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Proximate and anti-nutritional composition of underutilized and common *Vigna* species of Himachal Pradesh

Shweta*[1], Rajan Katoch[2] and Meena Kumari[3]
1. Department of Chemistry and Biochemistry, CSKHPKV, Palampur, India.
2. Department of Crop Improvement, CSKHPKV, Palampur, India.
3. Dr. Y.S. Parmar UHF, Nauni, Solan, India.
Correspondence Author*: Shweta, shwetakhosla14@gmail.com

ABSTRACT
In the present investigation entitled "Proximate and anti-nutritional composition of underutilized and common Vigna species of Himachal Pradesh", the dried mature seeds of five Vigna species viz. Vigna umbellata (BRS-2), Vigna unguiculata (LOBIA-1), Vigna radiata (SUKATI-1), Vigna angularis (HPU-51) and Vigna mungo (HIM-MASH) were procured from Crop Improvement Department, College of Agriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. The proximate and anti- nutritional composition of five Vigna species were determined by using standard procedures. Significant variations were observed in the proximate composition of five Vigna species viz. moisture content (8.85-12.17 per cent), crude protein content (20.48-24.45 per cent), fat content (0.44-1.78 per cent), crude fiber content (4.02-5.44 per cent), ash content (3.10-3.87 per cent) and carbohydrate content (54.97.09-59.09 per cent). Anti-nutritional components viz. saponin (1.09-2.90 per cent), phytic acid (473.30-645.40 mg/100g), trypsin inhibitor content (21.80-32.46 mg/g) and α amylase inhibitor content (614.33-1560.67 unit/g), revealed significant variations among Vigna species. Vigna mungo revealed highest whereas, Vigna radiata revealed lowest content of oligosaccharides. The cumulative grading revealed that Vigna radiata and Vigna angularis have lowest amount of anti-nutritional constituents and Vigna angularis have highest amount of protein content as compared to other species.
Key Words: *Vigna, underutilized, proximate composition, anti-nutrients, oligosaccharides.*

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INTRODUCTION

Grain legumes and pulses occupy an important place in the world food and nutrition. They are important constituents in the diets of a large number of people, especially in the developing countries, where animal proteins are scarce and expensive. The world wide productions of pulses are 67.7 million tons [1]. India is the largest producer as well as consumer of pulses. The area under pulses in the country is around 24.38 million hectares with a production of 14.52 million tons [2]. In Himachal Pradesh, total pulse production is 16 thousand tones [3]. Legumes contain a large number of genera, out of which *Vigna* species are widely grown and consumed throughout the world. The worldwide production of *Vigna* species is 20 million hectares annually [4]. In Himachal Pradesh, many economically important species of genus *Vigna* are cultivated. More popular and highly nutritious species include *Vigna unguiculata, Vigna radiata* and *Vigna mungo.* Apart from traditional legumes many other non-traditional underutilized legumes such as rice bean *(Vigna umbellata)* and adzuki bean (*Vigna angularis*) are also cultivated in some parts of Himachal Pradesh. *Vigna umbellata* and *Vigna angularis* have recently gained attention as highly nutritive pulses with sound productivity.

Rice bean [*Vigna umbellata* (Thunb) Ohwi and Ohashi] as a grain legume is attracting attention throughout the world as a potential source of high quality protein for the future for bridging the "protein gap" [5]. Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the important *Kharif* pulses grown in India. It is one of the important protein rich leguminous food sources in the tropics and subtropics region. The world wide productions of cowpea are 3.6 million tons [6]. Cowpea contains about 25% protein and it is rich in amino acids like lysine and tryptophan [7]. Mungbean [*Vigna radiata* (L.) R. Wilczek] is popular legume in Asian countries. It is short duration and warm seasonal crop. Its worldwide production is 6

million hectares per annum and 3 million hectare in India [8] and its productivity is 1.04 million metric tons. It is the grain legume of highest digestibility for direct human consumption. Adzuki bean [*Vigna angularis* (Willd.) Ohwi and Ohasi] is traditional pulse crop in East Asia and widely used as a source of protein for human nutrition, especially in developing countries [9]. In India, its cultivation is confined to North-eastern and Northern hill zones. Blackgram [(*Vigna mungo* (L.) Hepper] is grown in several parts of Asia, mainly in countries like India, Bangladesh, Pakistan, Burma and Ceylon. In India, it is grown as *Kharif* crop in Northern region and as *Rabi* crop in Southern part. The cultivation of blackgram in India covers 3.25 million hectares with the production of 1.45 million tons [10]. In Himachal Pradesh, the area under production is 11830 hectares, whereas average yield is 338 kg/hectare [11]. Black gram contains 20-25% protein which nearly doubles the amount of proteins in cereals. It is also rich in amino acids, minerals and vitamins.

