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REVIEW

Horse gram- an underutilized nutraceutical pulse crop: a review

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Abstract Horse gram is an underutilized pulse crop grown in wide range of adverse climatic conditions. It occupies an important place in human nutrition and has rich source of protein, minerals, and vitamins. Besides nutritional importance, it has been linked to reduced risk of various diseases due to presence of non-nutritive bioactive substances. These bioactive substances such as phytic acid, phenolic acid, fiber, enzymatic/proteinase inhibitors have significant metabolic and/or physiological effects. The importance of horse gram was well recognized by the folk/alternative/traditional medicine as a potential therapeutic agent to treat kidney stones, urinary diseases, piles, common cold, throat infection, fever etc. The inception of nutraceutical concept and increasing health consciousness the demand of nutraceutical and functional food is increased. In recent years, isolation and utilization of potential antioxidants from legumes including horse gram are increased as it decreases the risk of intestinal diseases, diabetes, coronary heart disease, prevention of dental caries etc. Keeping in view the increasing demand of food having nutraceutical values, the present review ascribed with recent scientific knowledge towards the possibilities of exploring the horse gram, as a source of food and nutraceuticals compounds.

Keywords Antinutritional compounds · Prebiotic · Phytochemicals · Antioxidant · Bioactive compounds · Oligosaccharides

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Introduction

Horse gram [*Macrotyloma uniflorum* Lam. (Verdc.)], previously *Dolichos biflorus*] is an underutilized (Aiyer 1990) and unexplored (Reddy et al. 2008) food legume. It is considered as a good source of protein, carbohydrates, energy (Bravo et al. 1998). It is tolerant to drought (Bhardwaj and Yadav 2012), salinity (Reddy et al. 1998) and heavy metal stresses (Reddy et al. 2005). Horse gram mainly grown in India, Africa, Australia, Burma, Malaysia, Mauritius, and the West Indies (Jeswani and Baldev 1990) under low soil fertility status with few inputs (Witcombe et al. 2008). It is adapted to wide range of temperature regimes (Smartt 1985) where other crops invariably fail to survive. In India, it is generally sown late in the rainy season by resource-poor farmers in marginal and drought-prone condition. However, sowing of seeds on the first fortnight of August and September recorded higher grain and straw yields than those sown on the first fortnight of October (Naik 2001), even some report indicated that delayed sowing beyond 28 August substantially reduced crop yield (Bajpai et al. 1990).

M. uniflorum is a short day and day neutral plant, matures in 120–180 days after planting, (Cook et al. 2005). The origin of the horse gram is not known. However, the region of maximum genetic diversity is considered to be in the Old World Tropics, especially in India and Himalayas (Zeven and de Wet 1982) and has diploid chromosome numbers of 2n=20, 22, 24 (Cook et al. 2005). It has been used as a food item for millennia. Archaeological investigations revealed that horse gram used as food around 2000 BC (Mehra 2000).

Ethnic uses of horse gram

The soup extract from *kulattha* (Horse gram), called *yusa*, was consumed commonly during the Sutra period (c. 1500–800

BC) are the rasams of today (Achaya 1998). Horse gram is widely grown for human food as a pulse and fodder crop for livestock (Cook et al. 2005) as well as green manure and medicinal crop. In rural areas, seeds of horsegram are consumed after parched followed by boiling or frying (Purseglove 1974) along with cooked rice, sorghum or pearl millet. Sprouted seeds, having high nutritional quality, are widely consumed by the indigenous tribal peoples (Bravo et al. 1999). Even now, in addition to its nutritive value, the consumption of sprouted seed become increasingly popular due to the excellent source to reduce the risk of various diseases and exerting health promoting effects (Paśko et al. 2009). In Indian traditional medicine, seeds of horse gram are used for treatment of urinary stones (Yadava and Vyas 1994; Ravishankar and Vishnupriya 2012), urinary diseases and piles (Yadava and Vyas 1994), regulate the abnormal menstrual cycle in women (Neelam 2007), act as astringent, tonic (Brink 2006), and also used to treat calculus afflictions, corpulence, hiccups, and worms (Chunekar and Pandey 1998). Furthermore, the cooked liquor of the horse gram seeds with spices is considered to be a potential remedy for the common cold, throat infection, fever and the soup said to generate heat (Perumal and Sellamuthu 2007).

