Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6 suppl 2, 2014

Research Article

ANTIOXIDANT ACTIVITY OF PROTEINS FROM FIFTEEN VARIETIES OF LEGUME SEEDS COMMONLY CONSUMED IN INDIA

C. PETCHIAMMAL, WAHEETA HOPPER*

Department of Bioinformatics, School of Bioengineering, Faculty of Engineering & Technology, SRM University, Kattankulathur, 603203, Tamil Nadu, India. Email: hod.bioinfo@ktr.srmuniv.ac.in; srmbioinforesearch@gmail.com

Received: 03 Dec 2013, Revised and Accepted: 04 Feb 2014

ABSTRACT

Objective: To evaluate the *in vitro* antioxidant potential of commonly consumed fifteen legume seeds.

Methods: The antioxidant potential of the fifteen legume seed proteins was evaluated by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, ferric reducing power and reducing power assays. The total protein content of legume seeds was analysed.

Results: Among the legumes tested, high total protein content and the DPPH scavenging activity were observed in horse gram (black), cowpea (brown), horse gram (brown), common bean (light pink and brown), masur (black) and green gram. The ferric reducing power activity was high in horse gram (black and brown), cowpea (brown), common bean (light pink and brown) and masur (black). Horse gram (black and brown) and cowpea (brown) exhibited high reducing power activity than the other legumes.

Conclusion: The study suggests that horse gram (black and brown), cowpea (brown), common bean, masur (black) and green gram have high protein content and good antioxidant activity. These legume seed proteins could be used as natural antioxidant, functional food and therapeutics for the benefit of human health.

Keywords: Legume seeds, Total protein content, Antioxidant activity.

INTRODUCTION

The legumes were used as therapeutic agents in the old traditional medicine. In Mediterranean regions, the grain legumes have traditionally been consumed for centuries and constitute an element of the Mediterranean diet [1]. The natural food antioxidants are readily accepted by consumers and do not require safety tests if they are components of food and are generally recognized as safe (GRAS) [2]. Legumes belong to the family leguminosae, one of the most important families in Dicotyledons, including around 700 genera and 20,000 species. Legumes are the second most important source of food and fodder, green manures and forages. In comparison of cereal grains, legumes are good source of proteins, dietary fibers, low glycemic indexes, low levels of fat (2-5%), and high amounts of carbohydrates (55-60%) [3]. Proteins can act as free radical reducing agents, metal ion chelators, free radical scavengers and thus prevent oxidative damage to biomolecules, such as DNA, lipids and proteins [4].

Recently, plant based proteins are increased in demand as food and hence, there are more studies on functional proteins from legumes such as chickpea, common beans, lentil, cowpea, lupins, pea and broad beans as alternative to soybean [5, 6, 7). The epidemiological evidence indicated that the consumption of dietary antioxidant such as legume seed proteins provided protective effects for several chronic diseases like cardiovascular diseases, cancer, obesity and diabetes, hypercholesterolemia [8, 9, 10]. The consumption of legumes (soy, beans, and peas) in Asian populations is excessive than the western populations [11]. The legumes are rich source for lysine and tryptophan but low in sulphur-containing amino acids, methionine and cysteine. Plant proteins are cheaper than the animal proteins; therefore, the people consume legume seeds worldwide as major source of protein and especially in the developing or poor countries were consumption of animal protein may be limited as a result of economics, social, cultural, or religious factors [12]. Recently, natural proteins as antioxidant antioxidants have attracted interest. Milk proteins such as lactoferrin and casein, soy proteins, mushroom proteins, egg albumin protein, maize zein, potato patatin and yam discorin have antioxidant activity [13, 14, 15 and 16]. The healthy diet including edible antioxidants can help human body to neutralize the free radicals and reduce the oxidative stress diseases [17]. The proteins owe their antioxidant activity to their constituent amino acids. The antioxidant activities of aromatic amino acids such as tyrosine, phenylalanine and tryptophan and the sulphur-containing amino acid, cysteine, are due to their ability to donate protons to free radicals [18].

