Annals of Medicine and Medical Research

Research Article
Published: 17 Dec, 2020

Bioactive Peptides as Antioxidant, Antibacterial, Anti-Diabetic, Antihypertensive, and as Anti-Cancerous Agents obtained from the Extract of *Poncirus trifoliata* Seeds

Ayaz Ahmad[1]* and Sahrish Khan[2]*

[1]Department of Biology, Govt. Degree College, Pakistan

[2]Department of Biotechnology, Quaid-e-Azam University, Pakistan

Abstract

The seeds of *Poncirus trifoliata* (Khatti) have 44.3% proteins and were selected as a source of bioactive peptides through the germination in this study. Bradford assay (protocol) was utilized to measure the protein contents in the aqueous extract of the sample plant. Antioxidant, antibacterial and anti diabetic pharmacological activities of the seed protein extract were tested through the set protocols. The protein extract of the seeds showed the antioxidant, antibacterial and anti diabetic activity and results came near the standard drugs. Antibacterial activity of the plant peptides which have rare contact with the human pathogenic organisms can combat the resistance against the antibiotics. It was observed that the bioactive peptides play dual role, it not only sensitize the cells for insulin also effect on the β cells, and enhance the production of insulin. Plant scientists are switching towards the natural sources which are safer, healthier and have therapeutic activity, because bioactive peptides are reported as antioxidant, antibacterial, anti diabetic, antihypertensive, and as anti cancerous agents.

Keywords: Bioactive peptides; Antioxidants; Antibacterial; Anti diabetic; Antihypertensive; Anti cancerous agents

OPEN ACCESS

Correspondence:
Ayaz Ahmad, Department of Biology, Govt. Degree College Block no. 17 D.G. Khan, Punjab, Pakistan, Tel: +923346789348; E-mail: ayaz_sarkani@yahoo.com
Sahrish Khan, Department of Biotechnology, Quaid-e-Azam University, Islamabad, Pakistan, E-mail: Sahrishkhan727@gmail.com

Received Date: *09 Nov 2020*
Accepted Date: *14 Dec 2020*
Published Date: *17 Dec 2020*

Citation:
Ahmad A, Khan S. Bioactive Peptides as Antioxidant, Antibacterial, Anti-Diabetic, Antihypertensive, and as Anti-Cancerous Agents obtained from the Extract of Poncirus trifoliata Seeds. Ann Med Medical Res. 2020; 3: 1032.

Introduction

Poncirus trifoliata

It is commonly known as (khatti). *Poncirus trifoliata* belongs to the family *Rutaceae*. The species is considered to belong to its own genus, *Poncirus*. The citrus fruits are abundantly grown in the different districts of Punjab, Pakistan. The seeds of *Poncirus trifoliata* are rich in proteins and we focused to study the pharmacological effects of the peptides extracted from the germinated seeds. Seeds were collected from the fruits in the month of February. Seeds germinated in moderate temperature 25°C to 30°C. We used the brown paper to grow the seeds and proper moisture was maintained for the germination of the seeds. The trick in germinating the seed that was used to get highest germination of the seeds was peeling out the seed coat of the seed that decreased the germination time and increase the germination rate. We mainly focused on the pharmacological activity of the bio peptides, used the simple method of extracting the protein from the germinated seeds. Water used as solvent for extraction. The extract of protein from the germinated seeds was applied to test the pharmacological activities like antioxidant, antibacterial and anti diabetic. In this regard first of all we estimated the protein concentration in the water extract of the germinated seeds with the help of the Bradford Assay, simple, rapid, relatively sensitive and inexpensive method for quantifying the protein concentration. Peptide and bioactive peptides are differentiated on the bases of the therapeutic or regulatory effect. The peptides those have therapeutic and regulatory effect in the body are classified as the bioactive peptides [1]. Grains, seeds and milk have proteins are the source of the bioactive peptides. These proteins can be converted into the peptides *in vitro* with the help of the proteolytic enzymes. The germinating seeds are also the source of peptides those are produced by the proteolytic enzymes which are activated during the germination of the seeds [2]. The resulted peptides may have therapeutic effect on the bases of these effects these peptides are called the bioactive peptides [3].