Owing to their nutritional and medicinal value, there is an increased demand to explore an underutilized legume (Chel-Guerrero et al. 2002; Arinathan et al. 2003) to alleviate malnutrition and reduce risk of various diseases in developing countries. Horse gram is an excellent source of protein (17.9–25.3 %), carbohydrates (51.9–60.9 %), essential amino acids, energy, low content of lipid (0.58–2.06 %), iron (Bravo et al. 1999; Sodani et al. 2004), molybdenum (Bravo et al. 1999), phosphorus, iron and vitamins such as carotene, thiamine, riboflavin, niacin and vitamin C (Sodani et al. 2004). The details of major nutrient available in horse gram are presented in Table 1.

Nutraceutical properties of horse gram

Pulse crops are excellent sources of protein, dietary fiber, micronutrients and phytochemicals (Messina 1999). In addition to nutrients, it can also supply many bioactive substances in small quantities which have significant metabolic and/or physiological effects. These bioactive substances are referred as non-nutrient bioactive compounds, viz. phenolic acids, flavonoids and high molecular tannins (Siddhuraju and Becker 2007). These compounds contain potential medicinal/nutraceutical properties and have inhibitory role in reduction of various diseases like, coronary heart diseases, diabetes, and obesity (Bazzano et al. 2001).

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 (Brower 1998). In simple terms, nutraceuticals are those foods or parts of foods that provide health and/or medical benefits including prevention, protection and treatment of a disease (Belem 1999). In view, of its medicinal synergy, economical status and no side effects, the nutraceuticals, functional or health foods have gained a wide interest during the last few decades (Raskin et al. 2002). The health promoting effects of phytochemicals and nutraceuticals and/or functional foods are likely due to a complex mix of biochemical and cellular interactions which together promote overall health of the individual (Dillard and German 2000). The clinical success of nutraceutical products (Acharya and Thomas 2007) coupled with increasing health consciousness results in rapid global growth of the nutraceuticals and functional food industry (Hasler 2000). The major chemical compounds recognized as potential health promoting benefits are the phenolics, flavonoids, alkaloids, carotenoids, prebiotics, phytosterols, tannins, fatty acids, terpenoids, saponins, and soluble and insoluble dietary fibers (Patwardhan et al. 2005).

Table 1 Major nutrients in different milled fractions of Horse Gram (Percent of dry matter)

Constituent	Cotyledon*	Embryonic axe*	Seed coat*
Moisture	5.8±0.31b	8.4±0.21a	3.9±0.05c
Protein	22.6±1.23a	18.6±0.90b	9.1±0.35c
Fat	1.8±0.06b	2.6±0.04a	0.6±0.02c
Ash	2.9±0.02b	2.2±0.04b	3.8±0.05a
Crude fiber	1.6±0.02c	11.2±0.26b	21.8±1.6a
Total carbohydrate[a]	66.9±2.6b	68.2±1.9b	82.6±1.1a
Soluble sugars	6.4±0.15a	4.8±0.19b	0.96±0.06c
Reducing sugar (mg/100 g)	538.3±16.2a	211.7±6.3b	108.6±5.1c
Non reducing sugar	5.86±0.15a	4.6±0.08b	0.85±0.03c
Dietary fiber	16.7±0.27c	22.6±0.18b	36.4±0.90a
Soluble	1.32±0.04b	3.1±0.04a	3.9±0.05a
Insoluble	15.38±0.16c	19.5±0.28b	32.5±1.1a

* Values are mean ± standard deviation of three independent determinations. Means with the same letter (a, b, c) within the same row do not differ ($P>0.05$)

[a] By difference as 100 - (moisture + protein + ash + fat)

Sreerama et al. (2010a)

Plant materials play major role in primary health care (Motsei et al. 2003). Scientist have claimed that the grain legumes effectively contribute to a balanced diet and can prevent widely diffused diseases, including type II diabetes and cardio vascular diseases (Leterme 2002). The extracts from *M. uniflorum* seeds had significant activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* (Gupta et al. 2005). Different parts of the horse gram plants are used for the treatment of heart conditions, asthma, bronchitis, leucoderma, urinary discharges and for treatment of kidney stones (Ghani 2003).