MATERIALS AND METHODS

Folin-Ciocalteu's phenol regent, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyl-s-triazine (TPTZ), bovine serum albumin were obtained from Sigma. All other chemicals/reagents and solvents used were of analytical grade.

Isolation of proteins from legumes

The legume seeds were collected from Tamil Nadu, India. The seeds were dried and made in to coarse powder. Protein isolation from was carried out according to the method described by Zhang et al [19]. The legume seeds were defatted with hexane in soxhlet apparatus for 6-12 hrs. The defatted flour was dried and protein was obtained by dispersing the flour in distilled water in the ratio of 1:10 (w/v). The pH was adjusted to 8.5 with 1N NaOH solution. The mixture was centrifuged at 5000g for 20 min at 4°C. The supernatant was adjusted to isoelectric point of pH 4.5 with 1N HCl, the precipitate formed was collected by centrifugation at 8000g at 4°C for 15 min. The pellet was lyophilized and stored at -20°C

Determination of protein content of the legumes

The protein content of lyophilized sample was determined by Folin-phenol method [20] using bovine serum albumin as standard.

In vitro antioxidant activity assays

DPPH Radical Scavenging Assay

The radical scavenging effect of legume proteins on DPPH free radical was measured according to the procedure previously reported [21]. To 1 mg/ml of legume samples, 2 ml of 0.1 mM of DPPH in 95% ethanol was added; the solution was mixed well and kept for 45 min at room temperature. The absorbance of the solution was measured at 517 nm. Ascorbic acid was used as the positive control. A lower absorbance represents a higher DPPH radical scavenging activity. The scavenging effect was expressed by the following equation:

DPPH radical scavenging activity (%) = (Ac – As)/ (Ac) x 100

(Ac) - Absorbance of the Control, (As) - Absorbance of the Sample

Reducing Power Assay

Reducing power of the legume protein was estimated according to Duh et al [22]. To 2.5 ml of legume sample (1mg/ml) in phosphate buffer (0.2 M, pH 7.6), 2.5 ml of 1% potassium ferricyanide was added. The reaction mixture was incubated at 50°C for 20 min, equal volume of 10% TCA was added to the reaction mixture, mixed by vortex and centrifuged at 1000 rpm for 10 min at 4°C. Supernatant, 2.5 ml, was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% of $FeCl_3$. The mixture was kept for incubation at room temperature for 30 min. The absorbance of the solution was measured at 700 nm. Ascorbic acid was used as standard and reducing power activity was expressed as µg AAE/mg of sample and increasing absorbance indicates greater reducing power.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out according to Benzie [23]. The method is based on the ability of the sample to reduce Fe^{3+} to Fe^{2+} ions. In the presence of the TPTZ, the Fe^{2+}-TPTZ complex exhibits a blue colour which is read at 593 nm. Legume samples 1mg/ml, was allowed to react with 2 ml of FRAP solution for 30 min at room temperature and the absorbance was measured at 593 nm. Ascorbic acid was used as standard and FRAP activity was expressed as µg AAE/mg of sample and increasing absorbance of sample indicates greater ferric reducing antioxidant power.

Statistical analysis

All experiments were performed in triplicates. All results were expressed as means ± standard deviation.

RESULTS AND DISCUSSION

Protein content in legumes

The legumes screened for antioxidant activity are listed in Table, 1. Traditionally, dietary protein is regarded as a source of energy and essential amino acids, which are needed for growth and maintenance of physiological functions for human. High protein content 18.74 - 24.94 %, was observed in horse gram (brown), common bean (light pink and brown), masur (black), cowpea (brown), horse gram (brown), masur (red), black gram and chick pea (green) (Table 2). Low level of protein content (15.35 - 17.12) was seen in chick pea (white and brown), cowpea (white), pea (white and green), green gram (Table 2). Hedley [24] reported the protein content of common bean (20.9 - 29.2 %), pea (18.3 - 31 %) and lentil (23 - 32 %). Protein content of lentil (25.1 - 29.2 %), chick pea (15.5 - 28.2 %) [25], cowpea (23.5-24.8%) [26] was reported.

