

Original Research Article

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## A Study on Protein, *in vitro* Protein Digestibility, Antioxidant Activity and Total Polyphenol Content of Horsegram Varieties

Aishwarya V. Patil\* and B. Kasturiba

Department of Food Science and Nutrition, College of Community Science, University of Agricultural Sciences, Dharwad, Karnataka, India

\*Corresponding author

### ABSTRACT

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Horsegram (*Macrotyloma uniflorum* (Lam) Verdc.) is minor legume, which belongs to family *Fabaceae*. It is a potential grain legume having excellent nutritional quality with better resilience property to adapt harsh environment conditions. Seven released varieties and five advanced breeding lines of horsegram were procured from All India Coordinated Research Project – Dryland Agriculture, Vijayapur. The horsegram were assessed for crude protein, *in vitro* protein digestibility, antioxidant activity and total polyphenols content. There was no significant difference among the varieties for crude protein, whereas *in vitro* protein digestibility, antioxidant activity and total polyphenol content varied significantly ( $p \leq 0.01$ ) among the varieties. The protein content, *in vitro* protein digestibility, antioxidant activity and total polyphenols content ranged from 23.10-28.21 g/100 g, 50.80-67.80 per cent, 80.24-89.41 per cent DPPH and 2.35-3.61 mgGAE/g respectively.

### Introduction

Food legumes constitute an important component of diet across the world and are the next important food crops after cereals. As a result, pulses have significant cultural and historical significance (Roy *et al.*, 2010). They play a vital role in sustainable agriculture and a key source of dietary nutrients for many of people in the developing countries. The legumes belongs to family Leguminosae (*Fabaceae*) which are eaten directly by humans, usually in the form of mature dry seeds, but sometimes in the form of immature seeds attached in the pod.

Dehulled pulses, also known as *dals*, are famous for their high quality protein content and are considered as meat substitutes for people in less developed countries. Legumes are good sources of protein that are cheap and widely available for human consumption. They are staple foods for many people in different parts of the world. Legume seeds have an average of twice as much protein as cereals and nutritive value of the proteins are usually high. They are widely cultivated and distributed in Africa, Asia, West Indies, Latin America and India. The legumes can be grown in marginal soils and in arid or semi arid regions. Their deep penetrating root

system enables them to withstand moisture stress (Bhokre and Joshi, 2015). Pulses are popular because animal protein is expensive and it is not consumed due to religious beliefs and moral values. In Indian diet, cereals (deficient in lysine) are usually eaten with pulses (poor source of methionine) to make a complete protein diet. Apart from being an important source of macro nutrients (carbohydrate, protein, fiber) and micro nutrients (calcium, magnesium, zinc, iron, potassium, and phosphorus), health benefits of consuming legumes are also related to the amount of dietary fiber and polyphenol content. There are a number of epidemiological studies related to high phenolic content in legumes for fewer incidences of diseases such as cancer, aging, diabetes, and heart diseases, which is related to the consumption of legumes (Kris-Etherton *et al.*, 2002). The dominant phenolic compounds present in leguminous seeds are flavanoids, phenolic acids, and procyanidins (Amarowicz and Pegg, 2008). They are also an important source of antioxidants, where they exhibit anti-inflammatory, cardio-protective, anti-microbial, vasodilatory effects, and anti-allergic properties. Presently, attention towards underutilized legumes is increasing for finding new alternative sources to meet the ever increasing demand for vegetable protein (Bhartiya *et al.*, 2015). In developing countries, primarily a handful of conventional legumes are dominating the production and market chains and are playing a crucial role in eradicating protein energy malnutrition still, some of the underutilized indigenous legumes like, horse gram has a great significance in the nutritional security of rural, tribal and underprivileged masses.

