In-Vitro Antioxidant and Pharmacognostic Studies of *Phaseolus vulgaris* (Linn) Seed Coat

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: Pulses have grown increasingly popular as a result of their high nutritional content and phytochemical content. However, before to eating, the seed coats of some legume food items were removed, resulting in the food losing its nutritious content. The study deals with the study of pharmacognostic & physico-chemical profile along with in-vitro estimation of bioactive compounds of *Phaseolus vulgaris* (Linn) seed coat.

Methodology: The morphology of kidney beans was evaluated. Seed length, width, thickness, and surface area were also determined. In physico-chemical parameters extractive value ash value, moisture content, swelling index were recorded. Phytochemical screening displayed the presence of alkaloids, flavonoids, phenol, amino acid, tannins, carbohydrates and saponins. HPTLC & in-vitro estimations were done.

Results: Morphology revealed dark brownish red seed, kidney to oval shaped, medium size and bland taste. Microscopically, the transverse section showed the presence of proteinaceous aleurone cells, macro-sclereids and starch granules with irregular oval shape in the cotyledon specify the energy reservoir of seeds. HPTLC showed the presence of flavonoids in *Phaseolus vulgaris* seed coats. The antioxidant profile revealed TFC (total flavonoid content) as 13.62 mg/g QE (quercetin equivalent) and TPC (total phenolic content) as 32.03 ± 1.50 mg/g GAE. IC50 value

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for vitamin C was found to be 369.03 µg/ml as compared to Phaseolus vulgaris seed coat 423.00µg/ml.

**Conclusion:** The study can serve as a valuable source of information and due to presence of phytoconstituents like flavonoid (quercetin) it could be considered for its neuroprotective activity.

**Keywords:** Red kidney bean; flavonoids; antioxidant; morphology; seed coat.

### 1. INTRODUCTION

Common bean (*Phaseolus vulgaris* Linn.) is the most significant pulse for direct human consumption among grain legumes [1]. People in a large number of countries, particularly in third-world countries, have discovered an alternative to animal protein. Consumers are increasing their protein consumption by choosing plant-based foods, notably legumes, due to a greater awareness of nutritional-dependent illness and the high cost of animal protein sources [2, 3]. The seed coat of pulse crops is routinely removed before to eating or the production of food products. This occurred owing to a lack of customer understanding and awareness of the nutritional significance of the seed's outer shell. Although various studies have shown that several common beans have antioxidant activity, much of the information has been limited to seed without its coat. Many scientific studies have shown that people who eat pulse foods have a lesser risk of developing chronic diseases like diabetes, cardiovascular disease, cancer, obesity [4] and digestive disorders [5], which can be attributed to the presence of naturally occurring powerful antioxidants and dietary fibres.

*Phaseolus vulgaris* (Red kidney bean) have excellent sources of proteins, energy, carbohydrates, minerals and vitamins. It contains flavonoids such as kaempferol [6], quercetin [7], naringin [8], rutin [9] etc. These flavonoids have protective effect in epilepsy. *Phaseolus vulgaris* is used as anti-oxidant, anti-inflammatory [10], anti-diabetes [11], anti-proliferative [12] and effective in neurodegenerative disease such as anti-parkinsonism [13]. To our knowledge, the nutritional value and antioxidant capacity of bean seed coatings are unexplored in relation to their nutraceutical value and health-promoting effects. The aim of this protocol was to explore the phytochemical, physico-chemical components and *in-vitro* antioxidant activity of *Phaseolus vulgaris* seed coat, that can serve as a basis of its future use as an bioactive compound in various diseases.

### 2. PHARMACOGNOSY

#### 2.1 Vernacular Name

Vernacular names are mentioned in Table 1 [14].

#### 2.2 Taxonomical classification

Taxonomical classification has been mentioned in Table 2.

#### 2.3 Nutritional Value

The seeds of *Phaseolus vulgaris* are nutritionally essential and have following crucial components shown in Table 3 [15].