Table 1 List of legumes for protein content and antioxidant activity

Common name	Synonyms	Botanical name	Indian name
Lentil (red)	Lentil	*Lens culinaris*	Masur
Lentil (Black)	Lentil	*Lens culinaris*	Masur
Pea (green)	Garden pea, Field pea	*Pisum sativum*	Mater vatana
Pea (white)	Garden pea, Field pea	*Pisum sativum*	Mater vatana
Chickpea (green)	Bengal gram, Indian pea	*Cicer arietinam*	Chenna
Chickpea (brown)	Bengal gram, Indian pea	*Cicer arietinam*	Chenna
Chickpea (white)	Bengal gram, Indian pea	*Cicer arietinam*	Chenna
Black gram (black)	Urad bean, Black bean	*Vigna mungo*	Urad
Green gram (green)	Green bean, Mung bean	*Vigna radiata*	Mung bean
Common bean (dark brown)	Kidney bean, French bean	*Phaseolus vulgaris*	Rajmah
Common bean (light pink)	Kidney bean, French bean	*Phaseolus vulgaris*	Rajmah
Cowpea (White)	Black eyed pea	*Vigna unguiculata*	Lobia
Cowpea (Brown)	Black eyed pea	*Vigna unguiculata*	Lobia
Horse gram (black)	*Dolichos biflorus*	*Macrotyloma uniflorum*	Muthira
Horse gram (brown)	*Dolichos biflorus*	*Macrotyloma uniflorum*	Muthira

Antioxidant activity of proteins

DPPH assay is based on an electron transfer reaction and hydrogen-atom abstraction and measurement of the reducing ability of antioxidants towards DPPH. High DPPH scavenging activity was observed in horse gram (black) 56.5%, cowpea (brown) 54.4 %, horse gram (brown) 53.3 %, common bean (light pink and dark brown) 39 % , 37.1%, masur (black) 34.7% and green gram 33.9 % (Table 2). Oboh [27] reported that *Cajanus cajan* (brown), *Sphenostylis sternocarpa* (brown) and *Vigna unguiculata* (brown) showed high scavenging activity than *Vigna unguiculata* and *Cajanus cajan* (white). High free radical scavenging ability and redox potential compared to other legumes could be attributed to their higher total protein content. The FRAP assay was originally developed by Benzie [23], to measure reducing power, the assay subsequently has been adapted and used for the assay of antioxidants in botanicals. High ferric reducing power activity was observed in horse gram (black and brown) 22.5 ± 0.09; 20.7 ± 0.21µg AAE/mg, cowpea (brown) 22.6 ± 0.02 µg AAE/mg, common bean (light pink and brown) 19.0 ± 0.107; 18.9 ± 0.08 µg AAE/mg and masur 13.7 ± 0.15 µg AAE/mg (black) (Table 2). Low range ferric reducing activity was exhibited in green gram 12.6 ± 0.28 µg AAE/mg, black gram 11.8 ± 0.17 µg AAE/mg and chickpea (green and brown) 8.89 ± 0.06 µg AAE/mg; 7.16 ± 0.15 µg AAE/mg. Very low ferric reducing power activity was observed in cowpea (white), pea (white), masur (red), and chick pea (white).

