Original Research Article

In-vitro Antioxidant and Pharmacognostic Studies of _Phaseolus vulgaris (Linn)_ seed coat

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.

Result: 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. IC_{50} value for vitamin C was found to be 369.03 µg/ml as compared to *Phaseolus vulgaris* seed coat 423.00 µg/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*, that can serve as a basis of its future use as a bioactive compound in epilepsy.

2. PHARMACOGNOSY

2.1 Vernacular name: Vernacular names are mentioned in Table 1 [14].

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

2.2 Taxonomical classification: Taxonomical classification has been mentioned in Table 2.

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

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

Table 3: Nutritional value of *Phaseolus vulgaris*

Basic components (mg/g)		**Fatty acid (mg/g)**	
Total lipids	10.60	Total saturated	1.54
Protein	225.30	Total monounsaturated	0.82
Carbohydrates	612.90	Total polyunsaturated	5.86
Essential minerals (mg/g)		**Vitamins (mg/g)**	
Macro-minerals		Ascorbic acid (C)	0.045
Calcium	0.83	Thiamine (B1)	0.00608
Magnesium	1.38	α-tocopherol (E)	0.0021
Potassium	13.59	Folate	0.00394
Phosphorus	4.06	Niacin (B3)	0.0211
Sodium	0.12	Phylloquinone (k)	0.056 µg/g
Micro-minerals		Pyridoxine (B6)	0.00397
Zinc	0.0279	Retinol	---
Iron	0.0669	Riboflavin (B2)	0.00212
Total dietary fibre	0.1520	**Caloric value**	3.37 kcal/g

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], hepato-protective [18], trypsin, α-amylase [19, 20], analgesic, anti-fertility**,** litholytic [21] and antidepressant [22].

3. MATERIAL 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 bean were evaluated for their physical quantitative characteristics that includes 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 (ϕ) 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 & Phytochemical screening

3.6.1 Soaking & 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 (Figure 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.