All these five *Vigna* species are rich not only in proteins, but also in other nutrients such as carbohydrate, starch, fiber, amino acids, vitamins and minerals which help to supplement cereal diets, improving their protein nutritive value [12]. Apart from its nutritional value, *Vigna* species also contain anti-nutritional factors such as saponin, protease inhibitors, phytic acid and oligosaccharides etc. Presence of anti-nutritional factors is one of the main drawbacks which limit the nutritional and food quality of *Vigna* species. Polyphenols and tannins inhibit the proteolytic enzymes and form insoluble complex with food proteins. They lower the digestibility of the food and protein quality [13]. Phytic acid is very reactive with positively charged ions such as minerals (especially Zn, Ca and Fe), thereby forming insoluble complexes that are less available for digestion and absorption [14, 15]. Presence of protease inhibitors in diet could lead to enlargement of the pancreas and also reduce the availability of sulphur containing amino acids [16]. Whereas, oligosaccharides present in legumes cause flatulence. There are number of treatments of grain legumes including soaking, dry and moist heat treatment, germination and fermentation which are able to eliminate some anti-nutrients and also increase the nutritional value [13]. Keeping in view the importance of the nutritive value of these grain legumes, the present study was carried out to evaluate the proximate composition and determine the anti-nutritional composition among five *Vigna* species. So further work can be carried out to reduce the level of anti-nutrients from these pulses and make them highly nutritive.

MATERIAL AND METHODS

The seeds of *Vigna* species were procured from Department of Crop Improvement, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. Dry mature seeds of *Vigna* species were cleaned and stored in air tight storage boxes. The samples were properly labelled and kept at room temperature for further analysis.

Proximate composition

Moisture content was determined by drying the samples for 8h at 65^0C in air oven until a constant weight was obtained [17]. Crude protein content was calculated from the nitrogen content (N % x 6.25) analyzed by the Kjeldahl method [17]. Crude fat content was determined using a Soxhlet apparatus [17]. Crude fibre and ash were estimated by the standard procedures of AOAC [17]. The carbohydrate content was estimated by the method given by Gopalan *et al.* [18].

Saponin

Three gram of finely powdered seed sample was put in a thimble and placed in soxhlet extractor for 10 hrs. Extraction was done with methanol (110 ml) at 20^0C. After 10 hrs the extract was concentrated to about 3-5 ml by gentle heating on the hot plate. The concentrated extract was brought to room temperature and 15-20 ml acetone was added drop wise with constant stirring till white precipitates appeared. The white precipitates were filtered and oven dried. Finally the weight of filter paper was measured along with the precipitates for the determination of saponin content [19].

Trypsin inhibitor activity

Sample was extracted with 25 ml 0.1 M phosphate buffer (pH 7.5). The ground sample was extracted in a refrigerator for 2 hrs with occasional shaking. The homogenate was centrifuged at 12,000 rpm for 20 minutes at 4-6^{o}C [20]. One micro litre of the extract was pipetted out in duplicate sets, one to serve as endogenous (E) and the other test (T). The volume was made to 2 ml with buffer in the endogenous set and 1 ml in the test set. 1 ml of trypsin solution (20 μg) was added to each tube in the test set. Pipetted out 1 ml of buffer and 1 ml of trypsin solution for standard (S). All the tubes were incubated in a water-bath at 37^0C. After 10 minutes, 2.5 ml of substrate (1mg BAPNA) was added to each tube. The reaction was allowed to proceed for 10 minutes at 37 ^{0}C. The reaction was stopped by adding 0.5 ml of 30 per cent glacial acetic acid. The absorbance was read at 410 nm [21].