High reducing power activity was seen in horse gram (brown and black) 50.4 ±0.85; 45.4 ±0.75 µg AAE/mg, cowpea (brown) 40.5 ±0.62 µg AAE/mg, masur (black) 37.2 ±0.50 µg AAE/mg, common bean (light pink and brown) 28.5 ±1.22; 28.4 ±0.5 µg AAE/mg, black gram 14.9 ±0.69 µg AAE/mg and green gram 14.8 ±0.9 µg AAE/mg (Table 2). Other legumes, chick pea (green, brown and white), pea (green), cowpea (white), masur (red) and pea (white) showed low reducing power activity. Number of antioxidant compounds have been isolated and characterized from dietary sources as effective radical scavengers. An antioxidant protein (32kDa) has been isolated from *Olanum torrum* seeds and antioxidant protein (35kDa) has been isolated from curry leaves (*Murraya koenigii* L) [28, 29]. Turmerin (14kDa), an antioxidant protein from turmeric (*Curcuma longa*) was effective as antioxidant and anti inflammatory [30]. Many vegetable proteins have comparatively strong antioxidant activities than soybean protein [31]. Oboh [27] reported that cowpea (brown) and common beans (brown and light pink) have good reducing power activity. Phenolic content of common bean and faba bean legumes have good antioxidant activity [32, 33]. Allhorn et al [34] reported that reducing property can be an antioxidation defense mechanism; this is possibly through the ability of the antioxidant compound to reduce transition metals.

This study suggests that legume seeds with high protein content, as in horse gram (brown and black), cowpea (brown), common bean (brown and light pink) and masur (black), also exhibited good DPPH scavenging activity, ferric reducing and reducing power activity.

CONCLUSIONS

Legume seed proteins exhibit free radical scavenging capacities. Horse gram (brown and black), cowpea (brown), common bean and masur (black) showed high protein content and also exhibited good DPPH scavenging activity, ferric reducing and reducing power activity. Comparatively, pea (white and green) and chick pea (white, green, brown) showed less antioxidant activity.

As legumes have high protein content with high antioxidant activity they can be used as a food supplement and natural antioxidant. Legume proteins are useful as therapeutics for health benefits of human.

ACKNOWLEDGEMENT

The authors thank to SRM University for the financial support and providing facilities.

Table 2 Total Protein content and antioxidant activities of 15 legume seeds

Plant name	DPPH scavenging Activity (%)	FRAP (µg AAE/mg of sample)	Reducing power Activity (µg AAE/mg of sample)
Lens culinaris (red)	16.1	3.49 ± 0.05	1.27 ± 0.70
Lens culinaris (black)	34.7	13.70 ± 0.15	37.20 ±0.50
Pisum sativam (green)	11.4	2.56 ± 0.08	2.05 ±0.63
Pisum sativam (white)	16.9	2.57 ± 0.06	0.61 ±0.41
Cicer arietinum (green)	12.3	8.89 ± 0.06	10.50 ±0.68
Cicer arietinum (brown)	14.6	7.16 ± 0.15	9.03 ±0.37
Cicer arietinum (white)	11.3	2.33 ± 0.05	3.50 ±0.16
Vigna mungo (black)	22.7	11.80 ± 0.17	14.90 ±0.69
Vigna radiata (green)	33.9	12.60 ± 0.28	14.80 ±0.90
Phaseolus vulgaris(brown)	39.1	18.90 ± 0.08	28.40 ±0.50
Phaseolus vulgaris(light pink)	35.0	19.00 ± 0.11	28.50 ±1.22
Vigna unguiculata (white)	11.1	4.92 ± 0.09	1.30 ±1.20
Vigna unguicualta (brown)	54.4	22.60 ± 0.02	40.50 ±0.62
Macrotyloma uniflorum (black)	53.3	20.70 ± 0.21	45.40 ±0.75
Macrotyloma uniflorum (brown)	56.5	22.50 ± 0.09	50.40 ±0.85
Ascorbic acid	86.1	22.60 ± 0.007	98.30 ± 0.55