A literature survey showed that Dolichin A and B, pyroglutaminylglutamine along with some flavonoids were isolated from horse gram (Handa et al. 1990; Kawsar et al. 2009). The ethanolic seed extract of *M. uniflorum* showed potential free radical scavengers (antioxidant) with significant scavenging activity of 64.01 %±1.78 at 500 μg/ml in Nitric Oxide radical Scavenging Assay, 74.42 %±2.37at 1,000 μg/ml in Hydroxyl method and 92.59 %±2.05at 250 μg/ml in Phosphomolybdate method as compared to that of standard (Ravishankar and Vishnu Priya 2012). Moreover, phytochemical studies revealed that Kaempferal-3-O-β-D-glucoside, β-sitosterol,stigmasterol (Kawsar et al. 2003) and phenolic compounds (Kawsar et al. 2008) were isolated from horse gram have the cytotoxicity and antimicrobial activities (Kawsar et al. 2008). Considering the nutritional and antinutritional aspects of horse gram, it has the greatest potential for further utilization as nutraceuticals, forage and food for malnourished areas of the world (Morris 2008). In the later sections nutraceutical properties of horse gram will be discussed under sub head: nutrient and non-nutrient bioactive compounds.

Nutrient bioactive compounds

Protein and amino acid

Grain legumes are important sources of food proteins and often they represent a necessary supplement to other protein sources (Duranti and Giusi 1997). Protein content in horse gram seed varies from 18.5 to 28.5 % (Savithramma and Shambulingappa 1996). Seed fraction of legume consist of cotyledon, seed coat and embryonic axe, which represent on an average of 89, 10 and 1 % of the total seed weight, respectively (Neelam 2007). The protein content of milled fraction of horse gram (Table 1) varies widely from 22.6 % in cotyledon to 9.1 % in seed coat fraction, whereas, embryonic axe contain higher protein than seed coat and lower than cotyledons (Sreerama et al. 2010b).

Proteins are classified on basis of their solubility as water-soluble albumins, salt-soluble globulins, alcohol-soluble prolamins, and acid- or alkaline-soluble glutelins (Utsumi 1992). Digestibility of raw and cooked legume protein varies from15–80 to 50–90 %, respectively, as compare to cereal seed protein (75–90 %) (Eggum and Beames 1983). The nutritional value of high protein foods are based on both protein quantity and quality.

In legume seeds, the major proteins are globulins (50–90 %) (Utsumi 1992), glutelins (10–20 %) and albumins (10–20 %) (Duranti 2006). In horse gram, out of the total protein content, albumin-globulin protein fraction contributes from 75.27 to 78.76 %, while glutelin and residual protein varies from 9.93–17.52 to 6.96–11.30 %, respectively (Yadav et al. 2004). It also has high lysine content, an essential amino acid (0.52 g g^{-1} of nitrogen) as compared to blackgram (0.40 g g^{-1} of N) and pigeonpea (0.48 g g^{-1} of N)(Gopalan et al. 1989). The other major amino acid found in horse gram seed are arginine, histidine, lysine, valine, leucine etc. but have primary limitation in methionine and tryptophan (sulfur-containing amino acids) (Thirumaran and Kanchana 2000).

Proteins and its peptides have been shown to inhibit the angiotensin-converting enzyme (ACE), antimicrobial activity, antioxidant activity, anti-carcinogenic activity, hypocholesterolemic effect, reduced serum triglycerides, increased lean muscle mass, protection against pathogens, regulation of blood glucose levels, and satiety effects (Chatterton et al. 2006; Severin and Xia 2005; Seyler et al. 2007). The branched-chain amino acids, leucine, isoleucine and valine participate in a variety of important biological functions in the brain, including those from tryptophan and tyrosine (Harris et al. 2005; Russo et al. 2003). There are many other types of protein found in legumes including various enzymes, protease inhibitors and lectins, which are collectively known as antinutritional compounds (ANCs).

Carbohydrate: starch, soluble sugar and dietary fiber

Carbohydrates are the major component of legumes, constitute from 50 to 70 % of the dry matter (Bravo et al. 1999) and are classified as digestible and non-digestible carbohydrates. The most abundant carbohydrates in legume seeds are starch and non-starch polysaccharides (dietary fiber), with smaller but significant amounts of oligosaccharides (Bravo et al. 1998). Starch is considered as partly digestible (Bravo et al. 1999) and those starches which are not digested in small intestine reaches the large intestine, where it is fermented by the colonic microflora and is known as resistant starch (Asp et al. 1996). Resistant starch, a non-digestible carbohydrate (Table 2) account 43.4 % of total carbohydrate in horse gram (Bravo et al. 1999). Fermentation of resistant starch yields relatively high amounts of butyrate and it is believed to exert a protective effect against colorectal cancer (Scheppach et al. 1995). This butyrate may reduce the risk of malignant changes in cells (Whitehead et al. 1986), increase in faecal bulking