Among legumes, horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.] is a minor legume crop of India and is of good nutritional quality (Pal *et al.*, 2015). It belongs to family *Fabaceae*, has potential nutritional and remedial properties with better climate

resilience to adapt to harsh environmental conditions. It is one of the most important under exploited food legume being grown almost all over the world including temperate and sub-tropical regions encompassing the countries particularly, India, China, Philippines, Bhutan, Pakistan, Sri Lanka and Australia (Durga, 2016; Bhartiya *et al.*, 2015). In India it is most extensively grown pulse in the south, the maximum area being in Andhra Pradesh, Karnataka and Tamil Nadu. It is grown mainly to furnish feed and fodder for cattle and horse. It makes excellent hay and is suitable as green manure. Antioxidant is significant for the defense mechanism in the human body and helps in the management with oxidative stress due to reactive oxygen species. Presently people are more concerned about antioxidant properties of food. This is primarily due to its protective action against harmful free radicals and thus reduces the risk of cardiovascular diseases and cancer, prevents oxidative damage of lipids and low-density lipoprotein. Antioxidants that exist, improve food quality and safety by blocking lipid peroxide. Horsegram contains a potential antioxidant activity and phenolic content. Hence the present study is undertaken to assess the protein, *in vitro* protein digestibility, total polyphenols content and antioxidant activity of horsegram varieties.

## **Materials and Methods**

### **Procurement of the horsegram varieties**

Twelve varieties of horsegram were procured from AICRP for Dryland Agriculture RARS, Vijayapur, Karnataka. Out of twelve varieties five were advanced breeding line of University of Agricultural Sciences, Dharwad, while remaining seven varieties were released for cultivation across different regions. The details regarding the samples are given in Table 1. The samples were cleaned before the study was carried out. All estimations were carried out in triplicates.

### **Crude protein**

The crude protein was estimated in Kelplus instrument of Pelican and crude protein was expressed in per cent (%) and calculated by multiplying with a factor of 6.25 (Anon., 2005).

Crude protein (%) =

$$\frac{(\text{Titre value} - \text{Blank}) \times \text{Normality of HCl} \times 14.007 \times 6.25}{\text{Weight of the sample (g)}} \times 100$$

### ***In vitro* protein digestibility of horsegram varieties**

Sample containing 100 mg of protein was treated with 0.1 N HCl containing 19.5 mg of pepsin at 37 °C for 3 hours. 0.5 N sodium hydroxide was used to neutralize the contents. After neutralizing phosphate buffer (25 ml) containing pancreatin (6 mg) was added and incubated at 37°C for 24 hours.

Then, volume was made upto 100ml using distilled water and aliquot of 50 ml was treated with 10 per cent trichloroacetic acid and left overnight for precipitation. The suspended contents were centrifuged and the residue was analysed for protein by microkjeldahl method (Mouliswar *et al.*, 1993). The amount of protein digested was calculated using the formula,

Digested protein =

$$\frac{\text{Total protein} - \text{Undigested protein}}{\text{Total protein}} \times 100$$

### **Antioxidant activity of horsegram varieties**

The principle used for estimating antioxidant activity was DPPH (2,2-diphenyl-1-picryl-

hydrazyl) free radical method is an antioxidant assay based on electron-transfer that produced violet solution in methanol. 1g of defatted sample was refluxed for half an hour using 10ml methanol, the extract was centrifuged and supernatant was used for estimation. 1ml of extract was mixed with 0.05 mg/ml of DPPH and mixed well to stand at room temperature at 30 min. Then the absorbance was measured at 517 nm (Dorman *et al.*, 2004).

The DPPH is estimated by,

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1) \times 100$$

Where,  $A_0$  was the absorbance of the negative control and  $A_1$  was the absorbance of reaction mixture or standards.

### **Polyphenol content of horsegram varieties**

Total polyphenols was estimated using Folin-Ciocalteu reagent. Blue coloured complex was formed in alkaline medium when phenols reacted with phosphomolybdic acid in Folin-Ciocalteu reagent (Sadashivam and Manicham, 2008).