The values presented mean ± SD

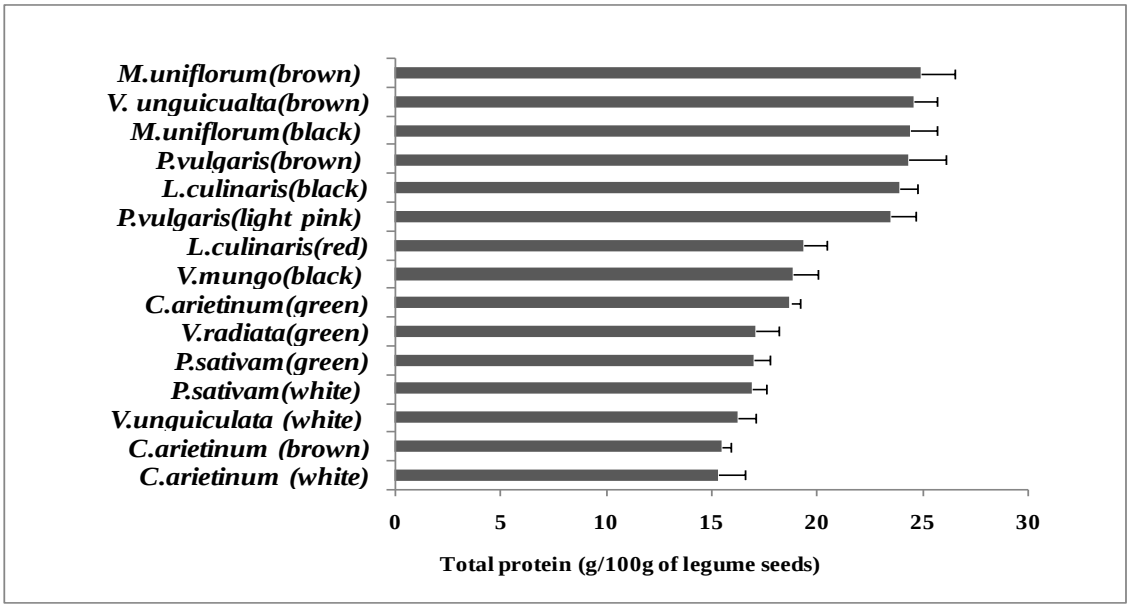

Fig. 1: Total protein content of legume seeds

REFERENCES

1. Guarrera PM. Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium). Fitoterapia 2005; 76(1): 1-25.
2. Rajalakshmi D and Narasimhan S. Food antioxidants; sources and methods of evaluation. In D.L Madhavi, S.S. Deshpande and D.K. Salunkhe (eds), Food Antioxidants. Marcel Dekker, New York. 1996; p. 65-83.
3. Rochfort S and Panozzo J. Phytochemicals for health, the role of pulses. J Agric Food Chem 2007; 55(20): 7981-7994.
4. Rice-Evans CA, Miller JM, and Paganga G. Structure antioxidant activity relationship of flavonoids and phenolic acids. Free Radic Biol Med 1996; 20: 933-956.
5. Bamdad F, Goli AH and Kadivar M. Preparation and characterization of proteinous film from lentil (*Lens culinaris*):

Edible film from lentil (*Lens culinaris*). Food res Int 2006; 39(1): 106-111.