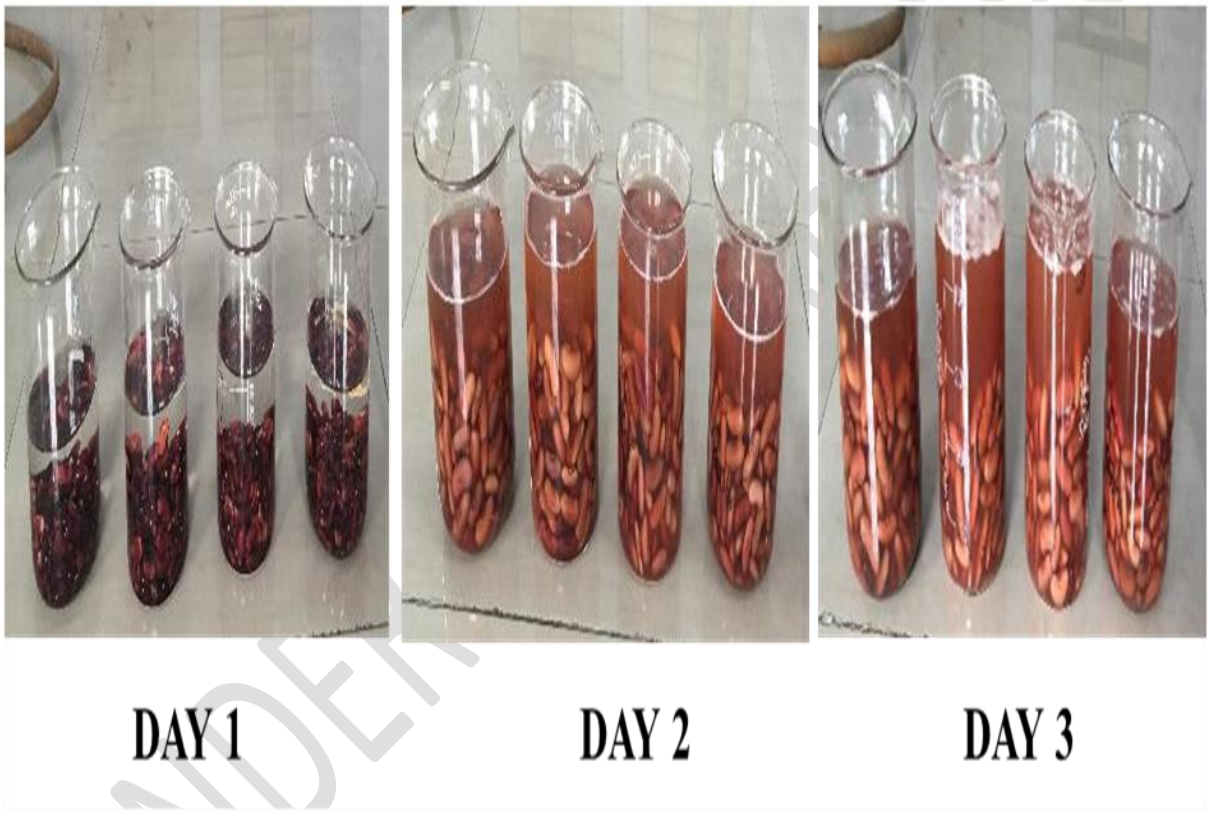

Figure 1: Soaking and extraction of red kidney bean

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 $NaNO_2$ & 0.3 ml $AlCl_3$ 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 Na_2CO_3 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 = $[(As - Ai)/As] \times 100$

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

4.1. 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

4.2. The Physical Quantitative Characteristics were depicted in table 4.

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 (ϕ)	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

4.3. 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.

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

4.4. 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).

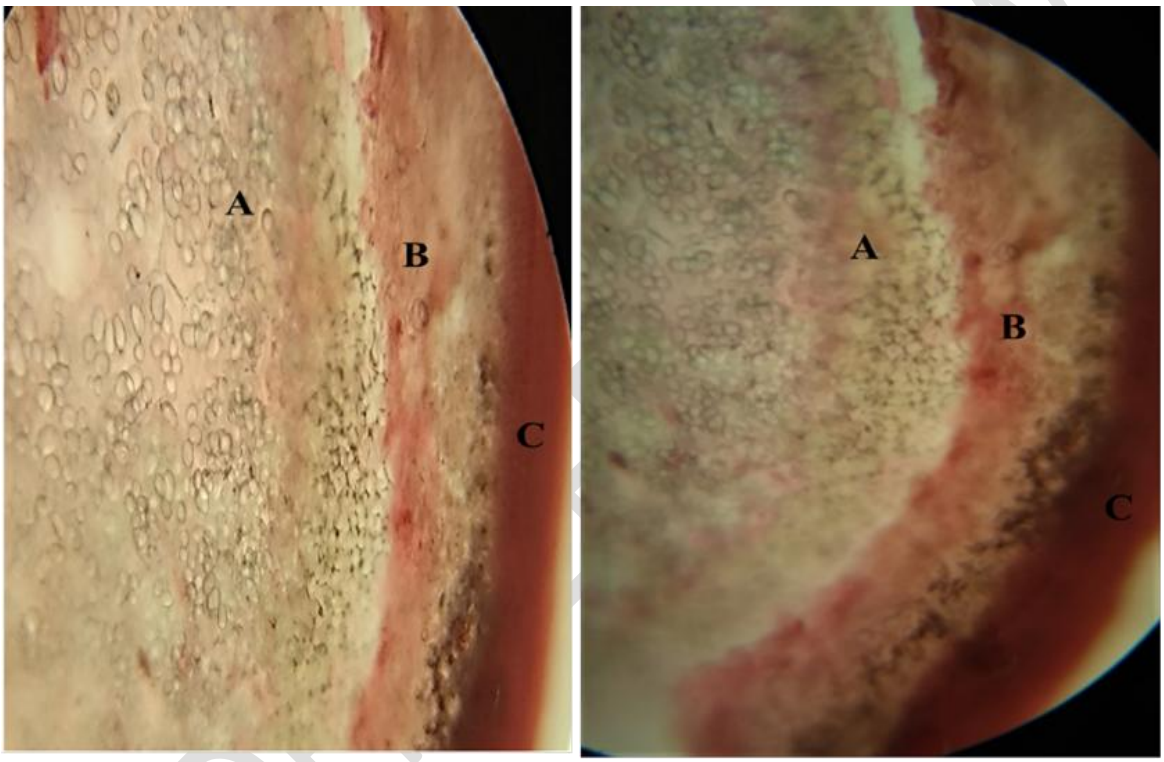

Figure 2: Optical light microscopy of red kidney bean (cross-section)

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.

