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Comparative Study of Bioactive components of underutilized and common *Vigna* species prevalent in Himachal Pradesh

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ABSTRACT
In the present investigation entitled "Comparative Study of Bioactive components of underutilized and common Vigna species prevalent in 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 bioactive constituents of five Vigna species were determined by using standard procedures. Bioactive components viz. total phenols (549.56-1126.07 mg/100g), simple phenols (155.17-573.32 mg/100g), total tannins (394.32-687.98 mg/100g), condensed tannin (33.29-89.11 mg/100g), hydrolysed tannin (305.22-645.67 mg/100g), flavonoid (236.11-557.41 µgCE/100g) andphytic acid (473.30-645.40 mg/100g) revealed significant variations among Vigna species.Vigna umbellata showed highest content of ascorbic acid, whereas Vigna unguiculata showed lowest content of ascorbic acid.Maximum antioxidant activity observed by Vigna angularis and minimum activity was observed by Vigna unguiculata.
Keywords: Vigna species, Bioactive componenets

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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 produce many primary and secondary metabolites, which involve for the treatment of various diseases, such as on consumption of legumes are significantly associated with 22% and 11% lower risk of coronary heart disease and cardiovascular disease, respectively [4]. 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 [5].

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" [6]. 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[7]. Cowpea contains about 25% protein and it is rich in amino acids like lysine and tryptophan [8]. 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 [9] and its productivity is 1.04 million metric tons. It is the grain legume of highest digestibility for direct human consumption. The sprouts of mungbean are also excellent source of nutrients and bioactive components, which promote the health and lower the risk of various diseases [10]. 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 [11]. 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 [12]. In Himachal Pradesh, the area under production is 11830 hectares, whereas average yield is 338 kg/hectare [13]. 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.These 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 [14]. *Vigna mungo*are used as cooling astringent, diet during fever, poultice for abscesses, soap alternative, affections of liver and cough [15].

Apart from its nutritional value, *Vigna* species also contain anti-nutritional factors such as phenolic compounds, tannin, phytic acid, protease inhibitors, oligosaccharides etc. Besides negative input of anti-nutritional factors, they also have some beneficial attributes. Polyphenolic compounds such as phenols and flavonoids, phytic acids and saponinshave antioxidative properties which are beneficial for human health. Protease inhibitors *viz*. α- amylase inhibitor and trypsin inhibitor inhibit the activity of trypsin enzyme in the gut of seed eating insects. Therefore, protease inhibitors have great potential as a tool to engineer resistance of crop plants against pests for crop improvement [16]. Recently, legumes are gaining interest because they are excellent sources of bioactive compounds, which play a significant role as a nutraceuticals, pharmaceuticals, pesticides and industrial products. The aim of present research paper is to evaluate the phytochemical properties of five different *Vigna* species.

MATERIAL AND METHODS

The seeds of *Vigna* species were procured from Department of Crop Improvement, CSK Himachal Pradesh KrishiVishvavidyalaya, 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.

Phenolic components

Total phenols, simple phenols, condensed tannin, hydrolysed tannin and tannin were estimated by the method given byMakkar et al. (1993) [17] .Sample was taken with 10 ml of 70 per centacetone. Extraction was carried out in shaking water bath for 2 hrs at 37^0C. After extraction, the contents were centrifuged at 10,000 rpm for 20 minutes and filtrate was used for the estimation of total phenol, simple phenol and condensed tannin. From the filtrate, 0.1 ml aliquot was taken for total phenol estimation. To this 2.5 ml of 20 per centNa_2CO_3 was added followed by 0.5 ml of FolinCiocalteau reagent. After 40 minutes incubation, absorbance was recorded at 725 nm. For the estimation of simple phenol, 1.0 ml polyvinyl pyrrolidone (100 mg/ml) was added to 1.0 ml of filtrate and the solution was vortexed for 15 min. under ice cold conditions. From this solution 0.2 ml aliquot was taken and add2.5 ml of 20 per cent Na_2CO_3 was added followed by addition of 0.5 ml FCR. The solution was incubated for 2 hrs at room temperature. The precipitates were filtered and the absorbance of the filtrate was recorded at 725 nm.Tannin content was determined by subtracting simple phenol from total phenol. Condensed tanninwere determined by taking 1.0 ml of filtrate and 3.0 ml n-butanolHCl solution was added. After the addition of 1.0 ml ferric ammonium sulphate solution, absorbance was read at 550 nm. Hydrolysable tannins were calculated after subtracting condensed tannins from the total tannins. Tannic acid (0.1mg/ml) was used as the standard for all the above estimation.

