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Comparative study on phenolic content, flavonoid content, and antioxidant activities of five species of the genus *Phaseolus*

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ABSTRACT

The current study was designed to assess five species of the genus *Phaseolus* for phenolic content, flavonoid content, and antioxidant ability. The antioxidant capacity of the sample extracts was assessed using different antioxidant models such as ferric reducing antioxidant power (FRAP), DPPH free radical scavenging, phosphomolybdenum reducing power, ferrous ion chelating activity, hydrogen peroxide radical scavenging, hydroxyl radical scavenging, deoxyribose degradation, and β-carotene bleaching assays. The results obtained discovered that the concentration of phenolics and flavonoids in the studied species ranged from 1.11 to 4.01mg TAE/g plant material and 0.11 to 1.16 mg QE/g plant material. The antioxidant activity of the extracts varied in a wide range in the different antioxidant assays depending on the genotype as well as the polarity of the solvents used to obtain the extracts. Ethanolic and aqueous extracts exhibited the maximum amount of phenolics and flavonoids among the solvents. The species studied exhibited a significant range of phenolics, flavonoids, and antioxidant capacity. Hence, the present investigation can provide a new direction by utilizing *Phaseolus* species to formulate cost-effective, eco-friendly, and value-added therapeutic products.

1. Introduction

Phaseolus (Fabaceae) is one of the most important genera encompassing herbaceous to woody annuals and perennial vines comprising about 70 species all indigenous to the Americas, mainly Meso America. The species are a rich source of carbohydrates and proteins and a significant source of vitamin B complexes such as riboflavin, thiamine, niacin, and folic acid. It also provides zinc, copper, iron, phosphorous, calcium, potassium, and magnesium additionally has a high fiber content (Rocha-Guzman and Gallegos-Infante, 2007). It is also an imperative source of polyphenols such as flavonoids, isoflavones, lignans, and tannins. These compounds offer a protective role in humans owing to their strong tendency to scavenge free radicals (Câmara et. al., 2013; Bezuhla et. al., 2018).

The seeds of *Phaseolus* species were expended by humans worldwide as a dynamic source of proteins and fiber (Onyilagha and Islam, 2009). The consumption of seeds has previously been associated with a reduced risk of cardiovascular diseases, diabetes, and even certain types of cancer (Curran, 2012; Hayat et al., 2014). Some researchers reported that legume seeds are medicinally important due to their antioxidant, anticancer, antimicrobial, antiobesity, cardioprotective, hepatoprotective, and antiproliferative activities (Zhu et. al., 2012; Guajardo-Flores et al., 2013; Zou and Chang 2014). Several articles have appeared in the literature focused on the antioxidant potential of common beans (Amarowicz et al., 2008; Aknod et. al., 2011). Some studies evaluated the seeds of the most economically important species, *P. vulgaris*, for the presence of seed coat anthocyanin glycosides (Choung et al., 2003) and polyphenols (Espinosa-Alonso et al., 2006). The findings of Suárez-Martínez et al. (2016) reported *P. vulgaris* as a nutraceutical source for human health with favorable effects against cancer because of the antimutagenic and antiproliferative properties of their phenolic compounds, lectins, and protease inhibitors. The accessible literature revealed that *P.*

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vulgaris was explored pharmacologically and largely overlooked at the biological and biochemical levels among the various *Phaseolus* species. The beneficial effects of other species are not well investigated. Therefore, the current research was designed to evaluate five different species of the genus Phaseolus for the presence of phenolics, flavonoids, and their potential antioxidative effect.

2. Materials and methods

2.1. Plant material collection

The *Phaseolus* species, namely *P. vulgaris*, *P. aureus*, *P. mungo*, *P. trilobus*, and *P. aconitifolius,* were procured from the local market of Vijayapura district of Karnataka and authenticated by Dr. Sidanand Kambhar.

2.2. Preparation of extracts

The seed extracts of different species of *Phaseolus* were prepared in four different solvents (distilled water, ethanol, methanol, and acetone). 2.5 grams of dried seeds were treated with 25 ml of distilled water, and then the extract obtained was agitated and left overnight in a shaker. The extracts acquired were then centrifuged at 8.000 rpm for 20 minutes, and the supernatant was collected. The final volume of the collected supernatants was attuned to 25 ml by adding more distilled water. A similar technique was used to prepare ethanol, methanol, and acetone extracts. All the prepared extracts were preserved in a refrigerator at 4 °C, and for the tests, one percent extracts were used.

2.3. Total phenolic content (TPC)

The total phenolic content in the seed extracts was examined by the Folin-Ciocalteu method (Wolfe et al., 2003). The reaction mix was prepared by adding 125 µl of Folin-Ciocalteu reagent and 1.25 ml of Na_2CO_3 solution to an aliquot of the sample extracts (0.125 ml). The reaction mixture was then placed at normal temperature for 90 min, and the absorbance of each sample was recorded at 760 nm. All the samples were analyzed in 3 replicates, and the average value of the absorbance was attained. A calibration graph was plotted using tannic acid reference compound (10 µg/ml to 100 µg/ml, R^2 = 0.992). The results were interpreted as mg tannic acid equivalents (TAE)/g of sample.