### **Results and Discussion**

Table 2 depicts the crude protein among the horsegram varieties. There was no significant difference in crude protein among the varieties. Protein content ranged from 23.10-28.21 g/100 g. Highest protein content was observed in VHG44 (28.21 g/100 g), followed by VHG13-01 (27.68 g/100 g) and VHG15 (27.02 g/100 g) and DHG01 had lowest protein content. On comparison of proximate composition of released varieties and advanced breeding lines of horsegram, crude protein varied significantly ( $p \leq 0.01$ ) between released varieties and advanced breeding lines of horsegram varieties. Crude protein was higher in advanced breeding lines than released varieties (Table 3). The study conducted by Bhokre *et al.*, (2015) showed

similar values for crude protein. The study conducted by Hiremath *et al.*, (2000) recorded slightly lower values for crude protein. The study conducted by Bhokre *et al.*, (2015) showed similar values for crude protein. The reason attributed to this may be due to locality, varietal differences, agronomical condition in which they are grown and climatic conditions.

The *in vitro* protein digestibility is presented in Figure 1. The *in vitro* protein digestibility varied significantly ( $p \leq 0.01$ ) among the varieties. The *in vitro* protein digestibility among varieties ranged from 50.80-67.80 per cent. VH44 (67.80 %) had highest *in vitro* protein digestibility followed by VH13-01 (65.67 %) and VH938 (62.57 %). AK44 (50.80 %) had lowest *in vitro* protein digestibility among the horsegram varieties. Table 4 depicted the Comparison of *in vitro* protein digestibility between the released and advanced breeding lines of horsegram. There was a significant difference ( $p \leq 0.01$ ) between the released and advanced breeding lines of horsegram.

Higher values of *in vitro* protein digestibility was observed in advanced breeding lines (63.62 %), when compared to released varieties (56.05 %) of horsegram varieties. The study conducted by Khatoon and Prakash (2006) showed similar results and Sreerama *et al.*, (2008) showed lower values of *in vitro* protein digestibility. This may be due to the presence of antinutritional factors.

Figure 2 depicts the total antioxidant activity of horsegram varieties. Total antioxidant activity varied significantly ( $p \leq 0.01$ ) among the varieties. The total antioxidant activity ranged from 80.24-89.41 per cent DPPH.

Lowest antioxidant activity was observed in AK42 (80.24 per cent DPPH). The varieties VH44 (89.41 per cent DPPH) had highest total antioxidant activity followed by VH935 (89.28 per cent DPPH) and CRHG22 (87.71 per cent DPPH). Comparison of total antioxidant activity of released varieties and advanced breeding lines of horsegram is presented in Table 5.

**Table.1** Horsegram varieties selected for study

| Horsegram varieties                      |  |
|--|--|
| Released varieties                       | Advanced breeding lines                |
| AK 42- ARJIA KULTHI 42                   | VHG 13-01- VIJAYAPUR HORSEGRAM - 13-01 |
| AK 44- ARJIA KULTHI 44                   | VHG 15- VIJAYAPUR HORSEGRAM -15        |
| CRHG 22- CRIDA HORSEGRAM 22              | VHG 44- VIJAYAPUR HORSEGRAM -44        |
| CRHG 23- CRIDA HORSEGRAM 23              | VHG 935- VIJAYAPUR HORSEGRAM -935      |
| DHG 01- DANTEWADA HORSEGRAM 01           | VHG 938-- VIJAYAPUR HORSEGRAM - 938    |
| GPM 06- GERMPLASM SELECTION 06           |  |
| KBHG 01- KARNATAKA BIJAPUR HORSEGRAM- 01 |  |