Alpha amylase inhibitory activity

Sample was extracted with 5 ml of buffer A and centrifuged at 10,000 rpm for 10 minutes. After centrifugation, pellet was re-suspended in 5 ml of buffer B. The above solution was placed for 30 minutes

in shaking water bath and centrifuged at 10,000 rpm for 20 minutes. Supernatant was then incubated at 70˚C for 20 minutes. After the protein precipitation the content were centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected for the estimation of α- amylase inhibitor content. Two hundred microlitres of α-AI extract was pre-incubated with 50 µl of the amylase for 30 minutes and 1 ml (1%) starch solution was added before further incubating at 37^0C for 10 min. The reaction was stopped by adding 1 ml DNS reagent and then the contents were heated in a boiling-water bath for 5 min. One ml potassium sodium tartrate (40%) solution was added while the contents were still warm and volume in each tube was made up to 10 ml. A blank was set without α-AI extract, another one without amylase enzyme and replaced by equal quantities of extraction buffer. The absorbance was measured at 530 nm. The reducing sugar released from starch was estimated as maltose (0.5 mg/ml) equivalent from the standard graph [22].

Inhibitory activity (%) = $(A_{control}-A_{sample}) / A_{control} \times 100$

Phytic acid

Sample was taken with 50 ml of 3 % TCA and mixed it for 30 minutes. The suspension was centrifuged and took 10 ml aliquot and 4 ml of $FeCl_3$ was added. The contents were heated for 45 minutes. Then centrifuged it and precipitates were washed twice by dispersing in 25 ml 3 per cent TCA and heated in boiling water for 5 to 10 minutes and centrifuged. The precipitates were dispersed in few ml of water and 3 ml of 1.5 N NaOH added. The final volume was made 30 ml with water and heated for 30 minutesand filtered it. The precipitates were then washed with 60-70 ml hot water. The precipitates were dissolved from the paper with 40 ml hot 3.2 N HNO_3. 5 ml of aliquot was transferred to 100 ml volumetric flask and diluted approximately to 70 ml. 20 ml of 1.5 M KSCN added and color was read immediately at 480 nm [23].

Oligosaccharide

Standard sugars (raffinose, stachyose and verbascose) were used for the preparation of standard curve. For the extraction of the sample, one gram finely ground seed sample was extracted with 10 ml of 70 per cent aqueous ethanol and kept overnight on shaking water bath. The contents were filtered through filter paper. Residue was further washed with 5 ml of 70 per cent aqueous ethanol. The filtrate obtained was pooled and vacuum dried at 45˚C. The concentration sugar syrup was dissolved in 5 ml of double distilled water. Separation of oligosaccharides was done by TLC. The glass plates were coated with silica gel G slurry and air dried. Spotting of sugar samples was done by using micropipettes. 5 µl aliquots of each sample were spotted separately. The plates were developed by using a solvent system of butanol, acetic acid, ethyl acetate and water in the ratio 40:25:30:40 and dried. The plates were sprayed with 1 per cent α- naphthanol reagent and dried in hot air oven. The separated spots were compared with standard sugar spots. Separated sugars that appeared were raffinose, stachyose and verbascose [24].

The sugar spots were scrapped from TLC plates and eluted in distilled water. The eluted individual oligosaccharides were estimated by the method of Tanaka et al. [25]. 1 ml of eluted sugar solution was treated with 1 ml of 0.2 M thiobarbituric acid and 1 ml of concentrated HCl. The tubes were boiled in water bath for exactly 6 min. After cooling, the oligosaccharide contents were quantified at 432 nm.

Data analyzed statistically by using analysis of variance [26].

RESULT

Proximate Composition

The proximate composition of different *Vigna* species is presented in Table 1. *Vigna umbellata* had maximum moisture content and *Vigna mungo* revealed minimum moisture content as compared to other species. The maximum value for ash content was observed in *Vigna unguiculata* (3.87 %) and minimum value for ash content was observed in *Vigna umbellata* (3.10 %). The crude fat content was recorded lowest in *Vigna angularis* and highest valiue in *Vigna radiata*. Whereas, crude fiber content showed variation from 4.02 to 5.44 per cent. The average crude protein content in *Vigna* species varied from 20.48 (*Vigna umbellata)* to 24.45 per cent (*Vigna angularis)*. *Vigna mungo* (59.09%) exhibited higher amount of carbohydrate content whereas, minimum amount of carbohydrate content was observed in *Vigna angularis* (54.97%).

Anti-nutritional Composition

Saponin acts as an anti-nutrient and reduces the nutritive value of pulses. *Vigna mungo* exhibited significantly higher value of saponin content whereas, *Vigna unguiculata* revealed minimum saponin content. Significant variation in saponin content are shown in figure 1.

