Polyphenols and Tannins in Horse Gram a Lesser Known Legume from a Drought Prone Area of Maharashtra

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Abstract

Horse gram is lesser known legume grown in some parts of world. The plant can withstand adverse climatic conditions. It is rich in protein, minerals, and vitamins. Apart from this it has range of medicinal properties and reported as a effective neutraceutical legume due to presence of anti-nutritive substances. Horse gram has been already in use as a traditional medicine for the treatment of kidney stones, urinary diseases, piles, common cold, throat infection, fever, intestinal diseases, diabetes, coronary heart disease, dental problems etc. In recent years, isolation and utilization of potential antioxidants from legumes including horse gram are increased as it decreases the risk of several diseases. In the present study horse gram was analysed from a drought prone area of Maharashtra with respect to phenolic compounds. A comparison was done with some commonly used legumes which show higher polyphenols and tannins in horse gram as compared to other commonly used legumes.

Keywords — *Horse gram, nutraceutical, antioxidant, anti - nutritive factors*

I. INTRODUCTION

Horse gram [Macrotyloma uniflorum Lam. (Verdc)], previously Dolichos biflorus] or kulthi is one of the lesser known legume. It is also called as gahat or kulath in India and used as food and fodder in India. It is a rich source of protein, carbohydrate and energy[1]. It is widely grown in India, Africa, Australia, Burma, Malaysia, Mauritius and West Indies [2]. This pulse is found to be useful for the treatment of kidney stones, urinary diseases [3] , piles [4] ,common cold, throat infection, fever[5] etc. According to traditional healers specialized in the treatment of kidney stone as the area under this crop is reducing, the number of patients suffering from kidney stones are increasing significantly. In developing countries in order to reduce risk of various diseases and malnutrition, there is an increased demand to explore this lesser known legume as alternative food [6,7].

The term "nutraceutical" introduced in 1989 by Stephen DeFelice can be defined as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease [8]. The phenolics, flavonoids, alkaloids, carotenoids, prebiotics, tannins, terpenoids, saponins, and soluble and insoluble dietary fibres etc are considered as the main health promoting agents[9]. These are also called as anti-nutritional factors. These anti-nutritional factors are now being considered as strong antioxidants [10] .These antinutrients help in reducing risk of intestinal diseases (gallstones, diverticulosis, constipation and colon cancer), coronary heart disease, dental caries and diabetes [11]. Recently many food and health scientists have started working on antioxidant properties of phenolic compounds and their uses in medicine [12]. Anti-inflammatory, cicatrizant, anti-HIV functions, anti-microbial and insecticide property of tannins have been proved [13].

Horse gram has potential to be used as nutraceuticals, forage and food for malnourished areas of the world [14]. Keeping in view the importance of nutraceutical applications, in the present study nutraceutical or anti-nutritive factors of raw and pressure cooked kulthi seeds with respect to polyphenols and tannins were analyzed.

II. MATERIALS AND METHODS

A. Sample collection and processing of Horse gram

One kg of Horse gram seeds was collected from a local market of a small town Narayangaon in Pune district of Maharashtra state. Collected sample was oven dried at 100^{o}C for an hour and powdered in a blender to pass through a 0.8 mm sieve. All analysis was carried out in triplicate and average results are reported here.

B. Pressure cooking

Sample seeds were cooked in water (1:10 w/v) at 125^{o}C until soft. The soft seeds were grinded in a blender.

C. Determination of total polyphenols

Total polyphenols were estimated by the Prussian Blue spectrophotometric method [15]. In brief the method includes mixing of 60 mg of ground sample in 3 mL of methanol. The mixture was then filtered. The filtrate was mixed with 50 mL of distilled water

and analyzed within 1 h. 1 ml of this filtrate was mixed with 3 mL of 0.1 M ferric chloride (prepared in 0.1 M HCl). To this mixture 3.0 mL of freshly prepared 0.008 M potassium ferricyanide ($K_3Fe(CN)_6$) was added. The solution was kept for 10 minutes and the absorbance was measured on a VIS spectrophotometer (Systronic Visible Spectro 105) at 720 nm. Standardization was done with catechin. Results were expressed in terms of catechin equivalents.