Table 2 Soluble sugar and starch fraction of raw horse gram (g/100 g dry matter)

Soluble sugar and starch fractions	g/100 g dry matter[a]
Oligosaccharides	3.69±0.24
Sucrose	1.21±0.12
Maltose	0.53±0.06
Glucose	0.00±0.00
Xylose	0.64±0.09
Galactose	0.08±0.01
Arabinose	0.12±0.03
Fructose	0.03±0.01
Inositol	0.04±0.00
Total soluble sugar	6.38
Total starch	36.0±1.17
Digestible starch (% of TS) [b]	30.8 (85)
Resistant starch	5.21±0.64
IDF-RS[c]	1.22±0.03

[a] Mean values ± STD (n=3)

[b] Calculated by difference as TS-RS(Total starch-Resistant starch)

[c] *IDF-RS* Resistant starch associated to insoluble dietary fiber

Bravo et al. (1999)

Table 3 Composition of the dietary fiber fractions of raw horse gram (g/100 g dry matter)

Dietary fiber fractions	Insoluble dietary fiber (IDF)	Soluble dietary fiber (SDF)	Total dietary fiber (TDF)
Fucose	nd	nd	nd
Arabinose	4.14±0.08	0.17±0.03	4.30±0.05
Xylose	1.93±0.10	nd	1.93±0.10
Mannose	0.11±0.01	0.22±0.05	0.33±0.04
Galactose	0.41±0.02	0.11±0.03	0.52±0.04
Glucose	7.46±0.53	0.11±0.01	7.57±0.52
Total neutral sugars	14.06±0.78	0.60±0.08	14.65±0.73
Uronic acids	1.94±0.10	0.26±0.03	2.20±0.08
Klason lignin	5.61±0.39	–	5.61±0.39
Total dietary fiber	21.61±0.05	0.86±0.03	22.47±0.07

nd not detected

Bravo et al. (1999)

(Cummings et al. 1992) and lower faecal pH (Malhotra 1982). Resistant starch enriched food have beneficial for management of diabetes due to reduced postprandial glycaemic and insulinaemic response (Granfeldt et al. 1994).

Soluble sugar in horse gram consists of oligosaccharides, disaccharides (sucrose and maltose) and monosaccharides (glucose, galactose, arabinose, fructose and inositol). Oligosaccharides are the main constituent sugars of the soluble fraction, of most of the pulse crop, including horse gram. The oligosaccharide content in horse gram is 3.69 %. Small amount of maltose and monosaccharide was also detected in horse gram. Moreover, total carbohydrate, soluble sugars, reducing sugars, and non-reducing sugars were present in higher amounts in cotyledon fractions (Table 2).

Plant foods are the only sources of dietary fibers. The fiber content of human foods, from plant sources ranges from trace amounts to almost 50 % of dry weight (Anderson and Bridge 1988). Dietary fiber refers all polysaccharide which is resistant to digestion and absorption in small intestine but complete and partial fermentation in large intestine (Prosky and De Vries 1991). More recently, it includes oligosaccharides, such as inulin, and resistant starches (Jones et al. 2006). Major dietary fiber includes cellulose, hemicelluloses, pectin, arabinoxylans, betaglucan, glucomannans, plant gums and mucilages and hydrocolloids, which are principally found in the plant cell wall (Cummings and Stephen 2007). Pulses are good sources of both insoluble and soluble dietary fiber (Ramulu and Udayasekhararao 1997). Horse gram contains both soluble and non-soluble fiber (Table 3). Seeds of *M. uniflorum* contain more insoluble dietary fiber than kidney bean (*Phaseolus aconitifolius*) (Kawale et al. 2005). Crude fiber content was higher in seed coat fractions than in embryonic axe and cotyledon fractions. Furthermore, seed coat fractions of legumes with high fiber and low protein may be useful in food product formulations to improve gastrointestinal health and satiety changes (Sreerama et al. 2010a, b).