Flavonoid

One gram of defatted sample was taken in 100 ml beaker. To this, 5 ml of 70 per cent ethanol was added and kept in water bath shaker at 65^0C for 1 hr. The contents were centrifuged at 8,000 rpm for 15 minutes.0.5 ml of extract was taken and to this 2 ml of dH_2O was added. It was followed with the addition of 0.15 ml of 5 per cent $NaNO_2$ and incubated for 6 min. After this 0.15 ml of 10 per cent $AlCl_3$ was added and again incubated for 6 min. To this 2 ml of 4 per cent NaOH was added and final volume was made 5 ml with dH_2O. After 15 minutes the absorbance was measured at 510 nm [18].

Phytic acid

Phytic acid content was estimated by the method given by Wheeler and Ferrel(1971) [19]. 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.

Ascorbic acid

For the extraction of ascorbic acid, air-dried powdered sample was ground with 4% oxalic acid. Bromine water was added drop by drop to 10ml of the filtrate until it turned orange-yellow. The filtrate was made up to 25ml with 4% oxalic acid and used for ascorbic acid estimation. 2 ml of the extract was made up to 3ml with distilled H_2O in a test tube. One millilitre of 2% 2, 4-dinitrophenyl hydrazine reagent and a few drops of thiourea were added. After 3h incubation at 37°C, 7ml of 80% H_2SO_4 was added to dissolve the osazone crystals and the absorbance was measured at 540nm against a reagent blank [20].

Antioxidant activity

Antioxidant activity of *Vigna* species were evaluated on the basis of free radical scavenging activity of aqueous extracts of samples using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay[21].

To a known volume (20,40,60,80,100 µl) of ascorbic acid (2mg in 50 ml of methanol)and sample solution taken in different sets of test tubes respectively, methanol was added to make the final volume of 3 ml in each test tube. 1 ml of 200 µM DPPH solution prepared in methanol was added to the test tubes containing methanolic solutions of ascorbic acid and sample solution. The contents were mixed thoroughly with the help of vortex and allowed to incubate at 30^0C for 30 min. in the dark. Absorbance was measured at 517 nm with the help of Merck SpectroquantPharo 100 spectrophotometer. DPPH free radical scavenging activity (% inhibition) was calculated with the help of following equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100 \quad \text{------- (i)}$$

Graphs were plotted between per centInhibition and concentration of ascorbic acid to evaluate the values of slope and y-intercepts.

IC_{50} value (the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%) for ascorbic acid in methanol was evaluated using the following equation:

IC_{50}Value = (50–y-intercept)/slope -------- (ii)

RESULTS AND DISCUSSION

Plants having high phenolic content are not considered consumer friendly as they result in impaired nutritional quality, lower digestibility and reduction of food consumption. The variability in total phenolic content in *Vigna* species was evaluated and pertinent data on this aspect is presented in Table 1. The total phenolic content among different *Vigna* species revealed variations from 549.56 mg/100g to 1126.07 mg/100g. The minimum content for total phenol was observed in *Vigna unguiculata*and maximum content for total phenol was recorded in *Vigna angularis.* Saharan et al. (2002) [22] reported phenolic content ranging from 750.00 to 1698.00 mg/100g in seeds of *Vigna umbellata*. Whereas, Adeyemi and Olorunsanya (2012) [23] observed 0.20 to 1.38 per cent total phenol content in *Vigna unguiculata*.