6. Horax R, Hettiarachchy NS, Chen P and Jalaluddin M. Preparation and characterization of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). J Food sci 2004; 69(2): 114-118.
7. Makri E, Papalamprou E, and Doxastakis G. Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, pea, and broad bean) in admixture with polysaccharides. Food Hydrocolloids 2005; 19(3): 583-594.
8. Chau CF, Cheung PCK and Wong YS. Hypocholesterolemic effects of protein concentrate from three Chinese indigenous legume seeds. J Agric Food Chem 1998; 46: 3698-3701.
9. Troszynska A, Estrella I, Lopez-Amores ML and Hernandez T. Antioxidant activity of pea (*Pisum sativum* L) seed coat acetone extract. LWT Food Sci Technol 2002; 35: 158-164.
10. Castle EP and Thrasher JB. The role of phytoestrogen in prostate cancer. Urol Clin North Am 2002; 29: 71-81.
11. Messina M, McCaskill-Stevens W and Lampe JW. Addressing the soy and breast cancer relationship: review, commentary, and workshop proceedings. J Natl Cancer Inst 2006; 98(18): 1275-1284.
12. Romani AP, Vignolini C, Galardi N, Mulinacci S, Benedettelli and Heimler. Germplasm characterization of zolfino landraces (*Phaseolus vulgaris* L.) by flavonoid content. J Agric Food Chem 2004; 52: 3838-3842.
13. Chiue H, Kusano T and Iwami K. Deamidation-induced fragmentation of maize zein, and its linked reduction in fatty acid-binding capacity as well as antioxidative effect. Food Chem 1997; 58(1): 111-117.
14. Hou WC, Lee MH, Chen HJ, Liang WL, Han CH, Liu YW and Lin YH. Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas Decne*) tuber. J Agric Food Chem 2001; 49(10): 4956-4960.
15. Liu YW, Han CH, Lee MH, Hsu FL and Hou WC. Patatin, the tuber storage protein of potato (*Solanum tuberosum* L.), exhibits antioxidant activity in vitro. J Agric Food Chem 2003; 51(15): 4389-4393.
16. Zhao L, Zhao G, Hui B, Zhao Z, Tong J and Hu X. Effect of Selenium on Increasing the Antioxidant Activity of Protein Extracts from a Selenium-enriched Mushroom Species of the *Ganoderma* Genus. J Food Sci 2004; 69(3): 184-188.
17. Beidokhti MN and Prakash HS. Antioxidant and anti inflammatory potential of selected medicinal plants of Lamiaceae family. Int J Pharm Pharm Sci 2012; 5:100-104.
18. Rajapakse NE, Mendis WK, Jung JY, Je, Kim SK. Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties. Food Res Int 2005; 38: 175-182.
19. Zhang T, Jiang B and Wang Z. Gelation properties of chickpea protein isolates. Food Hydrocolloids 2007; 21: 280-286.
20. Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193(1): 265-275.
21. Yu L, Haley S, Perret J, Harris M, Wilson J and Qian M. Free radical scavenging properties of wheat extracts. J Agric Food Chem 2002; 50(6): 1619-1624.
22. Duh PD, Yen GC, Yen WJ and Chang LW. Antioxidant effects of water extracts from barley (*Hordeum vulgare L.*) prepared under different roasting temperatures**.** J Agric Food Chem 2001; 49: 1455-1463.
23. Benzie IF. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Meth Enzymol 1999; 299: 15-27.
24. Hedley CL. Introduction in Carbohydrates in grain legume seeds. Hedley CL [ed.], CABI Publishing, New York, 2001; P 1-11.
25. Wang N, Hatcher DW, Toews R and Gawalko EJ. Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). LWT-Food Sci Technol 2009; 42(4): 842-848.
26. Kabas O, Yilmaz E, Ozmerzi A and Akinci I. Some physical and nutritional properties of cowpea seed (*Vigna sinensis* L.). J Food Eng 2007; 79(4): 1405-1409.
27. Oboh G. Antioxidant properties of some commonly consumed and underutilized tropical legumes. Eur Food Res Technol 2006; 224(1): 61-65.
28. Sivapriya M and Leela S. Isolation and purification of a novel antioxidant protein from the water extract of Sundakai (*Solanum torvum*) seeds. Food Chem 2007; 104(2): 510-517.
29. Ningappa MB and Srinivas L. Purification and characterization of 35kDa antioxidant protein from curry leaves (*Murraya koenigii*. L.). Toxicol *in vitro* 2008; 22(3): 699-709.
30. Chethankumar M and Srinivas L. New biological activity against phospholipase A2 by Turmerin, a protein from *Curcuma longa* L. Biol chem 2008; 389(3): 299-303.
31. Mesa MD, Silván JM, Olza J, Gil Á, and del Castillo MD. Antioxidant properties of soy protein-fructooligosaccharide glycation systems and its hydrolyzates. Food Res Int 2008; 41(6): 606-615.
32. Sreeramulu D, Reddy CVK and Raghunath M. Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India. Indian J Biochem Biophys 2009; 46(1): 112-115.
33. Latha S and Daniel M. Phenolic antioxidants of some common pulses. J Food Sci Technol 2001; 38(3): 272-273.
34. Allhorn M, Klapyta A and Akerstrom B. Redox properties of the lipocalin alpha1-microglobulin: reduction of cytochrome c, hemoglobin, and free iron. Free Radic Biol Med 2005; 38: 557-567