#### 2.4 Phytoconstituents

For various pharmacological activities, the main phenolic compounds in common beans can be summarized as phenolic acids, flavonoids, proanthocyanidins, and coumarins. In brief it contains Quercetin 3-O-glucoside, kaempferol 3-O-glucoside, myricetin, p-coumaric acid derivatives, ferulic acid derivatives, ferulic acid, caffeic acid, vanillin aldehyde [16], Catechin, epicatechin, epigallocatechin, quercetin, naringenin, chlorogenic acid, cichoric acid, coumaric acid, vanillic acid [12].

#### 2.5 Traditional Medicinal Use

The seeds of *Phaseolus vulgaris* are recorded as diuretic chiefly in kidney and heart disease. They are also effective in lenient cases of diarrhoea [17].

#### 2.6 Pharmacological Activities

The extract of *Phaseolus vulgaris* seeds are used as antioxidant and anti-inflammatory [10], anti-diabetic [11], anti-Parkinson [13], anti-proliferative [12], hepatoprotective [18], trypsin, α-amylase [19, 20], analgesic, anti-fertility, litholytic [21] and antidepressant [22].
Table 1. Vernacular name of *Phaseolus vulgaris*

| Languages     | Names                                |
|---------------|--------------------------------------|
| English       | Kidney bean                          |
| Hindi         | Rajma                                |
| Bengali       | Barbati Beej, Raajma                 |
| Telugu        | Chikkuduginjalu, nallachikkudu       |
| Kannada       | Capparadavare                         |
| Oriya         | Baragudi Chhuin, Rajma               |
| Malayalam     | Rajma                                |
| Tamil         | Sigappu Kaaramani                    |
| Urdu          | Lal lobia                            |
| Portuguese    | Feijao (dry), Feijao-vagem (green)   |
| Italian       | Fagiolo, Faxoe, Faisoe (Liguria), Fasoel |
| Spanish       | Caraota, Chaucha                     |

Table 2. Taxonomical classification of *Phaseolus vulgaris*

| Kingdom       | Plantae                                |
|---------------|----------------------------------------|
| Sub-kingdom   | Viridiplantae                          |
| Super-division| Embryophyta                            |
| Division      | Tracheophyta                           |
| Subdivision   | Spermatophtina                         |
| Class         | Magnoliopsida                          |
| Order         | Fabales                                |
| Family        | Fabaceae                               |
| Genus         | Phaseolus                              |
| Species       | *Phaseolus vulgaris* L                 |
| Synonym (s)   | *Phaseolus vulgaris* var. humilis, *Phaseolus aborigineus* Burkart |

Table 3. Nutritional value of *Phaseolus vulgaris*

| Basic components (mg/g) | Fatty acid (mg/g) |
|-------------------------|-------------------|
| Total lipids            | 10.60             |
| Protein                 | 225.30            |
| Carbohydrates           | 612.90            |
| Essential minerals (mg/g)|                   |
| Total saturated         | 1.54              |
| Total monounsaturated   | 0.82              |
| Total polyunsaturated   | 5.86              |
| Vitamins (mg/g)         |                   |
| Ascorbic acid (C)       | 0.045             |
| Thiamine (B1)           | 0.00608           |
| α-tocopherol (E)        | 0.0021            |
| Folate                  | 0.00394           |
| Niacin (B3)             | 0.0211            |
| Phylloquinone (k)       | 0.056 µg/g        |
| Pyridoxine (B6)         | 0.00397           |
| Retinol                 | ---               |
| Riboflavin (B2)         | 0.00212           |
| Total dietary fibre     | 0.1520            |
| Caloric value           | 3.37 kcal/g       |
3. MATERIALS AND METHODS

3.1. Procurement and Authentication

The seeds of *Phaseolus vulgaris* were identified and procured from the local market of Modinagar, Ghaziabad. The material was authenticated by Dr. Sunita Garg, Emeritus Scientist, CSIR-National Institute of Science Communication and Information resources (NISCAIR), Pusa Campus, New Delhi. A voucher specimen was deposited at RHMD, New Delhi.

3.2. Physical Qualitative Characteristics

Red kidney beans were evaluated for their physical qualitative characteristics that include following characteristics of seed viz, colour; shape; size; odour; taste and seed coat pattern.