Table 1 Proximate composition of five different *Vigna species*

Vigna species	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Fiber (%)	Crude protein (%)	Total Carbohydrate (%)
Vigna umbellata	12.17 [1]	3.10 [5*]	1.59 [2*]	5.44 [1*]	20.48 [3*]	57.22 [2*]
Vigna unguiculata	9.92 [3*]	3.87 [1*]	1.43 [3*]	4.85 [2*]	23.87 [1]	56.06 [3*]
Vigna radiata	11.95 [2]	3.72 [2*]	1.78 [1]	4.39 [4*]	23.10 [2]	55.06 [4]
Vigna angularis	12.02 [2]	3.52 [3*]	0.44 [4*]	4.60 [3*]	24.45 [1]	54.97 [4]
Vigna mungo	8.85 [4*]	3.32 [4*]	1.74 [1]	4.02 [5*]	22.98 [2]	59.09 [1*]
CD (5%)	0.12	0.06	0.07	0.13	0.73	0.60
CD* (1%)	0.169	0.08	0.10	0.18	1.04	0.85

*** Significant at CD 1%**

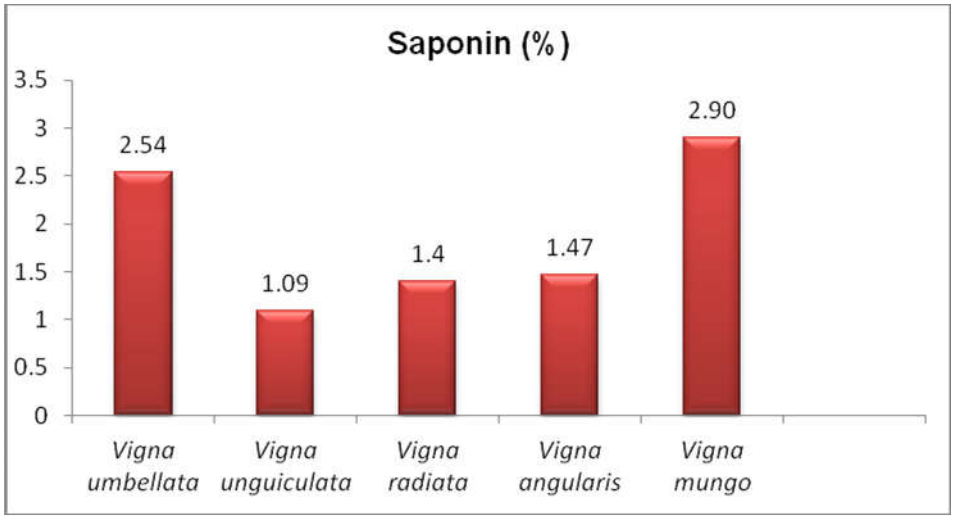

Figure 1. Saponin content in dry mature seeds of *Vigna* species

Vigna radiata exhibited significantly higher amount of trypsin inhibitor content among five *Vigna* species and lowest value was observed in *Vigna mungo*. Whereas, alpha amylase inhibitor content in *Vigna umbellata, Vigna unguiculata, Vigna radiata, Vigna angularis* and *Vigna mungo* were 1560.67 unit/g, 1035.66 unit/g, 986.70 unit/g, 614.33 unit/g and 853.00 unit/g, respectively. The results pertaining to the trypsin inhibitor content and alpha amylase inhibitor content in the seeds of *Vigna* species are presented in Table 2.

Table 2: Protease inhibitor content in dry mature seeds of five *Vigna* species

Vigna species	Trypsin Inhibitor Content (mg/g)	α-amylase Inhibitor (Unit/g)
Vigna umbellata	29.56 [2*]	1560.67 [1*]
Vigna unguiculata	26.58 [3*]	1035.66 [2*]
Vigna radiata	32.46 [1*]	986.70 [3*]
Vigna angularis	23.37 [4*]	614.33 [5*]
Vigna mungo	21.80 [5*]	853.00 [4*]
CD (5%)	0.80	24.88
CD (1%)	1.14	35.38

*** Significant at CD 1%**

The maximum content of phytic acid was revealed by *Vigna mungo* and the lower content was observed in *Vigna umbellata*. The phytic acid content of five *Vigna* species is shown in figure 2.