The biopeptides have shown the numerous pharmacological effects on the digestive, cardiovascular (antihypertensive), immune, endocrine (anti diabetic), and nervous system. The

bioactive peptides are separated from the different sources which come in existence by the action of proteolytic enzymes and exhibit their effect as antioxidants, antimicrobial, anticancer and as the immune modulator agents [4,5]. The protein present in the grains or seeds of those plants which are not still testified for their activities can provide breakthrough in this specific field of anti-diabetic therapy. There are also reported immune modulator and antihypertensive bioactive peptides. Bioactive peptides are emerging as the curing agent as they are showing the pharmacological activities in various pathological problems [6].

Material and Methods

Seed collection

Seeds were collected from the ripened fruit of *Poncirus trifoliata* from the District Mutan and Khanewal of province Punjab (Pakistan) from the garden of oranges (*Citrus cinencis*). Then the seeds were rinsed with the 0.1 sodium hypochlorite for 5 min and then washed with distilled water to remove the dirt [7,8]. The seeds of *Poncirus trifoliata* took long time to germinate, to reduce the germination time we applied the trick of removing seed coat and soaked them in the water for 5 h to 6 h and then transferred them on the sterile brown sheet. The seeds of *Poncirus trifoliata* took 8 to 14 days for growth. They do not grow at low temperature (winter season). The best time of their seedling is the month of April and fully ripened fruit of *Poncirus trifoliata* are also found at that time because the *Poncirus trifoliata* fruit is remained available for longer duration than the *Citrus cinensis* (orange). Seedling started at the 8th day when the seedling became prominent at the 10th day seeds were collected and referred to the cryogenic grinding, used the already chilled pestle and mortar. HCL 0.1 molar and 0.5 molar NaCl (pH alkali) for 2 h and then supernatant was collected [9]. The seeds by weight and volume of molar solution were used by 1:4 ratios. Then this supernatant is subjected to the 12000 rpm for 25 min and again the supernatant is collected and that supernatant is subjected to the dyeing binding method called the Bradford assay at 595 nm [10-17].

Results

The Bradford assay is utilized to measure the protein content in the aqueous extract of the *Poncirus trifoliata*. The stock solution (2 mg/ml) used to form following dilutions. Table 1 showing different concentrations of solutions used, Table 2 showing dilutions of aqueous extract of seeds were prepared to take the absorbance at 595 nm to fit in the graph of absorbance of different concentrations of the bovine serum albumin solution. Table 3 is showing the absorbance of different dilutions of stock solution of bovine serum albumin. The protein extraction is followed by the determination of the protein contents in the aqueous extract. We isolated the protein and then evaluated pharmacological activity of the extract. Simply used the aqueous protein extract to form the various dilution to find out the protein concentration. Table 4 showing the results of sample solution dilution and their absorbance were taken in triplicate on the bases of the concentration we have performed the antioxidant, antibacterial and anti-diabetic test. Dilution factor is 16 so the concentration of the protein extract solution was 12.8 mg/ml. Table 5 showing the absorbance of the extract different concentrations and percentage inhibition. Table 6 is showing the absorbance and percentage inhibition of different dilutions of ascorbic acid. Table 7 is showing the zone of inhibition of the aqueous extract of the *Poncirus trifoliata* in comparison of amoxicillin standard antibiotic. Minimum inhibitory concentration is 0.42. The dilution of 0.32 mg/ml did not show the zone of inhibition. The extract shows the convincing activity against the pathogenic bacteria rather the better effect as compare to standard antibiotic amoxicillin. The LOW minimum inhibitory concentration

Table 1: The different concentrations of solutions used.