3.3. Physical Quantitative Characteristics

Quantitative seed descriptors includes the physical evaluation [Table 4] of the following seven characteristics: average of 1 and 100 seed weight; seed length (L); seed thickness (T); seed width (W); diameter; volume and surface area. 1 and 100 seed weight were measured in six repetitions using a digital weighing balance. 10 randomly selected fully developed undamaged seeds were measured in six repetitions using a Vernier calliper (least count of 0.1mm). Length, Thickness and Width were measured from the highest, lowest aligned to hilum, and from hilum to the opposite side respectively. Various diameter means viz. Arithmetic (AMD), Geometric (GMD) and sphericity (ɸ) of kidney bean was calculated using equations [23]. Also, parameters like volume (V) and surface area (S) which depends on axial dimension (length) was calculated for single bean [24].

3.4. Physico-Chemical Parameters

Various physico-chemical parameters were estimated in triplicates, viz. moisture content, extractive values, ash values and swelling index.

3.5. Microscopy

The microscopy was done using optical microscope (Olympus vanoz-s-AH-2, Japan) with various optical magnification and images were captured employing a digital camera.

3.6. Extraction and Phytochemical Screening

3.6.1 Soaking and extraction procedure

The sample was collected and shade-dried at room temperature of about 25±2°C. 250g dry mature seeds of *Phaseolus vulgaris* were soaked overnight, for 16 h, in distilled water, on the proportion of 100 g per 300 mL of water [Fig. 1]. After soaking, seed coats were manually separated from cotyledons. Seed coats were further dried at room temperature, for an average period of 24 hours. Dried coats (7.97gm) were extracted without previous milling, with the ethanol: water (60:40, v/v) solution, followed by sonication. At the end using rotary flash evaporator under vacuum, the extract was concentrated to a semi-solid mass with the recovery of solvent. The traces of the solvents were separated by using lyophilizer.

![Fig. 1. Soaking and extraction of red kidney bean](image_url)
3.6.2 Preliminary phytochemical screening

Screening was performed as per standard protocol and results are depicted in Table 6 [25].

3.7. HPTLC of Bioactive Components

The ethanolic extract and flavonoid fraction were analyzed for the presence of flavonoids by comparing with the Rf value and spectral comparison with co-chromatographic standard compounds, Quercetin [26].

3.8. In-Vitro Estimation of Bioactive Components

3.8.1 Determination of total flavonoids (TF)

The total flavonoid content was confirmed according to the procedure given [27]. 1 ml extract of 1000 μg/ml concentration 4 ml of purified water was mixed and then 0.3 ml NaNO₂ & 0.3 ml AlCl₃ was added to solution after that mixture was incubated for 5 minutes at room temperature. Sodium Hydroxide (2ml) and purified water (2.4ml) was added to the incubated solution and the absorbance was measured at 510 nm with the help of spectrophotometer. Standard curve was used to determine Total Flavonoid content. Quercetin was used as standard and TF content was indicated as Quercetin equivalents (QE) in mg/g of dry sample.

3.8.2 Determination of total phenols

Total phenolic content was evaluated by using Folin-Ciocalteu (FC) reagent. The evaluation was carried out spectrophotometrically as stated by [28] with minimum moderation. In a test tube, 0.1 ml of extract (1mg/ml) was taken and then 1.9 ml distilled water and 1.0 ml of Folin-Ciocalteau’s reagent was added in a test tube, after that 1.0 ml of 100 g/L Na₂CO₃ was added to the solution. The mixture was incubated at room temperature for 2 hours and the absorbance of the solution was measured at 765 nm using spectrophotometer. The standard curve of gallic acid was used to estimation of Total Phenolic Content. The total phenolic compounds of the plant extracts were indicated as gallic acid equivalents (GAE) which showed the phenolic content equal to the gallic acid (mg/g) of dry material.

3.8.3 DPPH radical scavenging assay

According to [29] the free radical scavenging activity was evaluated with the help of an improved DPPH assay. 2.7 mL (0.2 mM) DPPH solution was added to 0.3 mL of the extract of different concentrations. Then, the mixture was shaken efficiently and incubated at room temperature for 1 h in dark before the absorbance was taken at 517 nm.

Percentage inhibition = [(As − Ai)/As] × 100

Where, As is the absorbance of pure DPPH and Ai is the absorbance of DPPH in the presence of different extracts. Vitamin C was used as reference.