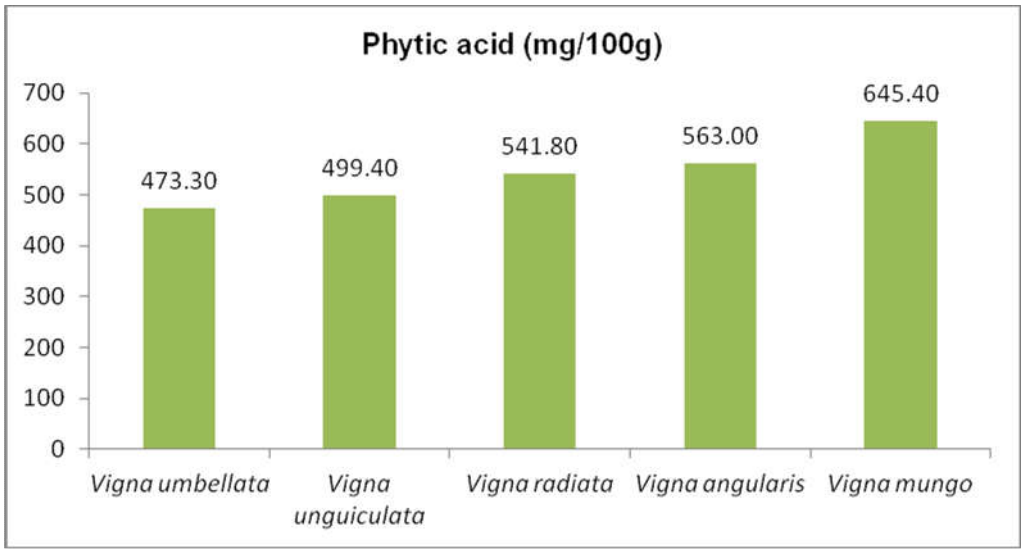

Figure 2 Phytic acid content in dry mature seeds of *Vigna* species

Three oligosaccharides were present in *Vigna* species, were revealed by TLC (Figure 3). In each *Vigna* species, three spots for raffinose, stachyose and verbascose appeared on TLC plate. Rf values of standard raffinose, stachyose and verbascose were 0.59 cm, 0.46 cm and 0.33 cm, respectively.