2.4. Total flavonoid content (TFC)

The total flavonoid content in the extracts was studied following Luximon-Ramma et al. (2002). The reaction mix was devised by adding 1.5 ml of extract to 1.5 ml of methanolic $AlCl_3$ (2%), and the samples were then retained at room temperature for 10 min. After that, the absorbance was recorded at 420 nm. The samples were prepared in triplicates, and the average value was calculated. A similar technique was followed for the reference compound quercetin, and the standard graph was plotted (10 µg/ml to 100µg/ml, R^2 = 0.920). The data obtained was articulated as milligram of quercetin equivalents (QE)/g of sample.

2.5. Ferric reducing antioxidant power assay (FRAP assay)

Each extract's ferric ion reducing ability was assessed following the method defined by Pulido et al. (2000). The reaction blend was prepared by adding an aliquot (100 µl) of seed extracts to 3 ml of FRAP reagent (1 part of 20 mM $FeCl_3.6H_2O$ solution, 1 part of 10 mM TPTZ solution, and 10 parts of 300 mM sodium acetate buffer at pH 3.6). After 15 min of reaction time at 37 °C, the absorbance was measured at 595 nm. Quantification was expressed by recording the absorbance in the calibration curve of the reference compound ascorbic acid (10 µg/ml to 100 µg/ml, R^2 = 0.980). The results were interpreted as milligrams of ascorbic acid equivalent per gram of the sample.

2.6. DPPH free radical scavenging assay

The extracts' DPPH free radical (1,1-diphenyl-2-picrylhydrazyl) scavenging ability was measured by using the technique devised by Aquino et al. (2001). The reaction medium containing seed extracts (25 µl) and 3 ml of 25 mM DPPH solution was incubated for 30 min in the dark at room temperature, and the absorbance was recorded at 515 nm against a blank. The DPPH radical scavenging ability as percent inhibition was calculated as Scavenging activity (%) = [(Ac-As)/Ac] x 100, where Ac is the absorbance of the control and As is the absorbance of the sample.

2.7. Phosphomolybdenum reducing power assay

The phosphomolybdenum reducing activity of the extracts was assessed by Prieto et al. (1999). A mixture was prepared by mixing 300 µl of the extract with 3 ml of the phosphomolybdate reagent (0.6 M sulphuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate) and placed in a water bath at 90 °C for 90 min. The mixture was chilled to normal temperature, and the absorbance was recorded at 695 nm against a blank. A calibration curve was obtained using ascorbic acid as a positive control (10 µg/ml to 100 µg/ml, R^2 = 0.953), and the results were reported as ascorbic acid equivalents (AAE) per gram of sample.

2.8. Ferrous ion chelating assay

The ferrous ion chelating activity was evaluated by following the procedure devised by Dinis et al. (1994). For the assay, 100 µl of 2 mM $FeCl_2$ and 300 µl of 5 mM ferrozine were mixed with sample extracts, and the blend was then equilibrated for 15 min at normal temperature. The absorbance of the reaction mixture was measured at 562 nm on a spectrophotometer. The tendency of the extracts to chelate transition metal ions was estimated by using the formula of inhibition percentage as employed for DPPH· free radical scavenging activity.

2.9. Hydrogen peroxide scavenging assay

The hydrogen peroxide scavenging capacity of the extracts was measured as per Zhao et al. (2006). An aliquot of 200 µl of sample and 200 µl of 40 mM H_2O_2 were mixed, followed by the addition of 200 µl of ammonium molybdate (3%), 200 µl of 2 M H_2SO_4, and 1.4 ml of 1.8 M KI. The reaction mixture was titrated against 5.09 mM NaS_2O_3 till the yellow color disappeared. The ability of the extracts to scavenge hydrogen peroxide was quantified as percentage (%) of the titer volume change [(Volume of control-Volume of the sample)/Volume of control] x 100.

2.10. Hydroxyl radical scavenging assay

The ability of the extracts to scavenge hydroxyl radicals was estimated following the method devised by Sadasivam and Manikam (1992). The reaction mix was prepared using 60 µl of 1.0 mM $FeCl_3$, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 0.15 ml of 0.17 M H_2O_2, 0.09 ml of 1mM 1,10-phenanthroline and 1.5 ml of extracts. The tubes were placed at room temperature for 10 min,

and the absorbance of the mixture was recorded at 560 nm. The hydroxyl radical scavenging capacity of the extracts was calculated using the equation: (Ac-As)/Ac x 100, where Ac is the absorbance of the control and As is the absorbance of the sample.

2.11. Deoxyribose degradation assay

Deoxyribose degradation activity of the extracts was evaluated by Halliwell et al. (1987) procedure, with some modifications. The assay mixtures, containing the samples, 50 µl of deoxyribose (50 mM), 50 µl of Na_2EDTA (1 mM), 300 µl of phosphate buffer (0.2M, pH 7.4), 50 µl of H_2O_2 (50 mM) and 50 µl of $FeCl_3$ (3.2 mM). The reaction was started by adding 50 µl of ascorbic acid (1.8 mM), and the final volume of the reaction medium was attuned to 800 µl with buffer. The reaction was completed by adding 250 µl of TCA (10%, w/w) after 20 min incubation at 50 °C. The color was then developed by adding 150 µl of TBA (5%, in 1.25% NaOH) and heating in an oven at 105 °C for 15 min. The reaction mixture was then allowed to cool at normal temperature, and the absorbance was measured at 530 nm against blank. Inhibition of deoxyribose degradation was estimated using the inhibition percentage formula as employed for DPPH· free radical scavenging activity.