4. RESULTS

The physical qualitative characteristics of seeds were evaluated as follows:

1) Seed colour – Dark brownish red
2) Seed shape – Kidney to oval shaped
3) Seed size – Medium
4) Seed coat Pattern – single colour on entire seed
5) Taste – Bland
6) Odour - None

The physical quantitative characteristics were depicted in Table 4.

4.1 Physico-Chemical Parameters

Various physico-chemical parameters were estimated [Table 5] in triplicates. It gives an idea about the quality and purity of crude drugs.

4.2 Microscopy

The cross section in Fig. 2 of soaked whole red kidney bean, visualizes the presence of three cell layers: the cotyledon (A), endosperm (B) and testa /seed coat (C) (helps from mechanical injury, predators & drying out).

The transverse section [Fig. 3] also showed the presence of proteinaceous aleurone cells (blue arrow), macro-sclereids (black arrow), which are important for the absorption of water by the seed are observed. The presence of starch granules (yellow arrow) with irregular oval shape in the cotyledon indicates the energy reserves of seeds.
Table 4. Physical Quantitative Characteristics of *Phaseolus vulgaris*

| Sr. No. | Parameters                                    | Mean ± S.E.M       |
|---------|-----------------------------------------------|--------------------|
| 1.      | Seed length (L in cm)                         | 2.090 ± 0.023      |
| 2.      | Seed thickness (T in cm)                      | 0.887 ± 0.010      |
| 3.      | Seed width (W in cm)                          | 1.150 ± 0.011      |
| 4.      | Arithmetic mean diameter (AMD in cm)          | 1.376 ± 0.012      |
| 5.      | Geometric mean diameter (GMD in cm)           | 1.287 ± 0.011      |
| 6.      | Volume (in cm$^3$)                            | 1.310 ± 0.035      |
| 7.      | Surface area (in cm$^2$)                      | 6.103 ± 0.110      |
| 8.      | Sphericity ($\phi$)                           | 0.616 ± 0.004      |
| 9.      | Weight variation within seed (one seed/g)     | 0.34 ± 0.03 – 0.72 ± 0.03 |
| 10.     | Weight of 100 seeds (in g)                    | 47.06 ± 0.373      |

Table 5. Physico-chemical Parameters of *Phaseolus vulgaris*

| Sr. No. | Parameters                        | Values (% w/w)       |
|---------|-----------------------------------|----------------------|
| A.      | Moisture Content                  | 1.03 ± 0.23          |
| B.      | Extractive values                |                      |
| 1.      | Alcohol soluble extractive value  | 10.46 ± 0.65         |
| 2.      | Water soluble extractive value    | 19.75 ± 0.41         |
| C.      | Ash Values                        |                      |
| 1.      | Total ash                         | 1.87 ± 0.02          |
| 2.      | Acid insoluble ash                | 0.33 ± 0.02          |
| 3.      | Water soluble ash                 | 0.82 ± 0.03          |
| D.      | Swelling index (in cm)            | 1.01 ± 0.06          |

Fig. 2. Optical light microscopy of red kidney bean (cross-section)
Fig. 3. Transverse-section of red kidney bean

Table 6. Phytochemical screening of Phaseolus vulgaris

| Bioactive Components | Result   |
|----------------------|----------|
| Alkaloids            | Present  |
| Carbohydrates       | Present  |
| Flavonoids          | Present  |
| Tannins             | Present  |
| Saponin             | Present  |
| Anthraquinone       | Absent   |
| Phenol              | Present  |
| Steroids            | Absent   |

The percentage yield was determined as follows

Percentage yield = Final weight of dried extract/Initial weight of powder × 100

Percentage yield = 23.30gm / 39gm × 100 = 58.97%.

4.3 Preliminary Phytochemical Screening

Screening was performed as per standard protocol and results are depicted in Table 6.