Significant variation in simple phenols content in different *Vigna species* was observed to range from 155.17 mg/100g (*Vigna unguiculata*) to 573.32 mg/100g (*Vigna angularis*). The level of simple phenol contents were in close observance with the values (0.18 to 0.35 per cent) reported by Katoch (2011) [24] in different genotypes of *Vigna umbellata*.

Table 1 Variation in phenolic content in dry mature seeds of five different *Vigna* species

***Vigna* species**	**Total phenol (mg/100g)**	**Simple phenol (mg/100g)**	**Tannin (mg/100g)**	**Condensed tannin (mg/100g)**	**Hydrolysed tannin (mg/100g)**
Vigna umbellata	793.83[3*]	215.88[2]	577.95[2]	33.29[5]	544.65[2]
Vigna unguiculata	549.56[5*]	155.17[3]	394.32[4*]	89.11[1*]	305.22[4*]
Vigna radiata	680.26[4*]	161.65[3*]	518.61[3]	69.54[2*]	449.07[3]
Vigna angularis	1126.07[1*]	573.32[1*]	552.75[2,3]	55.97[3*]	496.78[2,3]
Vigna mungo	903.80[2*]	215.82[2]	687.98[1*]	42.31[4]	645.67[1*]
CD (5%)	48.48	11.16	49.12	8.18	51.63
CD (1%)	68.94	15.87	69.87	11.64	73.43

*** Significant at CD 1%**

Each value represents mean of three replicates. In the same column, significant differences according to CRD are indicated by different numbers. Same numbers represent that their values are statistically at par

The tannin content varied from 394.32 (*Vigna unguiculata*) to 687.98 mg/100g (*Vigna mungo*) among five *Vigna* species. Saikia et al. [25], who observed tannin content in *Vigna umbellata* ranging from 513.00 to 572.00 mg/100g.Awasthi et al. (2011) [26] also observed 490.00 to 860.00 mg/100g of tannin content in *Vigna umbellata*.

The condensed tannins content in *Vigna* species showed variation from 33.29 to 89.11 mg/100g. The minimum value for condensed tannins was observed in *Vigna umbellata* and maximum value was exhibited by *Vigna unguiculata.*Priya [27] reported condensed tannin content in *Vigna umbellata* ranging from 0.03 to 0.10 per cent.

The hydrolysed tannin content in *Vigna* species revealed variations from 305.22 mg/100g (*Vigna unguiculata*) to 645.67 mg/100g (*Vigna mungo*). The hydrolysed tannin content revealed variations in *Vigna umbellata*(544.65 mg/100g), *Vigna unguiculata*(305.22 mg/100g), *Vigna radiata*(449.07 mg/100g), *Vigna angularis*(496.78 mg/100g) and *Vigna mungo* (645 mg/100g). Priya [27] observed variation in hydrolysed tannin content from 0.25 to 0.46 per cent in *Vigna umbellata*. Sood [28] also reported hydrolysed tannin content ranging from 0.17 to 1.83 per cent in *Vigna umbellata*.

Flavonoids are also secondary metabolites in plants with polyphenolic structure. Common flavonoid groups include aurones, xanthones and condensed tannins. Significant variations in flavonoid content were observed in five different *Vigna* species ranging from 236.11 (*Vigna unguiculata*) to 557.41 µgCE/g (*Vigna angularis*), shown in figure 1.