D. Determination of tannins

Tannins in horse gram seeds were determined as per standard method given in AOAC [16]. In this method 5g of seed powder was boiled in 400ml of water for 30 min and the content was diluted to 500 ml. 10 ml of the above diluted aliquot is mixed with 25 ml of Indigo carmine solution* and 750 ml of double distilled water. This mixture is than titrated. The potassium permanganate solution** was added from burette with stirring till the solution became light green and then bright yellow. The millilitre of potassium permanganate required was noted (Xml). The 100 ml of aliquot of the extract was mixed with 50 ml gelatine solution, 100 ml of acid sodium chloride solution and 10 g of kaolin. It was then shaken well for several minutes in a stopper flask. The mixture was allowed to settle and filtered. To 25 ml of aliquot of the filtrate were added 25 ml of Indigo carmine and 750 ml of distilled water, then it was titrated with potassium permanganate solution, as above the millilitre of potassium permanganate solution required was noted (Y ml). A millilitre of potassium permanganate solution used was subtracted from that obtained above (X-Y). It gives the quantity of potassium permanganate required to oxidize tannin.

1ml of 0.1 N oxalic acid ≡ 0.0042 g of tannin

III. RESULT AND DISCUSSION

The raw and pressure cooked samples of Horse gram were analysed for their polyphenols and tannin contents. The results are given in Table- 1.

A. Polyphenol content of horse gram

The total polyphenol content of raw and pressure cooked seeds were found to be 1420.3 and 533.1 mg/100g respectively. Table 1 shows a comparison of phenolic compounds of horse gram with other commonly used legumes [17]. Polyphenol and tannin content was more in horse gram as compared to the other legumes like chick pea, mung gram, pigeon pea and red lentil. Loss of polyphenols was seen in pressure cooked horse gram. The decrease in polyphenol content of horse gram after pressure cooking was due to thermal decomposition of these compounds. According to some researchers these compounds undergo thermal degradation during pressure cooking [18,19]. In pressure cooked horse gram the polyphenol content was found to be more than other legumes mentioned in table 1. It shows that even after cooking nutraceutical property of horse gram sample was more than commonly used legumes.

B. Tannin content of horse gram

Tannin content of raw and pressure cooked horse gram seeds was 651 and 236.1mg/100gm.Thus the values reported for tannin content lie well below the fatal dose (6000 mg/kg).

Tannins are phenolic compounds which interfere with protein metabolism. They mainly consist of oligomers and polymers. These phenolic compounds (tannin) are known to inhibit the activity of proteolytic and other digestive enzymes.

Antioxidants play vital role in reducing oxidative damage in the body. There are several synthetic antioxidants being used for this purpose but due to safety and toxicity problems now a days natural antioxidants are considered superior to synthetic antioxidants for protecting human body by free radicals [20]. Natural polyphenols and tannins reported to have higher antioxidant activity as compared to vitamins [21]. As the tannin content of horse gram was found higher than other commonly used legumes (Table 1), both in raw and pressure cooked variety, horse gram exhibited better neutraceutical properties than other legumes. Soaking and pressure cooking was reported to cause leaching of tannins and thus reduces tannin content of horse gram [22].

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* **Indigo carmine solution:** Solution containing 6g Indigo carmine and 50 ml concentrated sulphuric acid per liter.

** **Potassium permanganate solution:** 1.33g of potassium permanganate was dissolved in 1 litre of distilled water and its equivalent of 0.1N oxalic acid was obtained.

Table 1. Comparison of Horse gram with other commonly used legumes in terms of phenolic compounds (mg/100 g: mean ± SD)

Common name	Latin name	Polyphenols		Tannins	
		Raw	Pressure cooked	Raw	Pressure cooked
Chick pea	Cicer arietinum L.	274.5±24.79	198.2±4.83	236.6±28.31	154.0±10.78
Mung bean	Vigna radiata L.	512.5±31.44	304.2±28.02	395.9±9.82	221.7±25.76
Pigeon pea	Cajanus cajan L.	351.7±16.14	194.6±9.02	296.9±5.39	198.1±7.21
Red lentil	Lens culinaris	329.7±57.03	195.0±19.77	305.4±62.26	196.3±45.14
Horse gram	**Macrotyloma uniflorum**	**1420.3±2.1**	**533.12±9.2**	**651±23.12**	**236.1±11.14**

IV. CONCLUSION

Although polyphenols and tannins have anti-nutritional properties, the substantial reductions in their content are occurs through the use of simple house hold techniques such as pressure cooking. This is useful to regulate nutrient accessibility and proper digestion in human body. Nevertheless, the possible health benefits of these anti-nutritive factors in terms of their anti-oxidant activities cannot be overlooked. In the horse gram sample after pressure cooking polyphenol and tannin content was reduced to 533.12 and 236.1 mg/g these values were higher than other commonly used legumes. Hence, the potential health benefits of horse gram due to substantial content of polyphenols and tannin must be considered against other commonly used legumes due to their anti-oxidant properties. Further work is required to analyze types of polyphenol and tannins of horse gram in order to determine the nutritional importance of these compounds in food based on horse gram.