2.12. β-carotene bleaching assay

β-carotene bleaching activity of the sample was evaluated following the method described by Wettasinghe and Shahidi (1999). A stock solution of 1ml of β-carotene and linoleic acid was prepared with 0.2 mg of β-carotene in 1 ml of chloroform, 20 µl of linoleic acid, and 200 µl of 100% Tween 20. The chloroform was then vaporized at 40 °C for 10 min using a rotary evaporator, and then 100 ml of distilled water was added to the remainder. The distilled water was added gently to the mix to form a suspension. 3 ml of the suspension was taken out in separate tubes with 200 µl of samples, and immediately, the zero time absorbance was recorded at 470 nm (t = 0). Then, the tubes were retained in a water bath at 50 °C for 2 h; the absorbance was measured again at 470 nm (t = 120 min) against the blank. Inhibition percentage of bleaching was estimated using the following formula (Miller, 1971):

$$\text{Inhibition \%} = 1 - \left[\frac{A1_{(t=0)} - A1_{(t=120)}}{A0_{(t=0)} - A0_{(t=120)}} \right] \times 100$$

Where:
A1: Absorbance of the sample
A0: Absorbance of the control
t = 0: Absorbance at zero time
t = 120: Absorbance after 120 min

2.13. Statistical analysis

The experimentations were executed in triplicates, and the results were interpreted as average ± standard deviation. The One-way ANOVA test was employed to elucidate the mean difference for the different species, and the $p < 0.05$ values were considered significant. The results were further subjected to Pearson's correlation coefficient of phenolics and flavonoids with different antioxidants using the GraphPad InStat and MS Excel.

Table 1. Total phenolics and flavonoid content in different species of *Phaseolus*

Species	Total phenolic content (mg TAE/g plant material)				Total flavonoid content (mg QE/g plant material)			
	Aqueous	Ethanol	Methanol	Acetone	Aqueous	Ethanol	Methanol	Acetone
P. aconitifolius	2.12 ± 0.09	4.01 ± 0.06	1.41 ± 0.07	1.11 ± 0.06	0.17 ± 0.005	0.26 ± 0.008	0.17 ± 0.005	0.13 ± 0.004
P. aureus	2.27 ± 0.06	2.34 ± 0.11	1.90 ± 0.10	2.06 ± 0.04	0.65 ± 0.002	0.61 ± 0.003	0.60 ± 0.002	0.65 ± 0.005
P. mungo	3.48 ± 0.07	3.58 ± 0.11	2.16 ± 0.61	2.20 ± 0.04	0.98 ± 0.003	0.74 ± 0.005	0.54 ± 0.004	0.52 ± 0.005
P. trilobus	1.37 ± 0.10	3.27 ± 0.06	3.21 ± 0.06	2.09 ± 0.04	0.13 ± 0.003	0.36 ± 0.004	0.27 ± 0.002	0.11 ± 0.004
P. vulgaris	1.44 ± 0.35	1.73 ± 0.06	1.48 ± 0.06	1.18 ± 0.12	0.45 ± 0.004	1.16 ± 0.671	0.26 ± 0.003	0.51 ± 0.002

Values are expressed as mean ± SD of triplicate measurements.
mg TAE/ g plant material: Milligram tannic acid equivalent per gram plant material.
mg QE/g plant material: Milligram quercetin equivalent per gram plant material.

3. Results and discussion

3.1. Phenolic and flavonoid content

The phenolics and flavonoids are responsible for the antioxidant properties of many plants. These highly reactive compounds act by scavenging free radicals, preventing the activation of procarcinogens, or by binding carcinogens to macromolecules (Krishnaswamy, 1996). Hence, it is imperative to study different genotypes of *Phaseolus* for total phenolics and flavonoid content. The concentration of total phenolics in the sample extracts is inferred as tannic acid equivalent/g of sample (TAE), and the content of total flavonoids is articulated as quercetin equivalent/g of sample (QE). The content of phenolic compounds in the species ranged from 1.11 to 4.01 mg TAE/g (Table 1), and the significant results were observed in the relative order *P. aconitifolius* > *P. mungo* > *P.trilobus* > *P. aureus* > *P. vulgaris*. Comparison among the species displayed that the species *P. aconitifolius* (4.01 mg TAE/g) showed higher content of phenolics than that of other species, and among the solvents, ethanolic extracts exhibited the maximum content of phenolics as compared to other solvent extracts.