S No.	Concentration (Mg/ml)	Stock solution vol (ml)	Distilled water vol (ml)	Final vol (ml)
1	0.12	0.2	3.7	4
2	0.25	0.5	3.5	4
3	0.5	1	3	4
4	0.75	1.5	2.5	4
5	1	2	2	4
6	1.5	3	1	4
7	2	4	0	4

Table 2: The dilutions of aqueous extract of seeds were prepared to take the absorbance at 595 nm to fit in the graph of absorbance of different concentrations of the bovine serum albumin solution.

S No.	Extract solution vol (ml)	Dilution factor	Distilled water vol (ml)	Final vol (ml)
1	0.5	8	3.5	4
2	0.25	16	3.7	4
3	0.25	24	5.7	6

Table 3: The absorbance of different dilutions of stock solution of bovine serum albumin. The protein extraction is followed by the determination of the protein contents in the aqueous extract. We isolated the protein and then evaluated pharmacological activity of the extract. Simply used the aqueous protein extract to form the various dilution to find out the protein concentration.

S No.	Concentration (Mg/ml)	Absorbance 595 nm
1	0.12	0.55
2	0.25	0.56
3	0.5	0.61
4	0.75	0.67
5	1	0.73
6	1.5	0.84
7	2	0.97

Table 4: The results of sample solution dilution and their absorbance were taken in triplicate.

S No.	Sample solution vol (ml)	Dilution factor	Distilled H_2O (ml)	Final vol (ml)	Absorbance 595 nm
1	0.5	8	3.5	4	1.16
2	0.25	16	3.8	4	0.68
3	0.25	24	5.8	6	0.21

Table 5: The absorbance of the extract different concentrations and percentage inhibition.

S No.	Conc. of extract µg/ml	Absorbance of extract 517 nm	Percentage inhibition=Ctrl-abs/ctrl × 100
1	8	0.15	42.43
2	16	0.14	45.32
3	25.6	0.14	47.96
4	32	0.13	49.96
5	42.6	0.12	52.88
6	64	0.11	59.57
7	106	0.18	69.6
8	128	0.06	78.29

Table 6: The absorbance and percentage inhibition of different dilutions of ascorbic acid.

S No.	Conc. of ascorbic acid µg/ml	Absorbance 517 nm	Percentage inhibition= Ctrl-abs/ctrl × 100
1	10	0.14	45.5
2	20	0.13	49
3	30	0.12	51.42
4	40	0.12	53.84
5	50	0.11	56.26
6	60	0.1	59.76
7	70	0.09	64.18
8	80	0.08	66.83
9	90	0.08	69.21
10	100	0.07	71.59

Table 7: The zone of inhibition of the aqueous extract of the *Poncirus trifoliataa* in comparison of amoxicillin standard antibiotic.

S No.	Antibacterial solutions	*E. coli*	*S. aureus*	*B. subtilis*
1	Amoxicillin (1 mg/ml)	17 mm	14 mm	15 mm
2	Extract (1.28 mg/ml)	18 mm	15 mm	16 mm
3	Extract(0.64 mg/ml)	12 mm	10 mm	12 mm
4	Extract (0.42 mg/ml)	5 mm	4 mm	5 mm

Table 8: The results were taken by using the Accu-Check glucometer.

Groups	Day 0	Day 15	Day 30
Group 1	77.8 ± 2.70	79.75 ± 2.50	76.30 ± 2.60
Group 2	195.58 ± 4.20	152.60 ± 4.66	141.32 ± 3.27
Group 3	196.8 ± 5.65	147.40 ± 4.35	130.65 ± 3.75

also shows that the extract is potent. The protein extract of seeds show the good antioxidant and anti-diabetic activity but the best of them was the antibacterial activity. Table 8 the results were taken by using the Accu-Check Glucometer. The anti-diabetic activity of the seed protein extract shows the convincing activity in controlling the blood glucose level.

