aeruginosa (IC %)	% 0.4	% 1	% 1	% 0.6	%0.5	% 1	%100

***Table 5**: Inhibitory effect of fraction B_2 isolated from flaxseed protein extract on the growth of bacterial species, shown as IC %.*

Concentration.	***S. a.* (IC %)**	***S. e.* (IC %)**	***S .t.* (IC %)**	***E. c.* (8439) (IC %)**	***E. c.* (8739) (IC %)**	***E. f.* (IC %)**	***K. p.* (10031) (IC %)**	***K. p.* (100603) (IC %)**	***M. l.* (IC %)**	***P. a.* (IC %)**
(40 µg/ml)	%11	%10	%9	%5	%11	%6	%12	%13	%2	%2

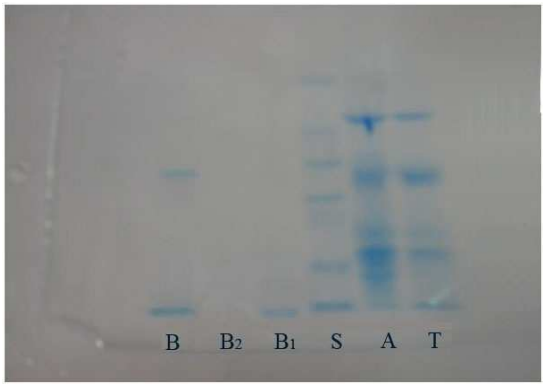

***Fig 3.** SDS-PAGE slab gel of flaxseed protein extract as well as the relevant isolated fractions .T; total extract, A: fraction A, B: fraction B, B_1: fraction B_1, B_2: fraction B_2, S: molecular mass marker proteins, SDS7 (from top to down: bovine albumin: 66000, egg albumin: 45000, glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle:36000, bovine carbonic anhydrase:29000, bovine pancreas tripsinogen:24000, soybean trypsin inhibitor:20100, bovine milk α-lactalbumin:14200).*

4. Discussion

Flaxseed contains dietary fibre (20-25 %), lipids (40-45 %) and protein (20-25 %) (9, 10). Fibre forms the main foodstuffs and includes soluble and insoluble substances. Insoluble fibre is made of cellulose, hemicellulose and lignin. Soluble fibre is composed of gum, sugar and pectin which form mucilage (8 % of dry weight) (8). Mucilage is obtained from aqueous extraction (10) and its compositions with high molecular weights may mask the behavior of the other macromolecules (21). Initially and before any protein isolation, it was necessary to remove oil as well as mucilage from the seed, since oil and especially mucilage severely interfere with the extraction and isolation of flaxseed protein (22). Mucilage, being a complex of polysaccharides, swells in aqueous medium and hinders protein isolation (23). Mucilage consists of two polysaccharide components, neutral and acidic. , which acidic part makes the major contribution (23, 24).Therefore, to remove mucilage the seeds were stirred in a warm alkaline solution (Sodium bicarbonate) for a period of time, followed by filtration and washing several times with distilled water (12).Oil extraction was performed with cold n-hexane on the dried ground meal in order to not damage the protein structure during oil removal. Protein extraction of flaxseed meal was carried out by dispersing the powder into an alkaline solution to dissolve protein content , and by centrifugation, a clear solution was brought to acidic pH to precipitate proteins accordingly (8, 16) . It should be mentioned that if de-oiling and de-mucilaging were not performed reasonably enough, the supernatant would not give a clear solution. Moreover, for further experiment, when such a precipitated protein sample was brought into buffer solution would give a turbid solution which could not pass through membrane filter in order to be sterilized and used for antibacterial tests. In designing microtitre-plate broth dilution method for antibacterial experiments, flaxseed protein samples were not serially diluted by MHB medium, but diluted with Tris buffer. This is because if protein sample dilution series were made by MHB medium, the final media of the plate well would give more nutrient content for more diluted protein samples and so more bacterial growth thus produced could make the interpretation of the results more complicated. Also, to fill the well with bacterial medium, MHB with two-fold concentrated was used in order to reach to the final MHB medium concentration in each plate well with an equal value to those of other wells. Also, by making an equal concentration of MHB medium in each well, the possibility of using flaxseed protein by microorganisms as a source of nutrient substance could be rejected because there were enough easily accessible hydrolyzed nutrients in MHB to be consumed by bacteria. In our experiments, antibacterial activity test for flaxseed proteins showed that with more diluted (decreased) protein extract concentration, more inhibition of bacterial growth was resulted specially for susceptible gram negative microorganisms. These may indicate that some hindering effect defer flaxseed protein

extract to be able to inhibit bacterial growth effectively. This phenomenon should not be very unusual as previous studies showed (4). For more isolated and purified protein samples, the inhibitory effect was increased accordingly, meanwhile the pattern of inhibition, i.e.; increased antibacterial activity versus decreased protein concentration, was repeated for the purified protein samples. This could be due to step by step dilution and removal of more mucilage from flaxseed protein content, which otherwise mask the behavioral (21), and so, antibacterial activity of the seed proteins. However, the mucilage content of flaxseed coat might not be removed completely from the proteins during extraction as well as column chromatography fractionation experiments. It should be mentioned that removing mucilage and oil from flaxseed protein extract has been a difficult task (16).

5. Conclusions

Flaxeed protein extract showed an antibacterial activity against the most test microorganisms specially gram negative bacteria. The activity was more pronounced (>50%) for the fractionated and isolated protein. Our experiment showed that the mucilage composition as carbohydrates would not contribute in inhibitory effect which some previous studies suggested (25). Despite that, carbohydrates may promote the microorganism growth as authors in the other report showed in their antifungal experiments (14). From our results it could be suggested that carbohydrate may, in fact, cover and inhibit antibacterial activity of flaxseed protein content.