Correspondingly, the content of flavonoids in the species ranged from 0.11 to 1.16 mg QE/g (Table 1). The species *P. vulgaris* (1.16 mg QE/g), *P. trilobus* (0.36 mg QE/g), and *P. aconitifolius* (0.26 mg QE/g) exhibited a maximum concentration of flavonoids in the ethanol extracts. In *P. mungo* (0.98mg QE/g) and *P. aureus* (0.65 mg QE/g), the content was observed to be maximum in the aqueous extracts. Among the species, *P. vulgaris* exhibited the highest flavonoids content compared to the other species. Among the solvents, the ethanol and aqueous extraction systems were the most suitable extraction systems as they exhibited the highest flavonoid contents compared to the other solvent systems. Thus, the results of the examined species for the phenolics and flavonoid content displayed the presence of an ample amount of phenolics and flavonoids contributing to its antioxidant activities. Certain reports have revealed that the main flavonoids in some varieties of *P. vulgaris* are kaempferol and quercetin, and both the compounds have been shown to decrease the risk of cardiovascular diseases and lung cancer (Espinosa-Alonso et al., 2006; Chávez-Mendoza and Sánchez, 2017). Various authors have reported that differences in phenolics and flavonoid composition among the species could be related to the color of the seed. However, detailed studies advocate that variability in phenolic content is more due to genotype than

seed color. In addition, environmental conditions can also affect the phenolics and flavonoid content of the seeds (Bezuhla et. al., 2018; Pitura and Arntfield, 2019; Rodríguez Madrera et al., 2020).

3.2. Ferric reducing antioxidant power (FRAP)

As estimated by FRAP assay, the ferric reducing ability of the *Phaseolus* species discovered that the activity varied among the species (Figure 1) and was found to be highest in *P. aureus* (22.0 mg AAE/g). However, a comparison of solvent extracts showed that all the species except *P. aureus* possessed the highest antioxidant activities in ethanol extracts compared to other solvent extracts. While *P. aureus* showed the maximum activity in the aqueous extract, the highest ferric reducing power of *P. aureus* is possible because of the presence of hydroxyl groups in the phenolic compounds, presumably acting as an electron donor. The antioxidant activities of phytochemicals comprising phenolics and flavonoids testified in the current study specified that the occurrence of reductants in the plant extracts of *Phaseolus* species triggered the reduction of the Fe^{3+}-TPTZ complex to the ferrous (Fe^{2+}) form, clearly suggesting that the extracts are significantly contributing to the observed antioxidant activity and the species possessed variable but considerable antioxidant and antiradical activities.

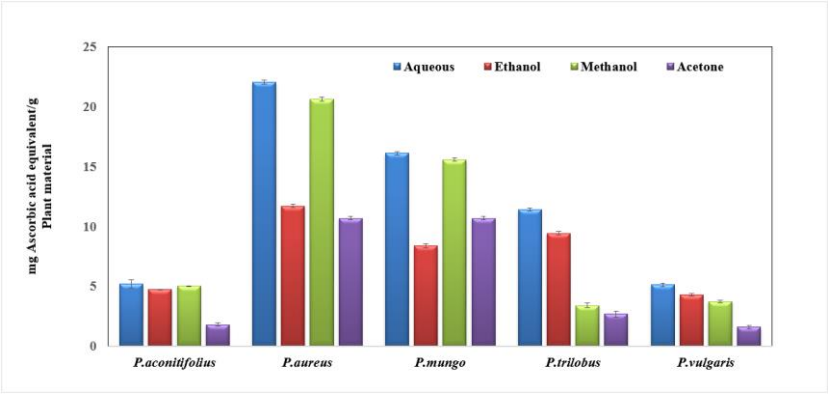

Figure 1. Ferric reducing antioxidant power (FRAP) of various *Phaseolus* species