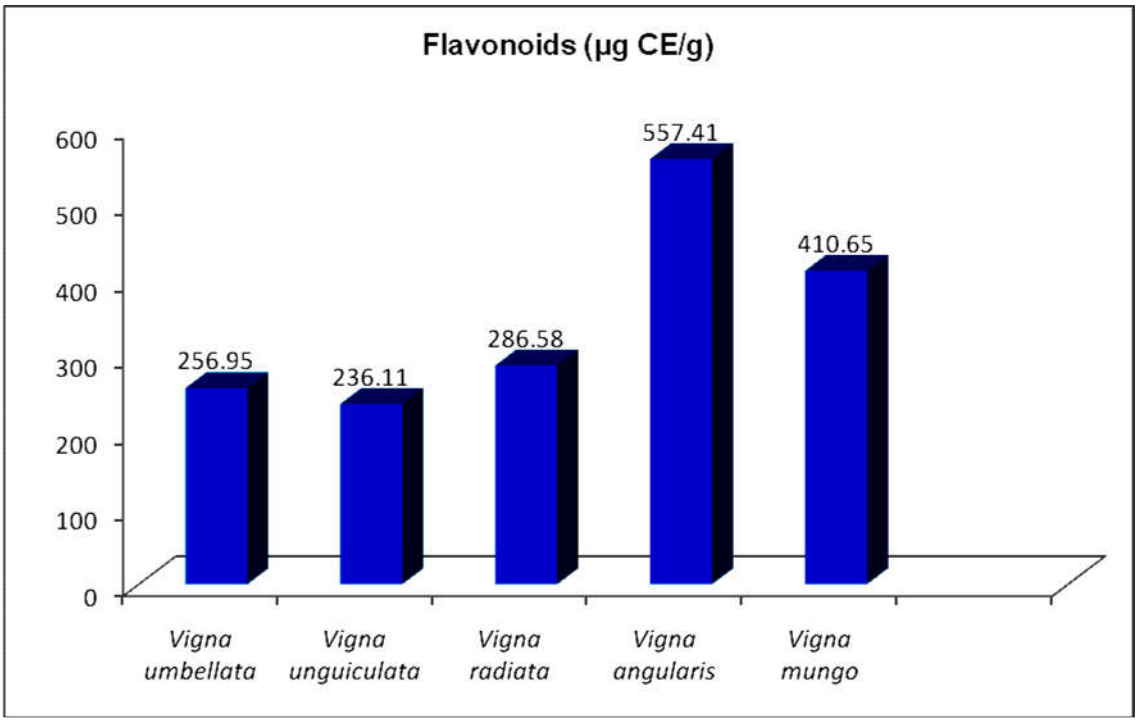

Figure 1.Flavonoid content in dry mature seeds of five *Vigna* species

The flavonoid content in *Vigna umbellata* was 256.95 µgCE/g. The flavonoid content in the present study was in close proximity to value reported by Yao et al. (2012) [29] who reported the variations from 55.95 to 294.52 µgCE/g in different varieties of *Vigna umbellata*. The flavonoid content in *Vigna unguiculata* was 236.11 µgCE/g. Adeyemi and Olorunsanya [23] also reported flavonoid content ranging from 0.06 to 0.28 per cent in *Vigna unguiculata*.

Phytic acid content in *Vigna* species varied significantly from 473.30 to 645.40 mg/100g. The maximum content of phytic acid was revealed by *Vigna mungo* and the lower content was observed in *Vigna umbellata*.Katoch [24] reported 3.20 to 6.40 per cent phytic acid content in *Vigna umbellata*. Farinu and Ingrao [30] also observed 5.10 to 10.27 g/kg phytic acid content in *Vigna unguiculata.* The phytic acid content of five *Vigna* species is shown in figure 2.