4.4 HPTLC of Bioactive Components

HPTLC study was performed to standardize the extract of Phaseolus vulgaris seed coats for the presence of flavonoids in Fig. 4. HPTLC fingerprinting was performed by using winCATS software. Sample application was executed by CAMAG Linomat 5 and inert gas spray and methanol as solvent type was used. After application chromatogram developed Twin Trough Chamber 20x10cm with Tol:EA:FA (10:3:1) at 60° C for 5 minutes. CAMAG TLC Scanner 3 was used to detect spots [Fig. 5]. The result from HPTLC fingerprinting scanned at 254nm and 366nm for extract by using CAMAG Reprostar 3 illumination instrument.

4.5 In-vitro Estimation of Bioactive Components

The total flavonoid content & Total phenolic content was confirmed and results were shown in Table 7. In-vitro antioxidant potential and % inhibition was depicted in Table 8 and Fig. 6.
Fig. 4. Spectral comparison of *Phaseolus vulgaris* seed coats with co-chromatographic standard, Quercetin

Fig. 5. HPTLC of active phytoconstituents in *Phaseolus vulgaris* seed coat

Table 7. *In-vitro* antioxidant potential of *Phaseolus vulgaris* seed coat

| *Phaseolus vulgaris* seed coat | Total Flavonoids Content (TFC in mg/g QE) | Total Phenolic Content (TPC in mg/g GAE) |
|-------------------------------|----------------------------------------|----------------------------------------|
|                               | 13.62 ± 0.49                          | 32.03 ± 1.50                           |

Table 8. *In-vitro* antioxidant potential of *Phaseolus vulgaris* seed coat

| IC<sub>50</sub> value | Concentration of Vitamin C (μg/ml) | Concentration of seed coat (μg/ml) |
|-----------------------|-----------------------------------|-----------------------------------|
|                       | 354.93 ± 7.37                     | 429 ± 4.07                        |
5. DISCUSSION

Medicinal plants are very useful and they have been used for more than hundred years by mankind in the prevention and treatment of numerous diseases [30]. From the above context, this study was designed to describe pharmacogностic profile and evaluate antioxidant activity of extract from *Phaseolus vulgaris*. In this study we discussed taxonomical classification, nutritional value in which plant seed contain higher amount of protein, lipids and carbohydrates and other essential components such as macro-minerals and micro-minerals, vitamins and dietary fibres [31].

This manuscript explains the morphology of red kidney bean as physical qualitative characterization, physical quantitative characterization and physico-chemical parameters. These physical characterizations of seed are significant for evaluating the product quality. The result for physical properties i.e. AMD, GMD, volume and surface area depends on axial dimension (length) of seed, whereas sphericity is depended on the lowest volume of the seed. Also, it helps various personals like plant breeders, machine manufacturers, food scientists, etc. Harvesting, grasping, shipment, detaching, aeration, examining, storing, filling and the other measurement prescribed machines and equipment and it is helpful to design relevant machine and equipment. Currently, there is no exclusive standard method is registered in prompting the physical dimensions of farming outcomes [32].

Microscopical examination of seed displayed three cell layers and showed the presence of proteinaceous cells, macro-sclerides and starch granule [33]. The highest percentage yield of hydroalcoholic extract of seed coat of *Phaseolus vulgaris* was obtained 58%. Phytochemical evaluation showed the existence of many bioactive compounds like alkaloids, glycosides, carbohydrates, tannins, saponin, steroids and several phenolic compounds such as flavonoids proteins. This study suggested hydroalcoholic extract of *Phaseolus vulgaris* have antioxidant potential owing to the presence of higher amount of phenol, flavonoids, saponins [34, 35]. In HPTLC, *Phaseolus vulgaris* exerted beneficial effects as compared with Quercetin as standard [36] and its Rf value was found 0.48. In antioxidant profile, higher value of total flavonoid and total phenol contents showed the presence of polyphenolic constituents and recommended its antioxidant action [37]. Scavenging of DPPH is one of the imperative parameters to assess the antioxidant effect of crude extracts. In this study extract exhibited higher percentage of DPPH scavenging activity and the study suggested plant extract contain flavonoids and related polyphenols [38].