**Table.2** Crude protein of horsegram varieties (g/100 g)

| Varieties                      | Crude protein              |
|--------------------------------|----------------------------|
| <b>Released Varieties</b>      |                            |
| AK42                           | 25.23 ± 0.05 <sup>c</sup>  |
| AK44                           | 25.43 ± 0.80 <sup>c</sup>  |
| DHG01                          | 23.10 ± 1.21 <sup>e</sup>  |
| GPM06                          | 23.36 ± 0.23 <sup>de</sup> |
| KBHG01                         | 24.63 ± 0.45 <sup>cd</sup> |
| CRHG22                         | 24.83 ± 0.58 <sup>cd</sup> |
| CRHG23                         | 23.66 ± 0.58 <sup>de</sup> |
| <b>Advanced breeding lines</b> |                            |
| VHG13-01                       | 27.68 ± 0.80 <sup>a</sup>  |
| VHG15                          | 27.02 ± 0.53 <sup>ab</sup> |
| VHG44                          | 28.21 ± 0.49 <sup>a</sup>  |
| VHG935                         | 26.93 ± 0.62 <sup>ab</sup> |
| VHG938                         | 26.13 ± 0.12 <sup>bc</sup> |
| <b>Mean ± SD</b>               | 25.52 ± 0.78               |
| <b>S.Em. ±</b>                 | 0.47                       |
| <b>C.D.</b>                    | NS                         |
| <b>F value</b>                 | 21.94                      |

Note: Values are mean of three replications, S.Em.: Standard error of mean, C.D.: Critical difference, NS-Non significant

**Table.3** Comparison of crude protein (g/100 g) of released varieties and advanced breeding lines of horsegram varieties

| Varieties               | Crude protein (%) |
|-------------------------|-------------------|
| Released varieties      | 24.51 ± 1.42      |
| Advanced breeding lines | 27.10 ± 0.93      |
| <b>t value</b>          | 6.57**            |

Note: Values are mean of three replications, \*\*Significant @ 1%,

**Table.4** Comparison of *in vitro* protein digestibility of released varieties and advanced breeding lines of horsegram varieties

| Varieties               | <i>In vitro</i> protein digestibility |
|-------------------------|---------------------------------------|
| Released varieties      | 56.05 ± 3.91                          |
| Advanced breeding lines | 63.62 ± 3.24                          |
| <b>t value</b>          | 6.07**                                |

Note: Values are mean of three replications, \*\*Significant @ 1%

**Table.5** Comparison of antioxidant activity of released varieties and advanced breeding lines of horsegram varieties

| Varieties               | Scavenging activity (%) |
|-------------------------|-------------------------|
| Released varieties      | 83.23 ± 2.92            |
| Advanced breeding lines | 87.52 ± 1.72            |
| <b>t value</b>          | 5.38**                  |

Note: Values are mean of three replications, \*\*Significant @ 1%

**Table.6** Comparison of total phenolic content of released varieties and advanced breeding lines of horsegram varieties

| Varieties               | Total polyphenols (mg/g) |
|-------------------------|--------------------------|
| Released varieties      | 2.85 ± 0.53              |
| Advanced breeding lines | 2.66 ± 0.50              |
| t value                 | NS                       |