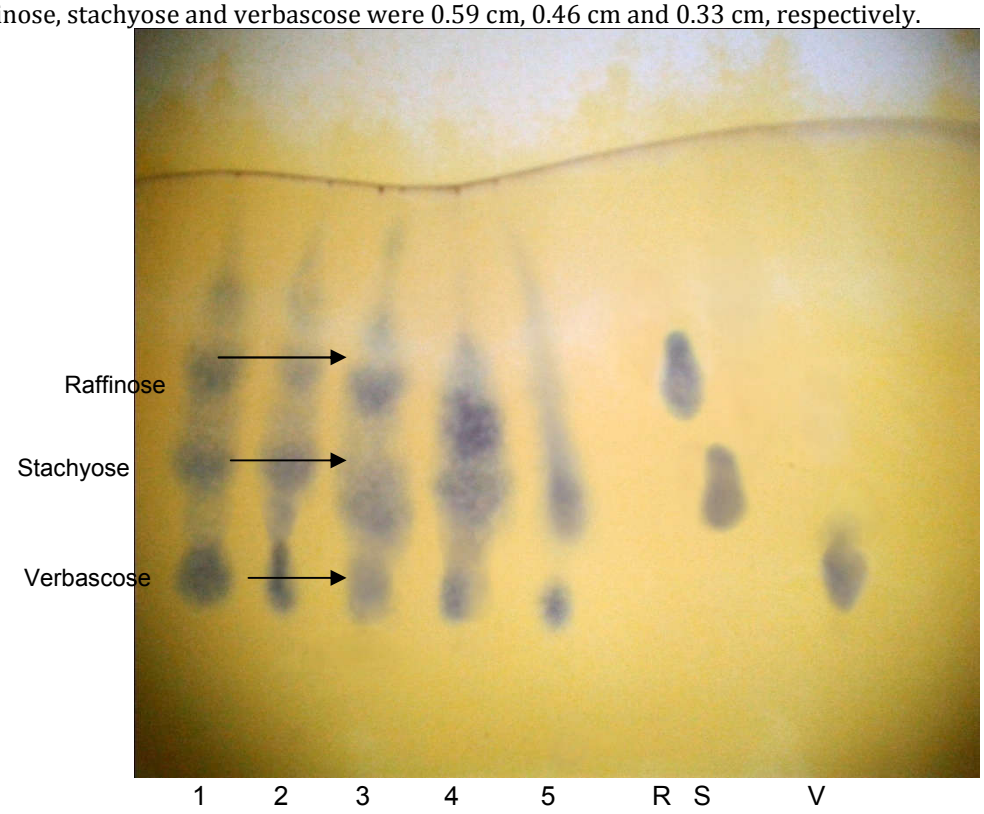

Figure 3 TLC profile of Oligosaccharides in five *Vigna* species

1= *Vigna umbellata*, 2= *Vigna angularis*, 3= *Vigna unguiculata*, 4= *Vigna mungo*, 5= *Vigna radiata*, R= Raffinose, S= Stachyose, V= Verbascose

The maximum value was recorded in *Vigna mungo* and *Vigna radiata* exhibited the lowest raffinose content. Stachyose content, *Vigna radiata* showed lowest and *Vigna mungo* showed highest. *Vigna mungo* exhibited higher value of verbascose and *Vigna radiata* revealed the minimum value for this parameter. The significant variability in the raffinose, stachyose and verbascose content of *Vigna* species are presented in Table 3.

Table 3 Oligosaccharides content in dry mature seeds of *Vigna* species

S. No.	*Vigna* species	Raffinose (%)	Stachyose (%)	Verbascose (%)
1	*Vigna umbellata*	0.84^{3*}	1.14^{3*}	2.05^{3*}
2	*Vigna unguiculata*	1.03^{2*}	1.42^{2*}	2.25^{2*}
3	*Vigna radiata*	0.58^{5*}	0.96^{5*}	1.59^{5*}
4	*Vigna angularis*	0.72^{4*}	1.05^{4*}	1.72^{4*}
5	*Vigna mungo*	1.18^{1*}	1.54^{1*}	2.41^{1*}
	CD (5%)	0.05	0.06	0.06
	CD (1%)	0.07	0.09	0.08

*** Significant at CD 1%**

DISCUSSION

The motive of this study to determine and comparison of the proximate and anti-nutritional composition of five different *Vigna* species and promote their cultivation in India. In case of proximate composition, we evaluated different proximate components such as moisture content, ash, crude fat, crude fiber, crude protein and carbohydrate content.

The moisture content shared variation from 8.85 to 12.17 per cent. The ash content in *Vigna species* varried significantly from 3.10 % to 3.87 %. The crude fat content among different species under study was recorded from 0.44 (*Vigna angularis*) to 1.78 (*Vigna radiata*) per cent. The crude fiber content showed variation from 4.02 to 5.44 per cent. Whereas, the crude fiber content varied from 4.02 % to 5.44 %. The average crude protein content in *Vigna* species varied from 20.48 (*Vigna umbellata)* to 24.45 per cent (*Vigna angularis)*. The total carbohydrate content in *Vigna umbellata, Vigna unguiculata, Vigna radiata, Vigna angularis* and *Vigna mungo* were 57.22 %, 56.06 %, 55.06 %, 54.97 % and 59.09 %, respectively. Thangadurai [27] observed 4.90 % crude fiber content, whereas, Olalekan and Bosede [28] reported carbohydrate content ranging from 56.60 to 57.83 per cent in cowpea. Kakati et al. [29] also reported crude fat content (1.60 to 1.67 % and1.26 to 1.54 %) and crude fiber content (4.07 to 4.12 % and 4.58 to 4.90 %), respectively, in *Vigna radiata* and *Vigna mungo*. Whereas, Awasthi et al. [30] analysed different genotypes of ricebean and observed that crude fat, crude fiber, crude protein and total carbohydrate content in ricebean varied between 0.48 to 1.15 %, 4.6 to 6.7 %, 17.9 to 19.4 % and 58.0 to 61.2 %, respectively. These reported values were in close proximity with the value of present research.

Anti-nutrients are natural or synthetic compounds which may interfere with the absorption of nutrients. These anti-nutrients either reduce the digestibility of pulses or cause toxic effects on their consumption [31].