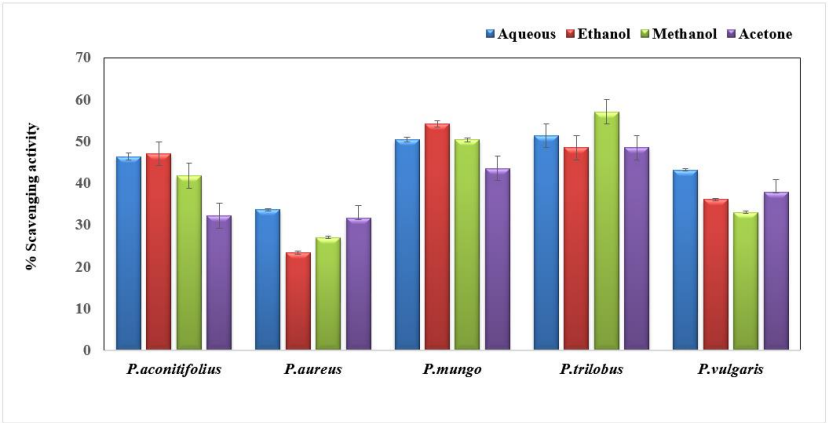

Figure 2. DPPH free radical scavenging activity of various *Phaseolus* species

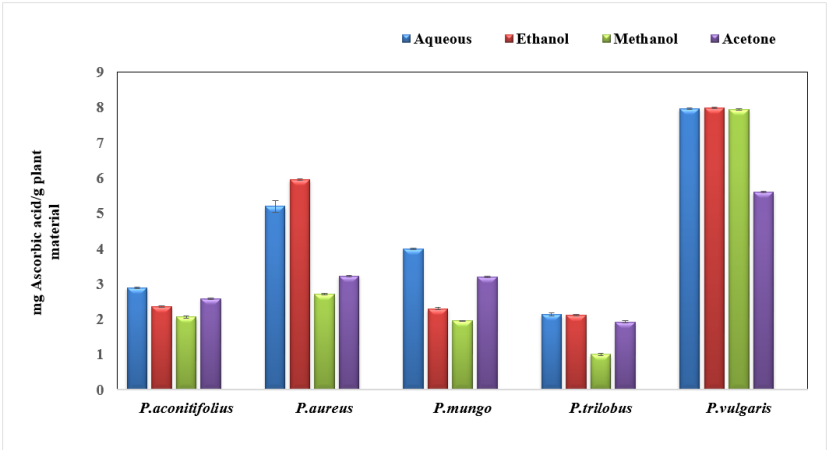

Figure 3. Phosphomolybdenum reducing power of various *Phaseolus* species

3.3. DPPH free radical scavenging activity

The DPPH free radical scavenging activity of the examined extracts displayed that *Phaseolus* species showed varied free radical scavenging activity (Figure 2). The scavenging effect of the species was found to be in the order: *P. trilobus* (57.0 %) > *P. mungo* (54.1 %) > *P. aconitifolius* (46.9 %) > *P. vulgaris* (43.1 %) > *P. aureus* (33.6 %). However, all the samples displayed the radical scavenging activity, yet, the methanol extract of *P. trilobus* exhibited relatively higher scavenging capability than other solvent extracts. Further, the ethanol and methanol extracts exhibited the highest activities among the solvents. Thus, the results indicated that all the extracts were facilitated as radical scavengers to some extent. Among the species, *P. trilobus* exhibited the highest scavenging activity; this high scavenging property of *P. trilobus* is presumably due to the presence of hydroxyl groups in the phenolic compounds that can offer the essential components as a radical scavenger (Gyamfi et al., 1999). Phytocompounds capable of implementing this response can be regarded as antioxidants and so radical scavengers (Dehpour et al., 2009).

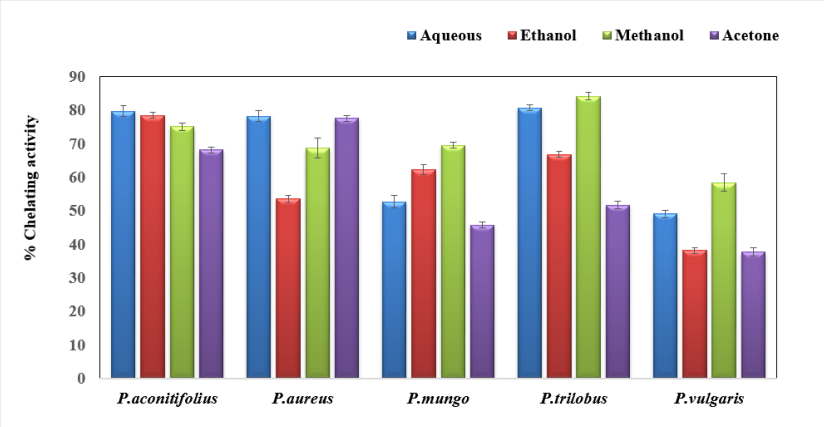

Figure 4. Ferrous ion chelating activity of various *Phaseolus* species

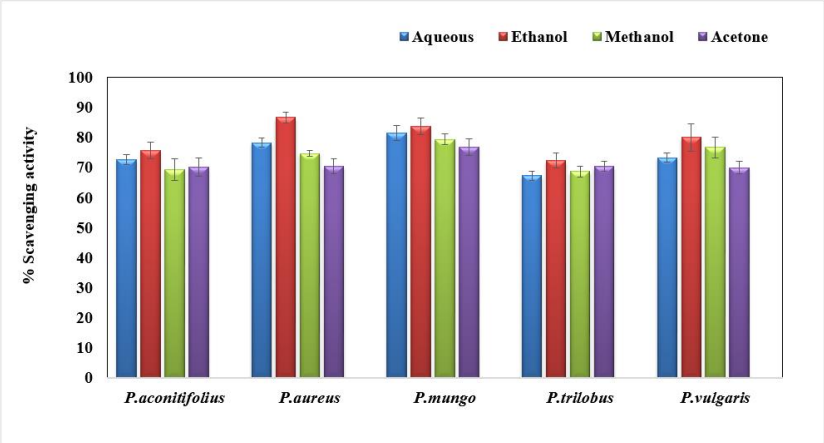

Figure 5. Hydrogen peroxide radical scavenging activity of various *Phaseolus* species