Note: Values are mean of three replications, NS-Non significant

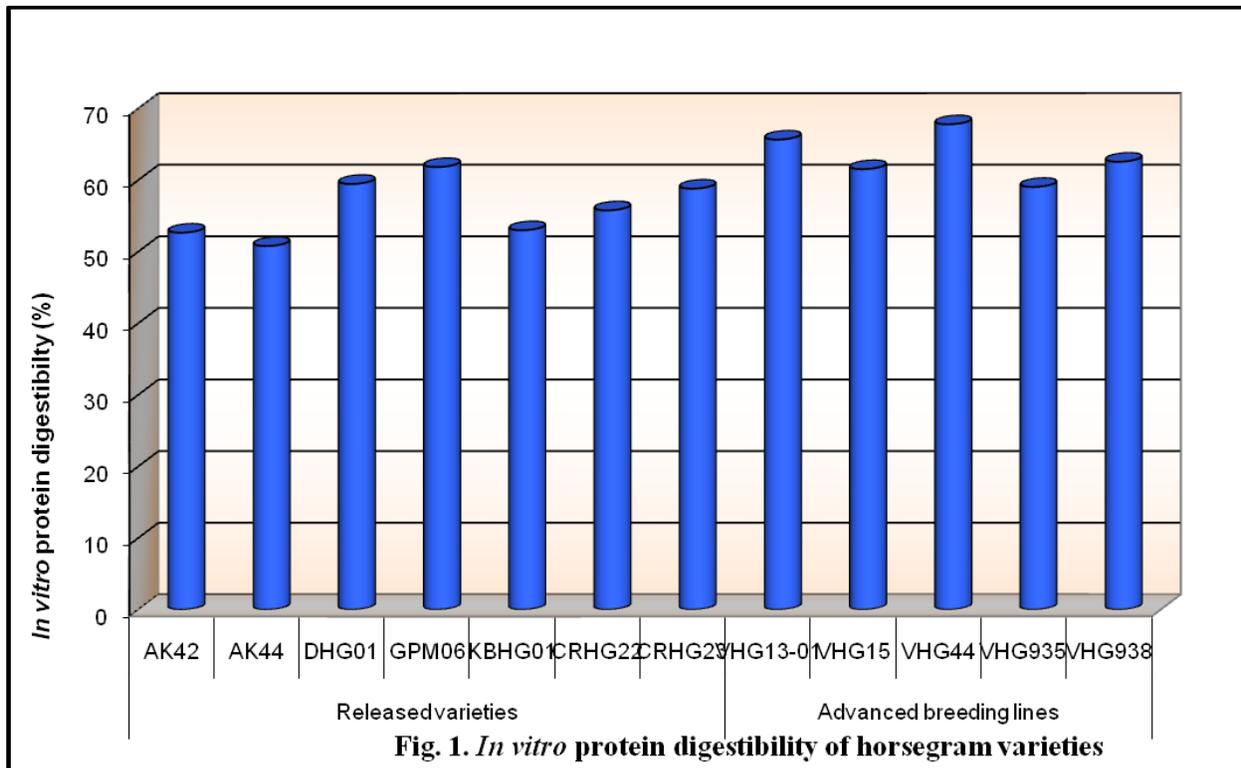
**Table.7** Correlation between antioxidant activity and total polyphenols content of horsegram varieties

|                      | Antioxidant activity | Total polyphenols |
|----------------------|----------------------|-------------------|
| Antioxidant activity | 1                    | 0.034             |
| Total polyphenol     |                      | 1                 |