Saponins are secondary plant metabolites, containing a carbohydrate moiety (mono/oligosaccharide) linked to a hydrophobic aglycone (sapogenin), which may be steroidal or triterpenoid in nature. Saponin acts as an anti-nutrient and reduces the nutritive value of pulses. The saponin content in five *Vigna* species revealed variations from 1.09 (*Vigna unguiculata*) to 2.90 per cent (*Vigna mungo*). Kaur and Kawatra [32] and Katoch et al. [33] also observed 25.50 g/kg and 1.20 to 3.10 mg/100g saponin content in *Vigna umbellata*, respectively. Whereas, El-Adawy [34] also observed 2.30 g/kg saponin content in *Vigna mungo*.

Trypsin inhibitors are present in considerable amounts in legumes, which are known to inhibit the trypsin activity in the insect gut and interfere with digestibility of dietary proteins and reduce their utilization ultimately leading to insect mortality. The seeds with high levels of these inhibitors are generally resistant to the insect infestation. The trypsin inhibitor content of *Vigna* species shared variations from 21.80 to 32.46 mg/g. Sood [35] reported trypsin inhibitor content in different cultivars of *Vigna umbellata* was ranging from 24.07 to 37.23 mg/g. These reported values were similar with the value of *Vigna umbellata* in present research.

α-amylase inhibitor interferes with the activity of amylase enzyme, which is responsible for digestion of starchy food. α-amylase inhibitors block the digestion process in insect pests and ultimately lead to the death of the insects. α-amylase inhibitors are considered as starch blockers due to their mode of inhibitory action. alpha amylase inhibitor content in *Vigna* species varied significantly from 614.33 Unit/g (*Vigna umbellata*) to 1560.67 Unit/g (*Vigna angularis*). Similar result was reported by Priya [36], who reported variation in α-amylase inhibitor content from 1469.00 to 3180.00 unit/g in *Vigna umbellata*.

Phytic acid acts as an antinutrient because it binds with enzymes of digestive tract and reduces the rate of digestion of starch and protein. It has strong binding affinity to important minerals such as calcium, iron and zinc and makes them unavailable to the body by reducing their absorption. Phytic acid content in *Vigna* species varied significantly from 473.30 to 645.40 mg/100g. Katoch [37] reported 3.20 to 6.40 per cent phytic acid content in *Vigna umbellata*. Farinu and Ingrao [38] also observed 5.10 to 10.27 g/kg

phytic acid content in *Vigna unguiculata. Whereas,* Suneja et al. [39] also observed phytic acid content in *Vigna mungo* from 1.70 to 9.00 mg/g. The phytic acid content in present research was in close proximity with reported values.

Indigestible substances especially flatulence induced oligosaccharides (α- galactosides e.g. raffinose, stachyose and verbascose) occur mainly in legume seeds. In our study showed three oligosaccharides spots observed in *Vigna* species such as raffinose, stachyose and verbascose by TLC plate method, Rf values of standard raffinose, stachyose and verbascose were 0.59 cm, 0.46 cm and 0.33 cm, respectively. Out of which *Vigna mungo* showed prominent spots whereas, lowest in *Vigna radiata*, these spots were small in size. Girigowda and Mulimani [40] reported 0.57 cm, 0.42 cm and 0.30 cm Rf values for raffinose, stachyose and verbascose, respectively.

Raffinose content in different *Vigna* species was observed from 0.58 to 1.18 per cent. Stachyose content in *Vigna* species varied significantly from 0.96 per cent (*Vigna radiata*) to 1.54 per cent (*Vigna mungo*). Among all *Vigna* species, content of verbascose showed variation from 1.59 to 2.41 per cent. Soris et al. [41] reported raffinose and stachyose content ranging from 1.12 to 1.22 g/100g and 0.94 to 1.02 g/100g, respectively, in *Vigna mungo*. Girigowda et al. [42] reported verbascose content ranging from 1.12 to 3.32 g/100g in *Vigna mungo*. The present research was in accordance with the values reported by these workers.

CONCLUSION

From the present comparison it may be conclude that *Vigna* species contain nutritional and antinutritional components. The cumulative grading revealed that *Vigna radiata* and *Vigna angularis* have lowest amount of anti-nutritional constituents and *Vigna angularis* have highest amount of protein content as compared to other species. So The results of the study could be utilized further for value addition and crop improvement programmes ensuring nutritional security.

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CONFLICT OF INTEREST

There is no conflict of interest.

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