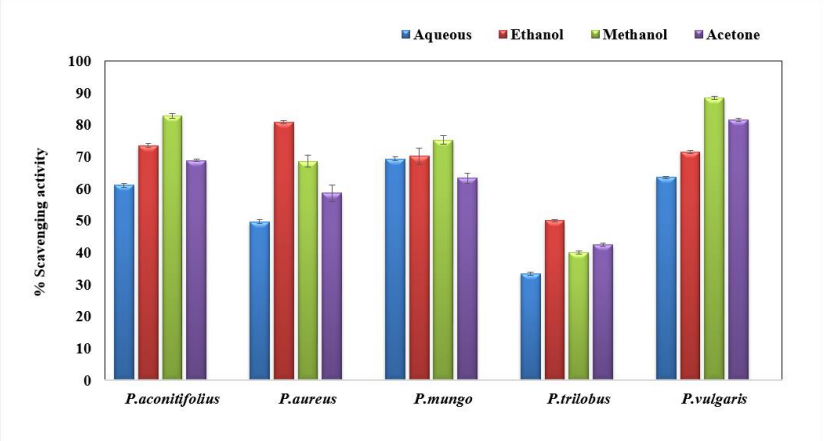

Figure 6. Hydroxyl radical scavenging activity of various *Phaseolus* species

3.4. Phosphomolybdenum reducing power

The reducing ability of the extracts estimated by phosphomolybdenum method revealed that the ethanol extracts of *P. vulgaris* (8.00 mg AAE/g), *P. aureus* (5.96 mg AAE/g), and *P. aconitifolius* (2.89 mg AAE/g) exhibited higher phosphomolybdenum reduction, while, in *P. mungo* (4.00 mg AAE /g) and *P.trilobus* (2.13 mg AAE/g) the highest reduction power was observed in aqueous and methanol extracts respectively (Figure 3). Thus different solvent extracts of different species possessed varied reducing power. Several studies have specified that the electron donation capacity of the phytochemical constituents present in the plants is linked with the antioxidant capacity (Lee et al., 2015; Gupta et al., 2016). The chemical constituents with reducing power revealed that they could donate electrons and reduce the oxidized intermediates of the lipid peroxidation process in such a way that they can act as primary and secondary antioxidants (Reische et al., 2008).

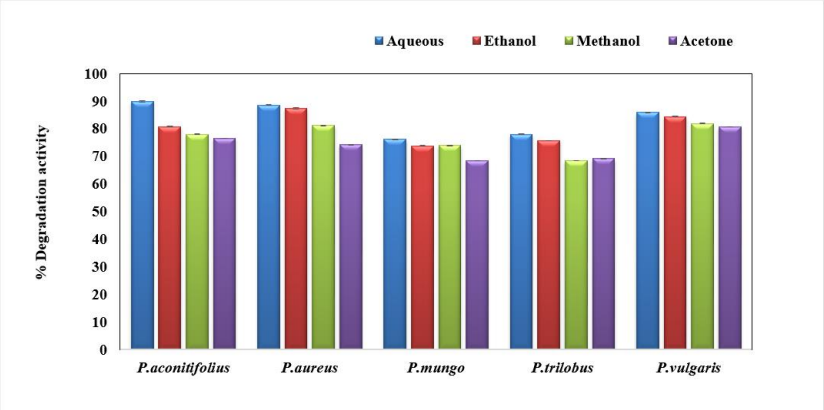

Figure 7. Deoxyribose degredation activity of various *Phaseolus* species

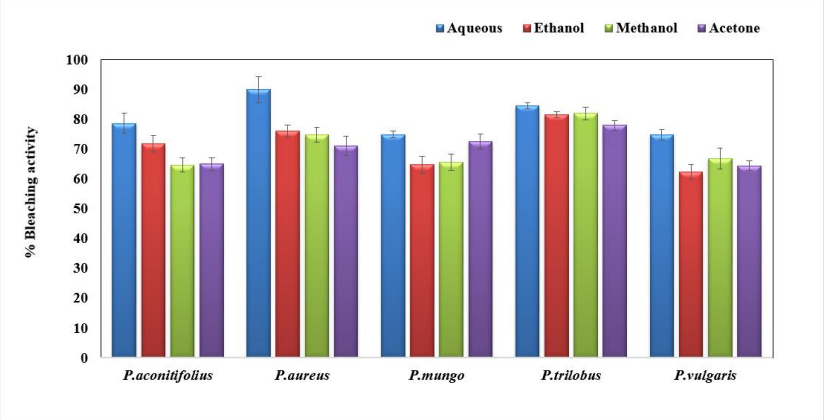

Figure 8. β-carotene bleaching activity of various *Phaseolus* species

3.5. Ferrous ion chelating activity

The assessment of *Phaseolus* species for chelating capacity revealed that the chelating effect of the extracts on ferrous ions ranged from 37.6% to 91.9% (Figure 4). The chelating capacity varied widely among the solvents; ethanol extracts of *P. vulgaris* chelated the least ferrous ions, whereas methanol extracts of *P. trilobus* chelated the most. Based on the chelating ability of the diverse species, the activity was observed as *P. trilobus* (84.1%) > *P. aconitifolius* (79.6%) > *P. aureus* (78.1%) > *P. mungo* (69.5%) > *P. vulgaris* (58.2%). Interestingly, the results revealed that the *Phaseolus* species exhibited potent chelating ability. The antioxidants prevailing in the plant extracts also form a coordination complex with the metal ions and thus, impeding the donation of electrons. This oxidation reaction gets detained, and no free radicals are formed. The degree to which phytochemicals can form metal ion complexes mainly depends on their chemical structures. Different phytochemicals have different chelating abilities (Arfan et al., 2012). The chelating compounds may also sterically hinder the formation of the metal hydroperoxide complexes (Reische et al., 2008).