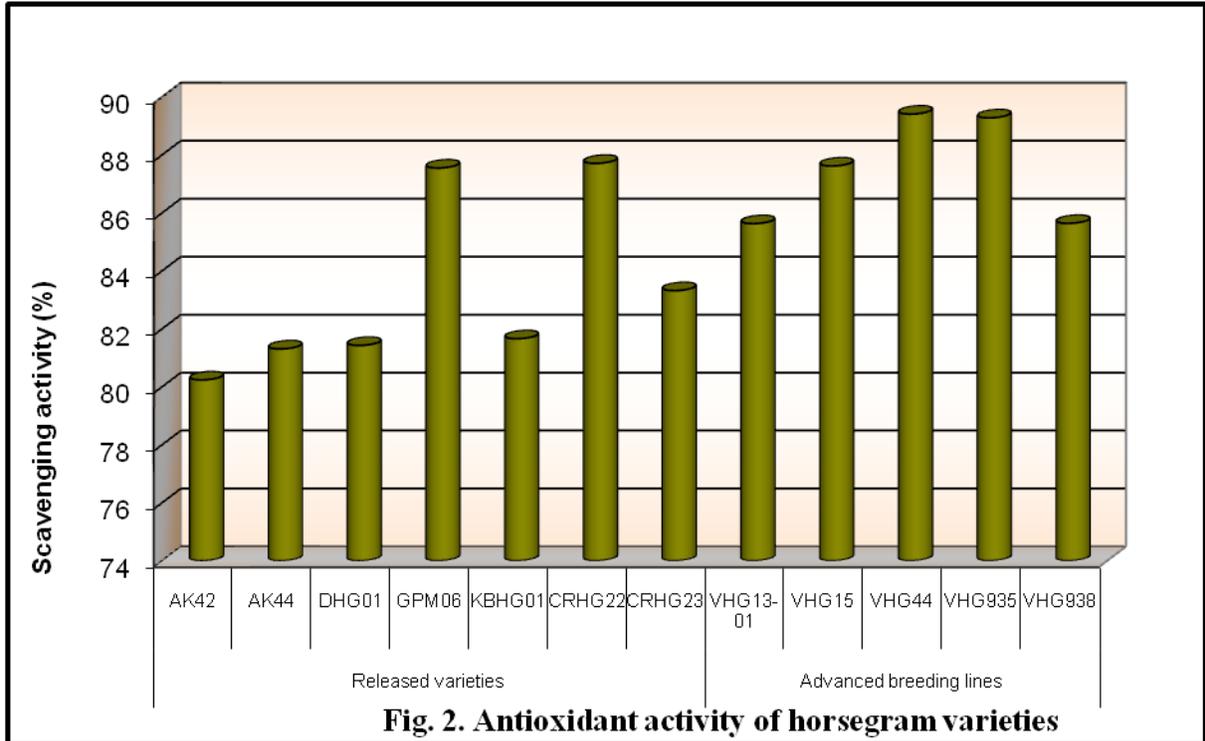
**Table.8** Correlation between antioxidant activity and protein content of horsegram varieties

|                      | Antioxidant activity | Protein |
|----------------------|----------------------|---------|
| Antioxidant activity | 1                    | 0.480** |
| Protein              |                      | 1       |