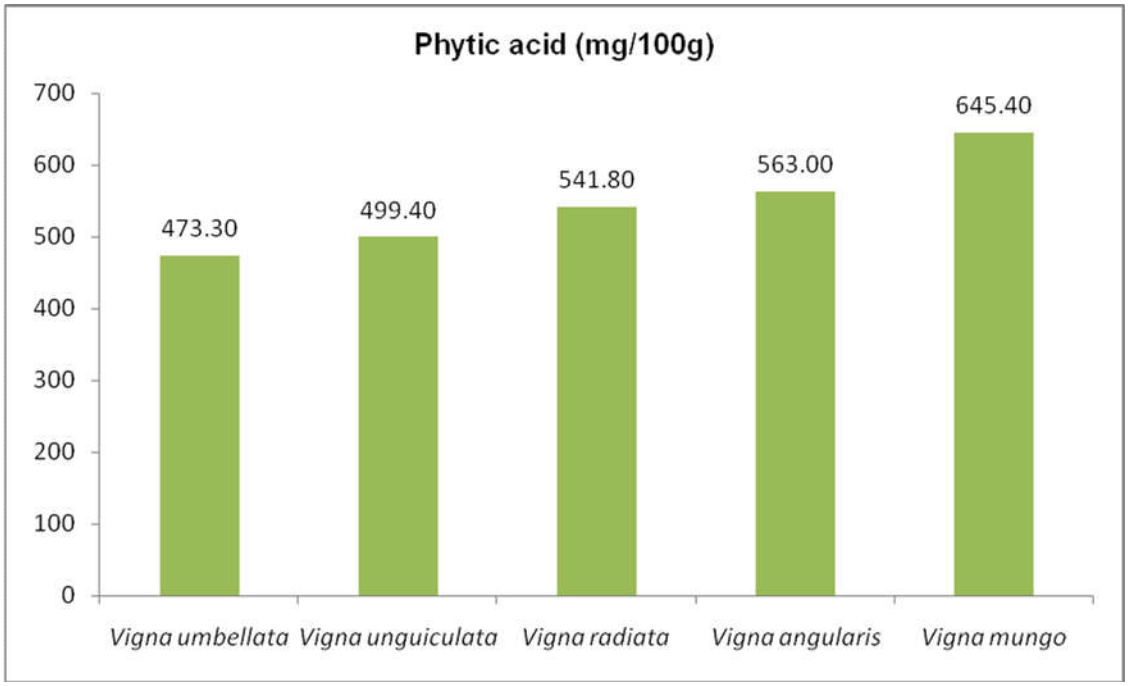

Figure 2Phytic acid content in dry mature seeds of *Vigna* species

Variation in ascorbic acid content was observed in different *Vigna* species are shown in Figure 3. Ascorbic acid content in *Vigna* species was ranged from 0.54 mg/100g (*Vigna unguiculata*) to 0.95 mg/100g (*Vigna umbellata*). Similar result was reported byOboh (2006) [31], who observed 0.50 to 0.90 mg/100g of ascorbic acid in *Vigna unguiculata.*

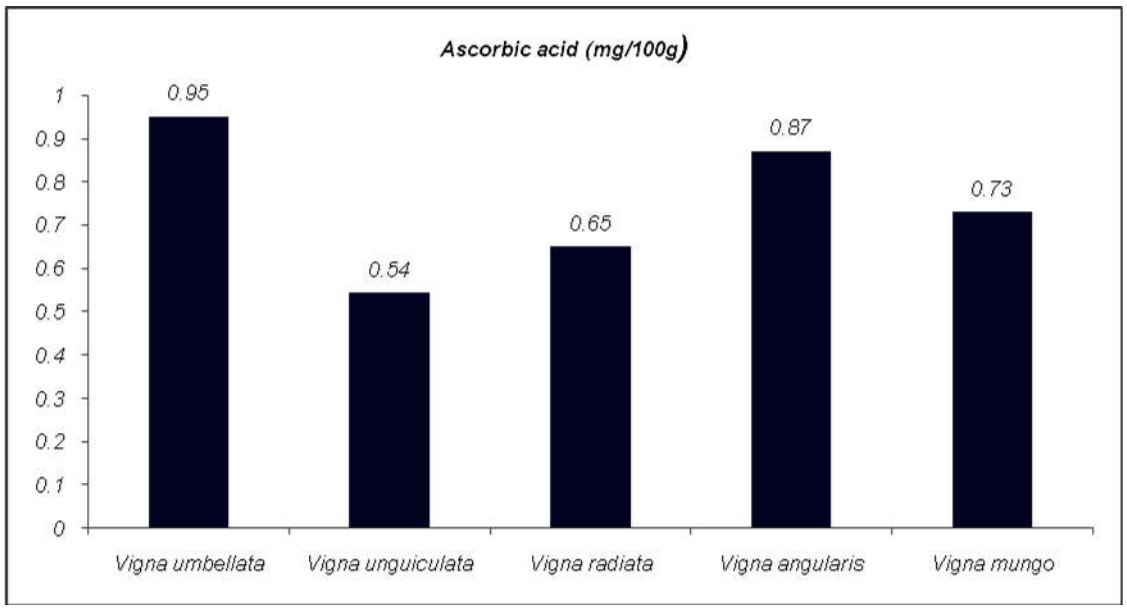

Fig 3. Variation in ascorbic acid content in *Vigna* species

Antioxidant activity

Free radicals are produced by the process of oxidation. These free radicals are very unstable molecules due to unpaired electron and cause oxidation of protein, nucleic acid, lipids and initiate many degenerative diseases. Antioxidant prevent or remove the damaging oxygen molecules from interacting with cellular molecules before they damage and lead to disease.