3.6. Hydrogen peroxide radical scavenging activity

The capability of *Phaseolus* species to scavenge hydrogen peroxide displayed varied scavenging activity in the different species (Figure 5) and was found to be in the order *P. aureus* (86.6%) > *P. mungo* (83.6%) > *P. vulgaris* (80.0%) > *P. aconitifolius* (75.6%) > *P. trilobus* (72.3%). The activity varied among the solvent extracts of different species and among the solvents the extracts prepared in ethanol showed the extreme scavenging activity in all the species as compared to other solvent systems confirming that ethanol extraction makes it possible to extract better or even preserve the molecules accountable for the antioxidant capacity of the studied species. As Shah et al. (2014) described, different extracts yield differently, depending upon the nature of solvents. The current study results indicated that the extracts might contain constituents capable of inhibiting the hydrogen peroxide. The secondary metabolites present in plants may have the capability to counteract the effect of hydrogen peroxide generation and thus averting the adverse effects of excess hydrogen peroxide production in the human body (Halliwell et al., 2000).

3.7. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging ability of the extracts is attributed to the antioxidant capacity of the extracts; therefore, it has been determined in different species of *Phaseolus* (Figure 6). It was observed from the figure that the activity varied amongst the species and the solvents. The activity was in the order: *P. vulgaris* (88.5%) > *P. aureus* (80.8%) > *P. aconitifolius* (82.8%) > *P. mungo* (75.3%) > *P. trilobus* (50.0%). Ethanol extracts from the individual species showed comparatively more scavenging activity than other solvent extracts. The current study results specified that the different solvent extracts of *Phaseolus* species revealed different effects on the scavenging of hydroxyl radicals. Accordingly, the outcomes of the current study specified that the *Phaseolus* species were found to be more potent in quenching superoxide anion radical and hindering deoxyribose degradation induced by hydroxyl radical.

Table 2. ANOVA for antioxidant activity among different solvent extracts of five *Phaseolus* species determined by different antioxidant assays

Antioxidant assays	df	MS	*p*
FRAP	4	122.5	0.0008
DPPH	4	335.3	6.9100
MoO_2P reduction	4	19.73	8.5800
Fe^{2+} chelation	4	579.4	0.0095
H_2O_2 scavenging	4	72.59	0.0185
·OH scavenging	4	743.9	0.0010
Deoxyribose degradation	4	110.0	0.0124
β-Carotene bleaching	4	154.3	0.0147

3.8. Deoxyribose degradation activity

As evaluated by deoxyribose assay, the scavenging capacity of Phaseolus species showed diverse degradation ability amongst the species (Figure 7). The percent degradation in the species was perceived as *P. aconitifolius* (89.92%) > *P. aureus* (88.62%) > *P. vulgaris* (85.89%) > *P. trilobus* (78.04%) > *P. mungo* (76.17%). The activity also varied among the solvents in all the species and was highest in the ethanol extract of *P. aconitifolius*, suggesting that certain hydroxyl radicals are scavenged by the antioxidants present in the sample. The phytochemicals present in the sample can inhibit deoxyribose degradation by hydroxyl radicals (You et al., 2007).

Table 3. Comparison between phytochemical constituents and antioxidant activities as represented by correlation coefficient

	P. vulgaris		*P. aureus*		*P. mungo*		*P. trilobus*		*P. aconitifolius*	
	TPC	TFC	TPC	TFC	TPC	TFC	TPC	TFC	TPC	TFC
FRAP	0.894	0.257	0.830	0.017	0.926	0.711	0.831	0.980	0.353	0.493
DPPH	0.967	0.536	0.646	0.055	0.489	0.238	0.574	0.347	0.536	0.561
PMR	0.687	0.027	0.966	0.011	0.815	0.952	0.806	0.410	0.505	0.327
FC	0.663	0.722	0.136	0.555	0.861	0.489	0.520	0.711	0.438	0.435
HPS	0.940	0.344	0.598	0.159	0.805	0.454	0.382	0.378	0.925	0.741
HRS	0.802	0.465	0.011	0.669	0.446	0.145	0.573	0.542	0.406	0.572
DD	0.742	0.512	0.940	0.237	0.396	0.563	0.889	0.744	0.998	0.945
β-CB	0.886	0.667	0.496	0.230	0.975	0.620	0.286	0.717	0.946	0.787

TPC = Total phenolic content; TFC = Total flavonoid content; FRAP = Ferric reducing antioxidant power; DPPH = DPPH radical scavenging; PMR= Phosphomolybdenum reduction; FC=Ferrous ion chelation; HPS=Hydrogen peroxide scavenging; HRS=Hydroxyl radical scavenging; DD=Dexyribose degradation; β-CB= β-Carotene bleaching activity

3.9. β-carotene bleaching activity

The *Phaseolus* species analyzed for the β-carotene bleaching activity revealed that the ethanol extracts of all the species exhibited the maximum activity (Figure 8). The comparison among the species specified that *P. aureus* (89.9%) showed the highest bleaching activity as compared to *P. vulgaris* (74.7%), *P. mungo* (74.9%), *P. trilobus* (84.5%), and *P. aconitifolius* (78.6%). The β-carotene bleaching activity differs in different species of *Phaseolus*. Moreover, several antioxidant compounds in the plants can also impede the amount of β-carotene bleaching by deactivating the linoleate-free radical and other free radicals formed in the system (Siramon and Ohtani, 2007). The rate of β-carotene bleaching can be slowed in the presence of antioxidants. We speculate that the β-carotene bleaching activity of the *Phaseolus* species studied is likely due to the presence of phytocompounds.