In human being, fibers primarily act on gastrointestinal tract, affecting different physiological effects like, alteration of the gastrointestinal transit time, satiety changes, influence on the levels of body cholesterol, after-meal serum glucose and insulin levels, flatulence and alteration in nutrient bioavailability (Lajolo et al. 2001). The main bioactive functions that have been attributed to dietary fibers are reduce constipation, modulation of blood glucose level (Spiller 2001; Redgwell and Fischer 2005), cholesterol reduction, prebiotic effects, prevention of certain cancers (Redgwell and Fischer 2005; Spiller 2001), cardiovascular diseases (CVD), diverticulosis, obesity (Spiller 2001) and lowers blood pressure (Brand et al. 1990). Similarly, Sharma and Kawatra (1995) also reported that soluble fiber also decreases serum cholesterol, reducing the risk of heart attack and colon cancer. Insoluble dietary fiber is required for normal, lower intestinal function in humans (Anderson et al. 1994).

Non-nutrient bioactive compounds

Pulses contain various molecules that can exert a broad range of biological actions in the human body, including both favourable and undesirable ones. The undesirable ones are to be considered as antinutrients or anti-nutritional factors (or compounds) (Thompson 1993). The antinutritional compounds found in pulse crops can be categorized as protein

ANCs and non-protein ANCs (Duranti and Gius 1997). Non-protein ANCs include alkaloids (Markievicz et al. 1988), phytic acid, phenolic compounds such as tannins (Davis 1981) and saponins (Hudson and El-Difrawi 1979). The presence of antinutritional factors, such as phenols, tannins (Cardador-Martinez et al. 2002), phytic acid (Urbano et al. 2000) and flatulence-causing oligosaccharides (Udensi et al. 2007) are now being considered as potential antioxidants. These antinutrients causes a number of positive health effects such as a decrease risk of intestinal diseases (gallstones, diverticulosis, constipation and colon cancer), coronary heart disease, prevention of dental caries and treatment of diabetes (Asp et al. 1996; Oku 1996). Saponins and another common class of antinutrients compounds have been reported to show hypocholesterolemic as well as anticarcinogenic effects (Koratkar and Rao 1997).

Phytic acid

Phytic acid or inositol hexaphosphate (IP_6) is a simple ringed carbohydrate with six phosphate groups attached to each carbon (a bioactive sugar molecule) and a major form of phosphorylated inositol (Norazalina et al. 2010). Phytic acid exists in the form of free acid, phytate, or phytin and all of these forms are interchangeable (Lori et al. 2001). It is widely distribute in legume seeds and it accounts for about 78 % of the total phosphorus in pulses (Chitra et al. 1995). The concentration of phytic acid in horse gram (Table 4) revealed a significant quantity in embryonic axe fractions, whereas, most of it concentration located in the cotyledon fractions. Phytic acid is considered as an anti-nutrient because it inhibit the digestibility of proteins, 'hard-to-cook' (Stanley and Aguilera 1985) and also reduces the bioavaibility of minerals such as calcium, zinc, iron, and magnesium (Sandberg 2002). On the other hand, it has potential antioxidant (Graf and Eaton 1990), anticarcinogenic (Turner et al. 2002; Shamsuddin et al. 1997), reduces the rate of cell proliferation, augmenting the immune response (Reddy 1999) and hypoglycemic or hypolipidemic (Rickard and Thompson 1997) activities. Phytate may have a stronger ability to quench free radicals because of its metal-chelating ability, which renders the prooxidant metal iron unavailable to participate in the Fenton reaction and to catalyze hydroxyl radical formation in vitro (Rao et al. 1991). Thus, phytate may prevent oxidative damage, such as lipid peroxidation (Ko and Godin 1991; Porres et al. 1999) and may thereby decrease the formation of atherosclerotic lesions.

Phenolic compounds

Phenolic compounds have a greatest beneficial interest on human health due to presence of antioxidant property, such as protection of oxidative damage (Sundaram et al. 2013). Natural polyphenols can range from simple molecules, such as phenolic acids, to highly polymerized compounds like tannins. Phenolic content of legumes varied in the range of 0.325–6.378 mgGAE(gallic acid equivalent)/g (Marathe et al. 2011). Marathe et al. (2011) reported that the horse gram is grouped under high phenolic acid content group (3.579±0.072 mgGAE/g). The principal phenolic compound (Table 5) of horse gram seed are flavonols (flavanoids) such as quercetin, kaempferol, and myricetin, vanillic, ρ-hydroxybenzoic, and ferulic acids (Sreerama et al. 2010a). The phenolic acids are a large family of secondary metabolites having either derivatives of benzoic acid (e.g. gallic, syringic and vanillic acid) or of cinnamic acid (e.g.caffeic, ferulic, sinapic and ρ -coumaric acid), which are commonly found as esters of caffeic and quinic acids (Kawsar et al. 2008) and are responsible for various beneficial effects in a multitude of diseases (Soobrattee et al. 2005). Sundaram et al. (2013) reported 0.101 g/100 g tannin in horse gram seed. Tannins are oligomeric, higher molecular weight polyphenolic compounds, occurring naturally in plants. Synthetic antioxidants are widely used to reduce oxidative damage, but due to safety and toxicity concern much attention has been focused on the