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REFERENCES

1. L Bravo ,P Siddhuraju and F Saura-Calixto 1998 Effect of various processing methods on the in vitro starch digestibility and resistant starch content of Indian pulses J. Agr. Food Chem. Vol **46,** pp.67.1998
2. J. R. Witcombe ,M. Billore , H.C.Singhal , N.B.Patel ,B. S.Tikka , D P Saini , L.K.Sharma , R.Sharma , S.K.Yadav and J.P.Yadavendra ."Improving the food security of low-resource farmers: introducing horsegram into maize-based cropping systems ".Expl .Agric. vol **44 ,pp**339,2008
3. K.Ravishankar and P.S..Vishnu Priya ." In Vitro antioxidant activity of ethanolic seed extracts of macrotyloma uniflorum and cucumismelo for therapeutic potential". Int. J. Res. Pharm. Chem. vol **2** ,pp 442,2012
4. N.D.Yadava and N. L. Vyas . In Arid legumes (Agro-Bios).1994
5. S.Perumal and M.Sellamuthu ."The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (Macrotyloma uniflorum (Lam.) Verdc.) seeds". Food Chem.vol. **105** pp 950'2007
6. L.Chel-Guerrero , V.Perez-Flores , D.Bentacur-Ancona and G.Davila-Ortiz G 2002." Functional properties of flours and protein isolates from Phaseolus lunatus and Canavalia ensiformis seeds "J. Agric. Food Chem.vol **50** ,pp 584,2002
7. V.Arinathan ,V. R. Mohan and A.J.De Britto ." Chemical composition of certain tribal pulses in south India". Int. J. Food Sci. Nutr.vol **54,pp** 209.2003
8. V.Brower . "Nutraceuticals: poised for a healthy slice of thehealthcare market?" Nat. Biotechnol.vol **16,pp** 728,1998
9. B.Patwardhan , D.Warude , V.Pushpangadan and N.Bhatt "Ayurveda and traditional Chinese medicine: a comparative overview Evid. Based Complement." Alternat. Med. Vol **2,** pp 465.2005
10. E A.Udensi ,F. C. Ekwu and J N.Isinguzo ."Antinutrient factors of vegetable cowpea (Sesquipedalis) seeds during thermal processing". Pakistan J. Nutr.vol **6** ,194.2007
11. N. G.Asp , J. M.Van Amelsvoort and J. G. Hautvast "Nutritional implications of resistant starch". Nutr. Res. Rev. vol **9** ,pp 1916.1996.
12. M. E.Alonso and A. O. E.Arellano ." Exhaustive extraction of phenolics and tannins from some sun-exposed forbs and shrubs of the tropical" Andes Ciencia. Vol **13** ,pp 429.2005
13. M.Furlan ,L.B. Mottaand , D.Y.Alves and C.D.Santos "Tannins: What do they represent in plant life?" In Petridis G. K. (Eds) Tannins: Types foods containing and nutrition p.251.2010
14. J.B.Morris ." Macrotyloma axillare and M. uniflorum: descriptor analysis, anthocyanin indexes, and potential uses "Genet. Resour. Crop Evol.vol **55,pp** 5.2008
15. M L.Price and L.G. Butler ."Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain" J. Agric. Food Chem.vol **25 ,pp** 1268.1977
16. AOAC: Official Methods of Analysis **240** 438 (Association of Official Analytical Chemists).1970
17. K.Shweta ,A.Shobha , B.Udipi and P. Ghugre ."Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking "Food Res. Int.vol **43** ,pp 526.2010
18. A.Kataria , B.M.Chauhan and D.Punia " Antinutrients and proteindigestibility (in vitro) of mungbean as affected by domestic processing andcooking "Food Chem. vol**32,pp** 9.1989
19. S.Jood,B.M.Chauhan and A.C.Kapoor ." Polyphenols of chickpea andblackgram as affected by domestic processing and cooking methods " J. Sci. Food Agric. Vol 39 ,pp145.1987.
20. C.K.Ramesh , R.Abdul ,B.T. Prabhakar ,B.R. Vijay Avin and S.J.Aditya Rao . " Antioxidant potentials in sprouts vs. seeds of Vigna radiate and Macrotyloma uniflorum". J. Appl. Pharm. Sci.vol1 **1** ,pp 99.2011
21. P.Siddhuraju and V.Becker V ." The antioxidant and free radical scavengingactivities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts" Food Chem . vol **101** pp10.2007
22. N.Sudha , J.M.Begum , K.G.Shambulingappa and C.K. Babu " Nutrients and some anti-nutrients in horsegram (Macrotyloma uniflorum (Lam.) Verdc) "Food Nutr. Bull. Vol **16** ,pp100.1995.