Conclusion

In our study it was concluded that the orange seeds or *Poncirus trifoliate* seeds which have the high contents of the protein can be utilized for therapeutic purposes. The seeds can be collected in the huge amount from the juice forming industries and even if we motivate the people can be collected from the consumers. As the research data is proving that the germinated seeds protein extract of *Poncirus trifoliata* having tremendous effect against the free radicals as an antioxidant and antibacterial activity against the pathogenic strains. Combating against the resisting bacteria can be predicted more surely when the bioactive peptide sourced seed not used as edible source and no work is performed in the context of bioactive peptides. On the bases of these results we concluded that bioactive peptides from *Poncirus trifoliata* not only helpful in controlling the blood glucose level even they can enhance the production of insulin from the β cells of pancreas and improving the sensitivity of the cells for insulin. *Poncirus trifoliata* protein extract showed the remarkable antioxidant, antibacterial and anti-diabetic activity.

References

1. Aliahmadi A, Roghanian R, Emtiazi G, Mirzajani F, Ghassempour A. Identification and primary characterization of a plant antimicrobial peptide with remarkable inhibitory effects against antibiotic resistant bacteria. African J Biotech. 2012;11(40):9672-6.
2. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.
3. Chaudhury A, Duvoor C, Dendi VSR, Kraleti S, Chada A, Ravilla R, et al. Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. Front Endocrinol. 2017;8:6.
4. Chi CF, Hu FY, Wang B, Li T, Ding GF. Antioxidant and anticancer peptides from the protein hydrolysate of blood clam (*Tegillarca granosa*) muscle. J Functional Foods. 2015;15:301-13.
5. Egorov AM, Ulyashova MM, Rubtsova MY. Bacterial enzymes and antibiotic resistance. Acta Naturae. 2018;10(4);33-48.
6. Giuliani A, Pirri G, Nicoletto S. Antimicrobial peptides: An overview of a promising class of therapeutics. Open Life Sciences. 2007;2(1):1-33.
7. Hall DG, Ammar ED, Bowman KD, Stover ED. Epifluorescence and stereomicroscopy of trichomes associated with resistant and susceptible host plant genotypes of the Asian citrus psyllid (Hemiptera: Liviidae), vector of citrus greening disease bacterium. J Microsc Ultrastruct. 2018;6(1):56-63.
8. Hatanaka T, Uraji M, Fujita A, Kawakami K. Anti-oxidation activities of rice-derived peptides and their inhibitory effects on dipeptidylpeptidase-IV. Int J Peptide Res Therap. 2015;21(4):479-5.
9. Kahanovitz L, Sluss PM, Russell SJ. Type1 Diabetes - A clinical perspective. Point Care. 2017;16(1):37-40.
10. Lambert PA. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. J R Soc Med. 2002;95(41):22-6.
11. Maiti D, Majumdar M. Impact of bioprocessing on phenolic content and antioxidant activity of spy seed to improve hypoglycemic functionality. Asian J Plant Sci Res. 2012;2(2):102-9.
12. Nongonierma AB, FitzGerald RJ. Susceptibility of milk protein-derived peptides to Dipeptidyl Peptidase IV (DPP-IV) hydrolysis. Food Chemistry. 2014;145:845-2.
13. Otto M. *Staphylococcus aureus* toxins. Curr Opin Microbiol. 2014:17:32-7.
14. Pan X, Zhao YQ, Hu FY, Wang B. Preparation and identification of antioxidant peptides from protein hydrolysate of skate cartilage. J Functional Foods. 2016;25:220-30.
15. Salman MT, Khan RA, Shukla I. Antibacterial activity of *Nigella sativa* Linn. Seeds against multiple antibiotics resistant clinical strains of *Staphylococcus aureus*. Int Arch Biomed Clin Res. 2016;2(2):96-9.
16. Taveira GB, Carvalho AO, Rodrigues R, Trindade FG, Da Cunha M, Gomes VM. Thionin-like peptide from Capsicum annuum fruits: Mechanism of action and synergism with fluconazole against Candida species. BMC Microbiol. 2016;16(1):12.
17. Zhang Y, Chen R, Ma H, Chen S. Isolation and identification of dipeptidyl peptidase IV-inhibitory peptides from trypsin/chymotrypsin-treated goat milk casein hydrolysates by 2D-TLC and LC–MS/MS. J of Agric Food Chem. 2015;63(40):8819-28.