Table 4 Concentration of antinutritional factors in different milled fractions of Horse Gram (Percent of dry matter)

	Antinutritional factor	Cotyledon *	Embryonic axe *	Seed coat *
1.	Phytic acid (mg/g)	8.42±0.41a	3.81±0.11b	1.02±0.09c
2.	Flatulence factors (mg/g)			
	a. Raffinose	6.35±0.26a	2.86±0.09b	0.96±0.03c
	b. Stachyose	14.84±0.91a	6.38±0.38b	0.70±0.05c
	c. Verbascose	3.75±0.26a	1.38±0.04b	1.05±0.04c
3.	Enzyme inhibitors (units/g)			
	Buffer extract			
	a. Trypsin inhibitor activity	9,856±16.1a	2,018±12.9b	1,134±8.2c
	b. α-amylase inhibitor activity	56.9±1.1a	12.3±0.4b	4.1±0.2c
	Methanol extract			
	a. Trypsin inhibitor activity	2,474±11.8c	3,663±13.1b	10,434±21.4a
	b. α-amylase inhibitor activity	632.2±2.4b	639.5±2.0b	937.3±2.7a

* Results are mean ± standard deviation of triplicate determinations. Mean values bearing different letters (a, b, c) in the same row are significantly different ($P<0.05$) on application of Duncan's multiple-range test

Sreerama et al. (2010a)

Table 5 Phenolic compounds in different milled fractions of horse gram[a]

	Horse gram fractions		
	Cotyledon	Embryonic axe	Seed coat
	Concentration (μg/g on dry weight basis)		
Flavonoids			
Quercetin	9.7±0.55c	113.4±6.0b	129.5±11.3a
Kaempferol	6.0±0.25c	67.4±3.7b	117.2±10.5a
Myricetin	2.4±0.07b	32.9±3.3a	35.5±5.2a
Daidzein	4.1±0.08b	22.2±1.3a	0.94±0.03c
Genistein	nd	44.7±3.22	nd
Phenolic acids			
Benzoic acid derivatives			
Gallic acid	26.9±1.3a	19.8±0.8b	5.5±0.06c
Protocatechuic acid	39.0±2.0a	11.8±0.4	23.1±1.2b
ρ-hydroxybenzoic acid	20.1±0.8b	13.1±0.5c	28.8±1.4a
Vanillic acid	58.4±3.3a	53.2±5.1a	42.4±3.6b
Syringic acid	18.4±1.0a	nd[b]	4.5±0.02b
Subtotal	162.8	97.9	104.3
Cinnamic acid derivatives			
Caffeic acid	88.9±5.0a	61.5±4.9b	10.6±0.6c
Chlorogenic acid	160.8±9.8a	26.8±2.1b	22.5±1.1 b,c
Ferullic acid	70.1±5.1a	31.4±2.4c	37.5±2.5b
Sinapic acid	9.7±0.09a	nd	3.7±0.02b
ρ-coumaric acid	40.9±3.2a	21.4±1.3c	24.5±1.8b
Subtotal	370.4	141.1	98.8
Total	533.2	239.0	203.1

[a] Data are expressed as mean (standard deviation of three independent determinations. Means with the same letters (a, b, c) within the same row do not differ significantly (P>0.05).[b] Below detection limit

Sreerama et al. (2010b)

use of natural antioxidants to protect the human body by free radicals (Ramesh et al. 2011).

Phenolic acids are believed to work synergistically to promote human health through a variety of different mechanisms such as impacting cellular processes associated with apoptosis, platelet aggregation, blood vessel dilation, enzyme activities associated with starch, protein, and/or lipid digestion, carcinogen activation, and detoxification (McDougall and Stewart 2005). The phenolic compounds have also been associated with colour, sensory qualities (Maga 1978) and organoleptic properties (flavor, astringency, and hardness) of foods (Bravo 1999; Tan 2000).