**Fig.1**



**Fig.2**



**Fig.3**

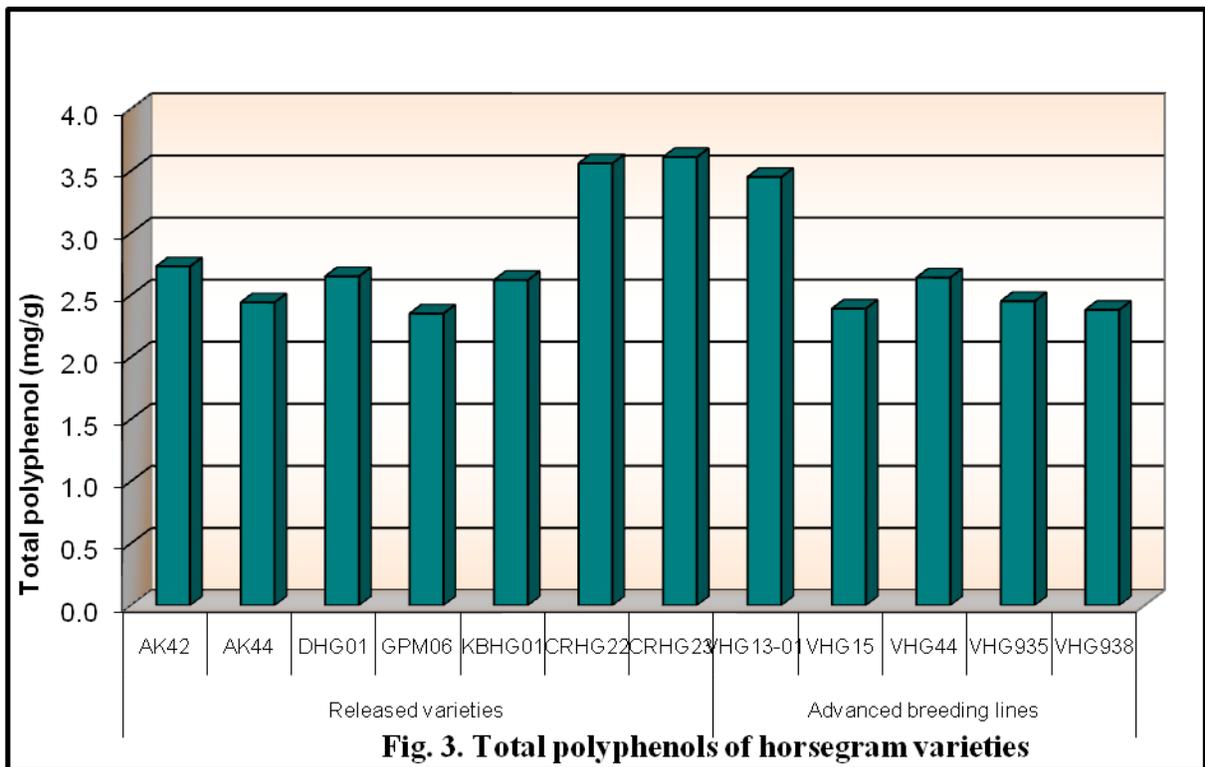


Figure 3 depicts the total polyphenol content of horsegram varieties. Total polyphenol content varied significantly ( $p \leq 0.01$ ) among the varieties. The total polyphenol content varied from 2.35-3.61 mgGAE/g. CRHG23 (3.61 mgGAE/g) had highest total polyphenol content followed by CRHG22 (3.56 mgGAE/g) and VHG13-01 (3.45 mgGAE/g). Lowest total polyphenol content was observed in GPM06 (2.35 mgGAE/g) among the horsegram varieties. Comparison of total polyphenol content of released varieties and advanced breeding lines of horsegram is presented in table 6. There was no significant difference between the groups for total polyphenol content. Released varieties had higher total polyphenols content (2.85 mgGAE/g) compared to advance breeding lines (2.66 mgGAE/g) of horsegram varieties. The results of present study were on par with the results of Pal *et al.*, (2015) and Siddhuraju and Manian (2007). The study conducted by Sundaram *et al.*, (2013) had slightly lower phenolic content in horsegram variety.

Table 7 depicts the correlation between total polyphenols and antioxidant activity content of horsegram varieties. The total polyphenol was positively correlated to antioxidant activity. Table 8 depicts the correlation between protein content and antioxidant activity of horsegram varieties. The protein content was positively correlated to antioxidant activity and it was statistically significant ( $p \leq 0.01$ ). There was a positive correlation between the total polyphenol to antioxidant activity in horsegram varieties. The reason for higher phenolic content may be due to dark colour and black variety of horsegram (Siddhuraju and Manian, 2007) and it also related to higher antioxidant activity.

In conclusion, there was no significant difference among the varieties for crude protein, whereas *in vitro* protein digestibility,

antioxidant activity and total polyphenol content varied significantly ( $p \leq 0.01$ ) among the varieties. When horsegram were compared between the groups *i.e.*, released varieties and advanced breeding lines, there was no significant difference between the groups for crude protein and antioxidant activity whereas there was a significant difference ( $p \leq 0.01$ ) for *in vitro* protein digestibility and total polyphenol content. Advanced breeding lines had higher values for crude protein, *in vitro* protein digestibility, antioxidant activity whereas released varieties had higher values for total polyphenol content. The protein content of horsegram varieties ranged from 23.10-28.21 g/100 g and *in vitro* protein digestibility ranged from 50.80-67.80 per cent. Highest protein content and *in vitro* protein digestibility was observed in VHG44 and VHG13-01. As horsegram is a underutilized legume and rich in nutrient, it can be utilized in product formulation. Advanced breeding lines had better nutrient composition *i.e.*, protein, *in vitro* protein digestibility, antioxidant activity and total polyphenol content than released varieties.

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