Vigna species contains different nutrients such as polyphenols, phytic acid, tannin and ascorbic acid, which act as antioxidant. These antioxidants play important role for the treatment of chronic diseases such as cancer, heart disease, stroke, rheumatoid arthritis and cataracts.

The data regarding variation in DPPH free radical scavenging activity of the aqueous extract of *Vigna*

species, in terms of mean of IC_{50} values are presented in Table 2. Significant variation in IC_{50} (mg/ml) value in *Vigna umbellata, Vigna unguiculata, Vigna radiata, Vigna angularis* and *Vigna mungo* was observed, which varied from 0.057 to 0.116 mg/ml.

Among five *Vigna* species, *Vigna unguiculata* exhibited significantly higher IC_{50} value and the lowest value was revealed by *Vigna angularis*. *Vigna umbellata* and *Vigna radiata* was statistically at par with each other at 5 per cent level of significance, while *Vigna mungo, Vigna angularis* and *Vigna radiata* were statatistially at par with each other at 1 per cent level of significance.

Those species, which revealed low IC_{50} value, had high DPPH free radical scavenging ability. *Vigna angularis* exhibited highest antioxidant activity among all species whereas, *Vigna unguiculata* revealed low antioxidant activity.

Vigna umbellata revealed 0.090 mg/ml IC_{50} value. *Vigna unguiculata* exhibited 0.116 mg/ml IC_{50} value, this value was in close proximity with the value reported by Zia-Ul-Haq et al. (2013) [32], who observed IC_{50} value 80.60 to 92.40 µg/ml in *Vigna unguiculata.* The IC_{50} value of *Vigna unguiculata* was to some extent higher than the reported values.

Table 2 Antioxidant activity in dry mature seeds of *Vigna* species

***Vigna* species**	**IC_{50} mg/ml**
Vigna umbellata	0.090[3]
Vigna unguiculata	0.116[1*]
Vigna radiata	0.086[3]
Vigna angularis	0.057[4*]
Vigna mungo	0.097[2]
CD (5%)	0.006
CD (1%)	0.008

*** Significant at CD 1%**

Each value represents mean of three replicates. In the same column, significant differences according to CRD are indicated by different numbers. Same numbers represent that their values are statistically at par.

In *Vigna radiata* 0.086 mg/ml IC_{50} value was observed, which was slightly higher than the result reported by Kim et al. (2012) [33], who observed 787.00 µg/ml IC_{50} value in *Vigna radiata*. *Vigna angularis* revealed lowest amount of IC_{50} value (0.057 mg/ml). *Vigna mungo* recorded 0.097 mg/ml IC_{50} value. Antioxidant activity was depending upon phenolic compound. Those species which had high phenolic compound showed high antioxidant activity [29]. *Vigna angularis* exhibited highest amount of total phenol and flavonoid content, so it showed high antioxidant activity.

CONCLUSION

From the present comparison it may be conclude that *Vigna* species are valuable source of protein, carbohydrate and mineral and vitamins, besides these important nutrients, *Vigna* species also contain biologically active components including phenols, phytic acid,flavanoids, ascorbic acid and also show antioxidant acivity.Though *Vigna* species has various medicinal applications and it can be used as a nutraceuticals. The cumulative grading revealed that *Vigna mungo*and *Vigna angularis*have highest amount of bioactive constituents and *Vigna unguiculata*have lowest amount as compared to other species. So the results of the study could be utilized further for value addition and crop improvement and to develop genetically engineered plants to control the amounts of specific bioactive compounds.

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