There is growing evidence that polyphenols found in plants have potential health benefits (Akin et al. 2012). Phenolic compound have antimicrobial (Taguri et al. 2006), antimutagenic, (Lairon and Amiot 1999), antiviral (Perez 2003), anticarcinogenic (Aaby et al. 2004), anti-inflammatory (Dos Santos et al. 2006), antiproliferative and vasodilatory actions (Lule and Xia 2005) and have potential preventive properties against cardiovascular diseases, hormonal related cancers (Gupta and Abu-Ghannam 2012). In addition, to their antioxidant properties, isoflavones and lignans exert a weak oestrogenic activity (Barnes et al. 2000), which may be implicated in several mechanisms protecting the human body and also have been suggested to reduce the risks of cancer and to lower serum cholesterol (Barnes et al. 2000). Some phenolic compounds, like the flavonoids, known to have antioxidant activities (Segev et al. 2010) and are used as antibiotics, anti-diarrheal, anti-ulcer and anti-inflammatory agents, and also for treatment of diseases such as hypertension, vascular fragility, allergies, and hypercholesterolemia (Bravo 1999). Furthermore, these flavonoids are known to possess antioxidant, anticancer, antiallergic and gastroprotective properties (Sreerama et al. 2012a). The dietary antioxidants are capable of blocking neuronal death in vitro and many therapeutic properties of neurodegenerative diseases, including Alzheimer's and Parkinson's diseases (Daniel 2003). The antioxidant activity of dietary polyphenols is considered to be much greater than that of the essential vitamins (Siddhuraju and Becker 2007). Hence, the evaluation and exploitation of phyto-nutrient compounds particularly phenolic acids, flavonoids and high molecular weight tannins of legumes assumed great significance (Duranti 2006).

Flatulence factors

Raffinose family oligosaccharides (raffinose, stachyose and verbascose) α-galactosyl derivates of sucrose, a low molecular weight causes accumulation of gas, discomfort, diarrhea, pain and cramps (Phillips 1993) after digestion. The formation of flatus in human is due to absence of an enzyme α-galactosidase in the digestive tract (Reddy et al. 1984). These oligosaccharides eventually fermented in the large bowel by the action of anaerobic bacteria of *clostridia* group to produce gases (CO_2, H_2, CH_4, H_2S and NH_2) and short-chain fatty acids (Dilis and Trichopoulou 2009). However, these oligosaccharides can be used as sources of non-digestible carbohydrates, which not only promote several beneficial physiological effects but can also selectively stimulate the growth of the colon microflora and act as prebiotics (Gupta and Abu-Ghannam 2012). In fact, many prebiotic i.e. non-digestible food ingredient, stimulate the growth and/or activity of bacteria in the digestive system such as ingredients in various food products like soft drinks, cookies, cereals, candies and infant foods (Nakakuki 2003).

The major flatulence factors in horse gram are presented in Table 4. The cotyledon fractions contain higher concentrations of oligosaccharides (raffinose, stachyose, and verbascose), accounting for 39 % of the total soluble sugars. Among the oligosaccharides, stachyose is recorded in higher amounts in both cotyledon and embryonic axe fractions, whereas, verbascose was the major oligosacharide in seed coat fractions

(1.05 mg/g) in horse gram) (Sreerama et al. 2010a). However, substantial amounts of raffinose and verbascose were also found in cotyledon and embryonic axe fractions.

The presence of flatulence causing raffinose family oligosaccharides (RFO) limits their biological value and acceptance as a regular food item (Reddy et al. 1984). However, it is well-known fact that the balance of intestinal bacterial flora is important for human health, especially bifidobacterium, which is facilitated by galacto-oligosaccharides (Alles et al. 1999) and thus invigorate human health.