6. CONCLUSION

*Phaseolus vulgaris* serve as decisive source of protein, minerals, vitamins, dietary fibres. This study described morphology, microscopy, HPTLC and in-vitro bioactive compound estimation. On the basis of result it is suggested that *Phaseolus vulgaris* have antioxidant property. In future, the present protocol may form the basis for the selection of plant species for further investigation in potent bioactive compounds for in-vivo activities.
NOTE
The study highlights the efficacy of "Herbal extract" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES
1. Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J. Beans (Phaseolus spp.) – Model food legumes. Plant and Soil. 2003;252:55–128.
2. Sasanam S, Paseephol T and Moongngarm A. Comparison of proximate compositions, resistant starch content, and pasting properties of different colored cowpeas (Vigna unguiculata) and red kidney bean (Phaseolus vulgaris). World Acad. Sci. Eng. Technol. 2011;5(9):553-557.
3. Moses O, Olawuni I, Iwouno JO. The proximate composition and functional properties of full-fat flour, and protein isolate of lima bean (Phaseolus lunatus). Sci. Rep. 2012;1(7):1-5.
4. Mudryj AN, Yu N, Hartman, TJ, Mitchell DC, Lawrence FR and Aukema HM. Pulse consumption in Canadian adults influences nutrient intakes. Br. J. Nutr. 2012;108(S1):S27-36.
5. Wang N, Hatcher DW, Tyler RT, Toews R, Gawalko EJ. Effect of cooking on the composition of beans (Phaseolus vulgaris L.) and chickpeas (Cicer arietinum L.). Food Res. Int. 2010;43(2):589-594.
6. Copmans D, Steurs G. Methylated flavonoids as anti-seizure agents: Naringenin 4, 7-dimethyl ether attenuates epileptic seizures in zebrafish and mouse models. Neurochem Int. 2018;112:124-133.
7. Sefil F, Ibrahim Kahraman, Nesilhan Pinar. Ameliorating effect of Quercetin on acute PTZ induced seizures in Rats. International Journal of Clinical and experimental medicine. 2014;7(9):2471-2477.
8. Kola PK, Akula A, Nissanka Rao LS, Danduga RSR. Protective effect of naringin on pentylenetetrazole (PTZ) induced kindling, Epilepsy Behav. 2017;75:114-126.
9. Nassiri M, Reza Martazavi, Fatemeh Samiee-Rad, Esmail Abbasi. The effect of rutin on the development of PTZ kindling and memory retrieval in rats. Epilepsy behave; 2010.
10. Akash, Sumitra S, Shailendra KS. Antioxidant and anti-inflammatory potential of kidney bean (Phaseolus vulgaris L). International research journal of pharmacy; 2018.
11. Luka CD, Olatunda A, Tijjani H, Olisa-Enewe IA. Effect of aqueous extract of Phaseolus vulgaris (red kidney bean) on alloxan induced diabetic wistar rats. International Journal of Science Invention Today. 2013;2(9):292-301.
12. Ombra MN, d’Acierno A, Nazzaro F, Riccardi R, Spigno P, Zaccardelli M, Fratianni F. Phenolic composition and antioxidant and antiproliferative activities of the extracts of twelve common bean (Phaseolus vulgaris L.) endemic ecotypes of Southern Italy before and after cooking. Oxidative Medicine and Cellular Longevity. 2016;13(9):82-98.
13. Rashmi A, Bhakti S, Yash N, Tejas G. Evaluation and comparision of Anti Parkinson activity of methanolic extract of Phaseolus vulgaris with L-DOPA. International journal of Pharmacology Research. 2015;103-108.
14. Porcher MH. Phaseolus vulgaris L. In: Multilingual multiscript plant name database; 2013. Available:http://www.plantnames.unimelb.edu.au/Sorting/Phaseolus.html [Cited 2016 14th Oct].
15. USDA. 2005. National Nutrient Database for Standard Reference Release 27: Basic Report 16032, Beans, Kidney, red, mature seeds, raw.
16. Lin LZ, Harnly JM, Pastor-Corrales MS, Luthria DL. The polyphenolic profiles of common bean (Phaseolus vulgaris L.). Food Chemistry. 2008;107(1):399–410.

17. Borkar A, More AD. Induced flower colour mutations in Phaseolus vulgaris Linn through physical and chemical mutagens. Adv Biore. 2010;1(1):22-8.