3.10. Statistical analysis

The significant mean differences amongst the various solvent extracts of five *Phaseolus* species were assessed by ANOVA, and a significant discrepancy with $p < 0.05$ was considered significant (Table 2). Further, Pearson's correlation coefficient correlation was obtained to describe the relationship of phenolics and flavonoids with antioxidant activities (Table 3). Individually, both positive and negative correlations were observed between phytochemical constituents and antioxidant methods in different species. In *P. vulgaris*, a significant correlation was observed between TPC against FRAP, DPPH, H_2O_2 scavenging, β-carotene bleaching, and ·OH scavenging activity, while TFC showed a good correlation only against ferrous ion chelating activity. The correlation observed between TPC, FRAP and DPPH are in accordance with the data reported by other authors for *P. vulgaris* (Rodríguez Madrera et al., 2021). In *P. aureus*, the correlation of TPC with phosphomolybdenum and deoxyribose degradation activity was significant; however, the coefficient of correlation observed amongst the TFC, and antioxidant activity was poor. In *P. mungo*, the correlation of TPC and TFC with FRAP, phosphomolybdenum reducing, ferrous ion chelating, H_2O_2 scavenging, and β-carotene bleaching activity was observed to be extremely significant. Interestingly, in *P. trilobus,* the correlation of TPC and TFC displayed a significant correlation with FRAP, phosphomolybdenum reducing, ferrous ion chelating, deoxyribose degradation, and β-carotene bleaching activity but was not significantly correlated with other antioxidants. Further, *P. aconitifolius* also showed a significant relationship of TPC and TFC with H_2O_2 scavenging, β-carotene bleaching activity, and deoxyribose degradation. The difference in the correlation between phytoconstituents and the antioxidant

activities specifies the diversity of the group of phytochemical compounds in the different species and their varied responses to different antioxidant methods to assess the antioxidant capacity.

The observed distinct radical scavenging activities of the species can be ascribed to the diverse chemical nature of several phytochemical compounds that may react with different types of free radicals in unique ways (Chun et al., 2003). The positive correlations observed specified that the phenolics and flavonoids are the main contributors to the observed antioxidant activity in the studied *Phaseolus* species. Further, the negative correlation designated that it could be related to other antioxidant compounds present in the species. This difference in correlation might be due to the difference in the stoichiometry of the reactions amongst the antioxidant compounds present in the extracts and the various radicals, which may be considered a reason for the difference in their scavenging ability (Khan et al., 2012). The results are in accordance with others, who have revealed the positive association between the total phenolic content and the antioxidant activity (Sultana et al., 2007; Kim et al., 2008; Oliveira et al., 2009; Chen et al., 2015). The results strongly suggest that the antioxidant activities of different *Phaseolus* species could be attributed to the presence of phytochemical constituents. Many species of the genus *Phaseolus* have also been reported to possess antioxidant activities (Sreeramulu et al., 2009; Capistrán-Carabarin et al., 2019; Alcázar-Valle et al., 2020). Among the antinutritional composites, testified in beans, are oligosaccharides, trypsin inhibitory activity, phytic acids, and lectins. However, the latest studies have stated that trypsin inhibitors and lectins could deliberate health benefits. For example, trypsin inhibitors could opine protection against rotavirus, impede some types of carcinogenesis, and could be used as chemopreventive agents (De Mejia et al., 2003), and lectins may decrease lymphoma growth and could be used as diagnostic markers for tumors, as well as help in the prevention of obesity (Chávez-Mendoza and Sánchez, 2017). Therefore, it can be assumed that the studied *Phaseolus* species have a high biological potential for preventing diseases caused by the free radicals.

4. Conclusions

The current study revealed that the *Phaseolus* species examined possessed significant antioxidant activities. Individually, notable differences were detected among the species, with ranges of variability in terms of phenolics, flavonoids, and antioxidant activity. However, different antioxidant test models showed different levels of antioxidant activity. However, in most of the assays, ethanol and aqueous extracts exhibited the highest antioxidant potential, and, among the species, *P. aureus*, *P. trilobus*, and *P. vulgaris* possessed the highest antioxidant activity. Further, the correlation study revealed that the antioxidant capacity of these species might be due to the existence of phytochemical constituents. Since the species studied have the capability of scavenging the free radicals, thereby; they might serve as potent antioxidants. Thus, the research reported herein highlighted the possible antioxidant capacity of these underutilized beans and their intake, which may contribute considerable amounts of antioxidants to the diet. Further studies are needed to isolate, identify, characterize and explicate the structure of the bioactive compounds.

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Conflict of interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Firdose R. Kolar: Conception, Design of study, Data analysis, Interpretation, Drafting manuscript, Final approval and accountability
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Supplementary File

None.

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