Proteinase/Enzyme inhibitors

Protease is a group of enzymes whose catalytic function is to hydrolyze (breakdown) peptide bonds of proteins. Proteases, is an indispensable for maintenance and survival of the organisms but have potentially damaging role in higher concentrations, thus its activities need to be regulated. Proteinase inhibitors play a vital role in regulating proteases (Muricken and Gowda 2010). Enzyme inhibitors are a storage protein (Richardson 1991) that binds to enzymes and decreases their activity however, on other hand it has defensive role against pests and pathogens and biomedical applications (Kennedy et al. 1993). There are ten families of proteinase inhibitors have been distinguished in plants, out of which 8 families belong to serine proteinase inhibitors while 9th and 10th belongs to metallo and cysteine inhibitor families, respectively (Richardson 1991). Trypsin, chymotrypsin, elastase, chymase, cathepsin G, plasmin, thrombin, and subtilisin are classical examples of functional serine proteinases (Losso 2008). Serine proteinase inhibitors are the Kunitz type inhibitors (bovine pancreatic and soybean trypsin inhibitors), the Bowman-Birk type inhibitors (BBIs), potato type I and II inhibitors, and the Kazal type inhibitors (pancreatic secretory trypsin inhibitors) (Losso 2008). Legume seeds generally contain BBIs (Richardson 1991), have single polypeptides with molecular masses in the range of 6–9 kDa (dalton) (Muricken and Gowda 2010). Bowman-Birk inhibitors (BBIs) are relatively stable to heat, acidic pH, resistance to various proteolytic enzymes (Ryan 1981) and have a tendency to self-associate forming dimers, trimers and tetramers in solution (Frokiaer et al. 1994). Protease inhibitor from the BBI, a double-headed inhibitor (Domoney et al. 1993) contains two active sites which inhibit the proteolytic enzymes trypsin and chymotrypsin.

Recently, four double headed Bowman-Birk type proteinase inhibitors which inhibits trypsin and chymotrypsin independently, were purified and characterized (Ramasarma and Rao 1991). The major BBI associated with horse gram is HGI-III that has 76-amino acid single-chain poly peptide with two independent inhibitory domains directed toward trypsin and chymotrypsin (Sreerama et al. 1997). HGI-III, though a single polypeptide of low molecular weight (~8,600 Da) and like other BBIs it undergoes self-association and exists as a dimer in solution (Kumar et al. 2004). In contrast to HGI-III, the three inhibitors of germinated horse gram seeds (HGGIs) are single polypeptides of 6,500–7,200 Da that exist as monomers and exhibit no such self-association (Kumar et al. 2002). HGI-III like other legume BBIs is stable at cooking temperatures (Muricken and Gowda 2010) and also exhibits remarkable stability to high temperature (95 °C) as well as extremes of pH from 2 to 12 (Sreerama et al. 1997). HGI-III contains seven inter-weaving disulfides and among these seven disulfides, four occur in the trypsin and three in the chymotrypsin reactive site domain, respectively (Kumar and Gowda 2013a, b). Proteinase inhibitors in horse gram (Table 4) revealed that buffer extracts showed higher proteinase inhibitors in cotyledon fractions than that of embryonic axe and seed coat fractions but methanol extracts showed higher proteinase inhibitors in seed coat fractions than that of cotyledon and embryonic axe fractions (Sreerama et al. 2010a). Horse gram having higher trypsin inhibitor activity (TIA) (9,856 TIA/g) could be used as functional food ingredients, similar to soybean BBI concentrate (Blanca et al. 2009).

BBIs have more recently been acknowledged to be helpful for human health, by their ability to suppress carcinogenesis (Chen et al. 2005; Clemente and Domoney 2006) due to intrinsic ability of BBI to inhibit serine proteases which involved in carcinogenesis (Kumar and Gowda 2013a). The BBIs have potential anti-inflammatory activity, against obesity and several degenerative and autoimmune diseases (Duranti 2006). BBI concentrate (BBIC) recently been used as treating ulcerative colitis (Gary et al. 2008) and multiple sclerosis (Gran et al. 2006). Horse gram having higher trypsin inhibitor activity (9,856 TIA/g) could be used as functional food ingredients similar to soybean BBI concentrate (Blanca et al. 2009).

Conclusions

After critical assessment of nutritional and therapeutic aspect of horse gram, it can now be concluded that-it is a rich source of nutrient and antinutrient content. The nutritional value of horse gram is comparable with other pulse crop. Horse gram has high levels of antioxidant and radical scavenging activities in addition to their traditional role of providing proteins and carbohydrates. It has rich source of various natural bioactive substances such as phytic acid, fiber, phenolic acid etc. These bioactive substances have immense potential for curing varieties of diseases such as common cold, throat infection, fever, urinary stones, asthma, bronchitis, leucoderma, etc. BBIs, the proteinase inhibitors have been identified to treat anti-inflammatory, obesity and several degenerative and autoimmune diseases. However, there is a dearth of information on the specific health beneficial components in this lesser known legume. Thus, considering its immense potential as health benefit it needs to exploit as a source of nutraceutical and food industries.

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