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References

[1] Pelegrini PB, Murad AM, Silva LP, dos Santos RCP, Costa FT, Tagliari PD, Bloch Jr C, Noronha EF, Miller RNG and Franco OL. Identification of a novel storage glycine-rich peptide from guava (*Psidium guajava*) seeds with activity against gram-negative bacteria. *Peptides (2008) 29: 1271-1279.*

[2] Flores T, Alape-Giro A, Flores-Dı´az M, and Flores HE. Ocatin. A novel tuber storage protein from the Andean tuber crop Oca with antibacterial and antifungal activities. *Plant Physiology* (*2002) 128: 1291–1302.*

[3] Tavares LS, Santos MdeO, Viccini LF, Moreira JS, Miller RNG and Franco OL. Biotechnological potential of antimicrobial peptides from flowers. *Peptides (2008) 29: 1842-1851.*

[4] Houshdar Tehrani MH, Fakhrehoseini E, Kamali Nejad M, Mehregan H and Mojdeh Hakemi-Vala M. Search for proteins in the liquid extract of edible mushroom, *Agaricusbisporus*, and studying their antibacterial effects. *Iranian Journal of Pharmaceutical Research (2002) 11: 145-150.*

[5] Wang S, Ng TB, Chen T, Lin D, Wu J, Rao P and Ye X. First report of a novel plant lysozyme with both antifungal and antibacterial activities. *Biochemical and Biophysical Research Communications (2005) 327: 820–827.*

[6] Ribeiroa SFF, Carvalho AO, Cunha MD, Rodrigues R, Cruz LP, Melo VMM, Vasconcelos IM, Melo EJT and Gomes VM. Isolation and characterization of novel peptides from chilli pepper seeds: Antimicrobial activities against pathogenic yeasts. *Toxicon (2007) 50: 600–611.*

[7] Lin P and Ng TB. A novel and exploitable antifungal peptide from kale (*Brassica alboglabra*) seeds. *Peptides (2008) 29:1664-1671.*

[8] Rubilar M, Gutiérrez C, Verdugo M, Shene C and Sineiro J. Flaxseed as a source of functional ingredients. *Journal of Soil Science and Plant Nutrition (2010) 10(3):373-377.*

[9] Anjum FM, Haider FM, Khan MI, Sohaib M and Arshad MS. Impact of extruded flaxseed meal supplemented diet on growth performance, oxidative stability and quality of broiler meat and meat products. *Lipids in Health and Disease (2013) 12:13.*

[10] Gutiérrez C, Rubilar M, Jara C, Verdugo M, Sineiro J and Shene C. Flaxseed and Flaxseed cake as a source of compounds for food industry. *Journal of Soil Science and Plant Nutrition (2010) 10 (4): 454 – 463.*

[11] Chung MWY, Lei B and Li-Chan ECY. Isolation and structural characterization of the major protein fraction from NorMan flaxseed (*Linum usitatissimum* L.). *Food Chemistry (2005) 90: 271–279.*

[12] Marambe PWMLHK, Shand PJ and Wanasundara JPD. An In-vitro investigation of selected biological activities of hydrolysed Flaxseed (*Linum usitatissimum* L.) proteins. *Journal of the American Oil Chemists' Society (2008) 85:1155–1164.*

[13] Sammour RH. Proteins of linseed (*Linum usitatissimum* L.), extraction and characterization by electrophoresis. *Botanical Bulletin of Academia Sinica (1999) 40:121-126*

[14] Xu Y, Hall III C and Wolf-Hall C**.** Antifungal activity stability of Flaxseed protein extract using response surface methodology. *Journal of Food Science (2008) 73(1):M9-M14.*

[15] Borgmeyer JR, Smith CE and Huynh OK. Isolation and characterization of 25 kDa antifungal protein from Flaxseeds. *Biochemical and Biophysical Research Communications (1992) 187(1):480-87.*

[16] Kankaanpaa-Anttila B and Anttila M. Flax preparation, its use and production. *United State Patent (1999) No.; 5,925,401.*

[17] Laemmli UK and Favre M. Gel electrophoresis of proteins. *Journal of Molecular Biology (1973) 80: 575-599.*

[18] Bradford MM. Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry (1976) 72:248-54.*

[19] Kumarasamy Y, Cox PJ, Jaspars M, Nahar L and Sarker SD. Screening seeds of Scottish plants for antibacterial activity. *Journal of Ethnopharmacology (2002) 83: 73-77.*

[20] National Committee for Clinical Laboratory Standards (NCCLS). Methods for Dilution Antimicrobial Susceptibility Tests for Bacterial that Grow Aerobically. *NCCLS Publication, wayne (2004) Document M7-A6.*

[21] Yang L and Zhang L. Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. *Carbohydrate Polymers (2009) 76, 349–361*.

[22] Smith AK, Johnsen VL and Beckel AC) Linseed proteins alkali dispersion and acid precipitation. *Industrial & Engineering Chemistry (1946) 38(3):353–356.*

[23] Wanasundara PKJPD and Shahidi F. Removal of flaxseed mucilage by chemical and enzymatic treatments. *Food Chemistry (1997) 59(1):47-55.*

[24] Hunt K and Jones JKN. The structure of linseed mucilage. Part II. *Canadian Journal of Chemistry (1962) 40:1266-1279*.

[25] Guilloux K, Gaillard I, Courtois J, Courtois B and Petit, E**.** Production of Arabinoxylan-oligosaccharides from Flaxseed (*Linum usitatissimum*). *Journal of Agricultural and Food Chemistry (2009) 57:11308-11313*.