18. Oomah BD, Corbe A and Balasubramanian P. Antioxidant and anti-inflammatory activities of bean (Phaseolus vulgaris L.) hulls. Journal of Agricultural and Food Chemistry. 2010;58(14):8225-8230.

19. Reynoso C, Ramos G, Loarca P. Bioactive components in common beans (Phaseolus vulgaris L.): Advances in Agricultural and Food Biotechnology. In: GuevaraGonzález RG, Torres-Pacheco I, ed. Research Signpost. Trivandrum, Kerala, India. 2006;217-236.

20. Marshall JJ, Lauda CM. Purification and properties of phaselamin, an inhibitor of alpha-amylase from the kidney bean Phaseolus vulgaris. Journal of Biological Chemistry. 1975;250(20):8030-8037.

21. Rana JH, Patel UR, Patel SJ, Lambole V, Shah, DP. Pharmacological activities of Phaseolus vulgaris: A review. Pharma Science Monitor. 2016;7(2):107-115.

22. Madhu S, Ramica S. Antidepressant activity of aqueous extract of Phaseolus vulgaris in rodent models of depression. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2014;4:118-124.

23. Mohsenin NN. Physical properties of plant animal material II. NEW YORK USA: Gordon and Breach Sci. 1986;841-881.

24. Jain RK, Bal D. Properties of pearl millet. J Agric Engg Res. 66 (2):85-91.

25. Kokate CK, Purohit AR, Gokhale SB. Pathway to Screen Phytochemical nature of Natural Drugs. Pharmacognosy, 42nd ed, Nirali Prakashan, Pune. 2008;56-61.

26. Harborne JB. Phytochemical methods, A guide to modern techniques of plant analysis. 3rd ed, Springer Pvt. Ltd. New Delhi, India. 1998;40-107.

27. Kim D, Jeong S, Lee C. Antioxidant capacity of phenolic phytochemicals from various cultivars of common bean. Food Chemistry. 2003;91(6):321-326.

28. Slinkard K, Singleton VL. Total phenol analysis: Automation and comparison with manual methods. Am J Enol Vitic. 1977;28:49-55.

29. Lillian B, Telma C, Paula B, Leticia ME, Isabel F. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food and Chemical Toxicology. 2008;46:2742-2747.

30. Baldivia DDS, Leite DF, De Castro DTH, Campos JF, Dos Santos UP, Paredes-Gamero EJ, Carollo CA, Silva DB, De Picoli SK, Dos Santos EL. Evaluation of in vitro antioxidant and antiproliferative properties of aqueous extract from stem bark of Stryphnodendron adstringens. Int J Mol Sci. 2018;19:2432.

31. Zainish MS, Salman A, Muhammad MH. Phaseolus vulgaris L.: Botany, medicinal uses, phytochemistry and pharmacology. World Journal of Pharmaceutical Research. 2016;5:1611-1615.

32. Waziri AN, Mittal JP. Design–related physical properties of selected agricultural products. Food and Agriculture Organization of the United Nations. 2013;14:59-62.

33. Joseph SG, Barry GS. Microstructure of black, green and red gram. Food Structure. 1993;12(2):155-162.

34. Peerzada AM, Ali HH, Naeem M, Latif M, Bukhari AH, Tanveer A. Cyperus rotundus L.: Traditional uses, phytochemistry, and pharmacological activities. J Ethnopharmacol. 2015;174:540-560.

35. Akhtar S, Ismail T, Fraternale D, Sestili P. Pomegranate peel and peel extracts: Chemistry and food extracts. Food Chem. 2015;174:417-425.

36. Mishra J, Singh RD, Jodon VS, Gusein MP, Aradhana. HPTLC profile of Quercetin content of common bean, American Journal of Food Technology. 2012;7(2):96-100.

37. Khorasani EA, Mat TR, Mohajer S, Banisalam B. Antioxidant activity of total phenolic and flavonoid content of various solvent extracts from in vivo and in vitro grown Trifolium pratense L. (Red Clover). BioMed Res Int. 2015;643285.
38. Nambooppha B, Photichai K, Wongsawan K, Chaummitri P. Quercetin manipulates the expression of genes involved in the reactive oxygen species (ROS) process in chicken heterophils. J Vet Med Sci. 2018;80:1204-1211.