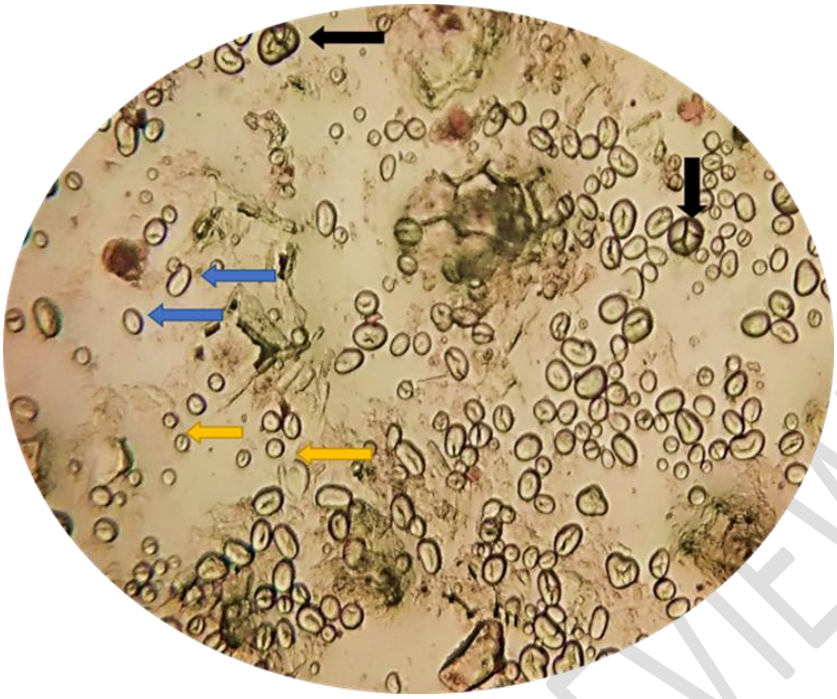

Figure 3: Transverse-section of red kidney bean

The percentage yield was determined as follows:

$$\text{Percentage yield} = \frac{\text{Final weight of dried extract}}{\text{Initial weight of powder}} \times 100$$

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

4.5. Preliminary phytochemical screening: Screening was performed as per standard protocol and results are depicted in Table 6.

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

4.6. HPTLC of bioactive components:

HPTLC study was performed to standardize the extract of *Phaseolus vulgaris* seed coats for the presence of flavonoids in Figure 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 (Figure 5). The result from HPTLC fingerprinting scanned at 254nm and 366nm for extract by using CAMAG Reprostar 3 illumination instrument.

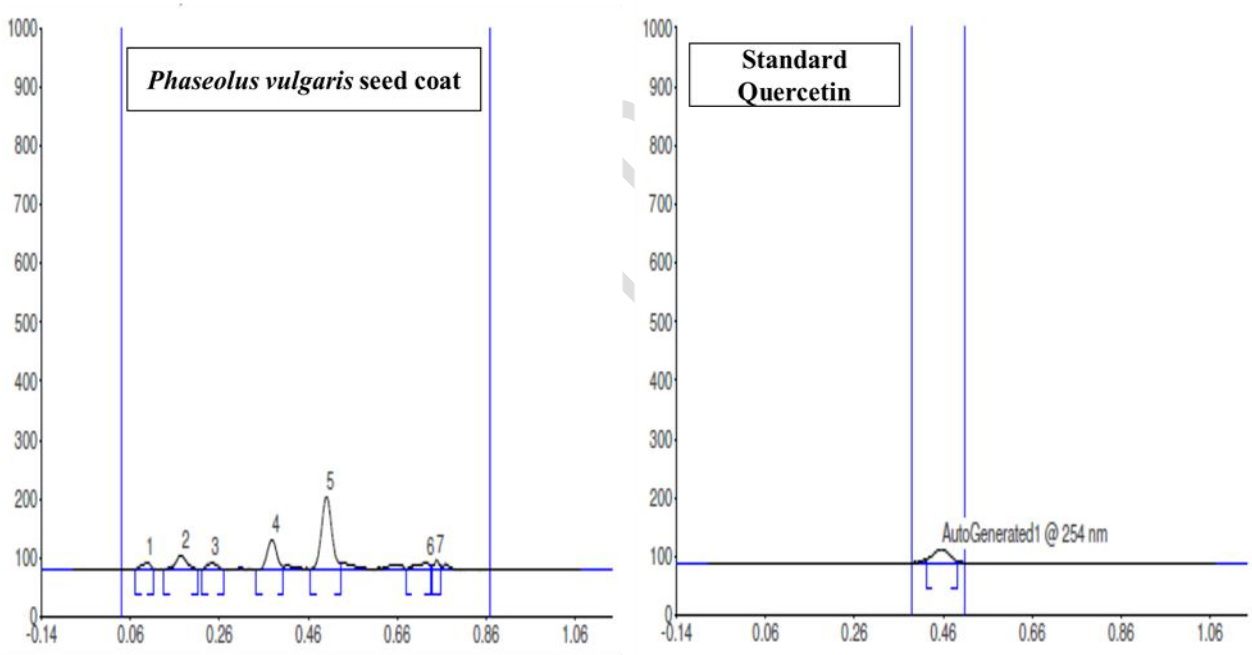

Figure 4: Spectral comparison of *Phaseolus vulgaris* seed coats with co-chromatographic standard, Quercetin.

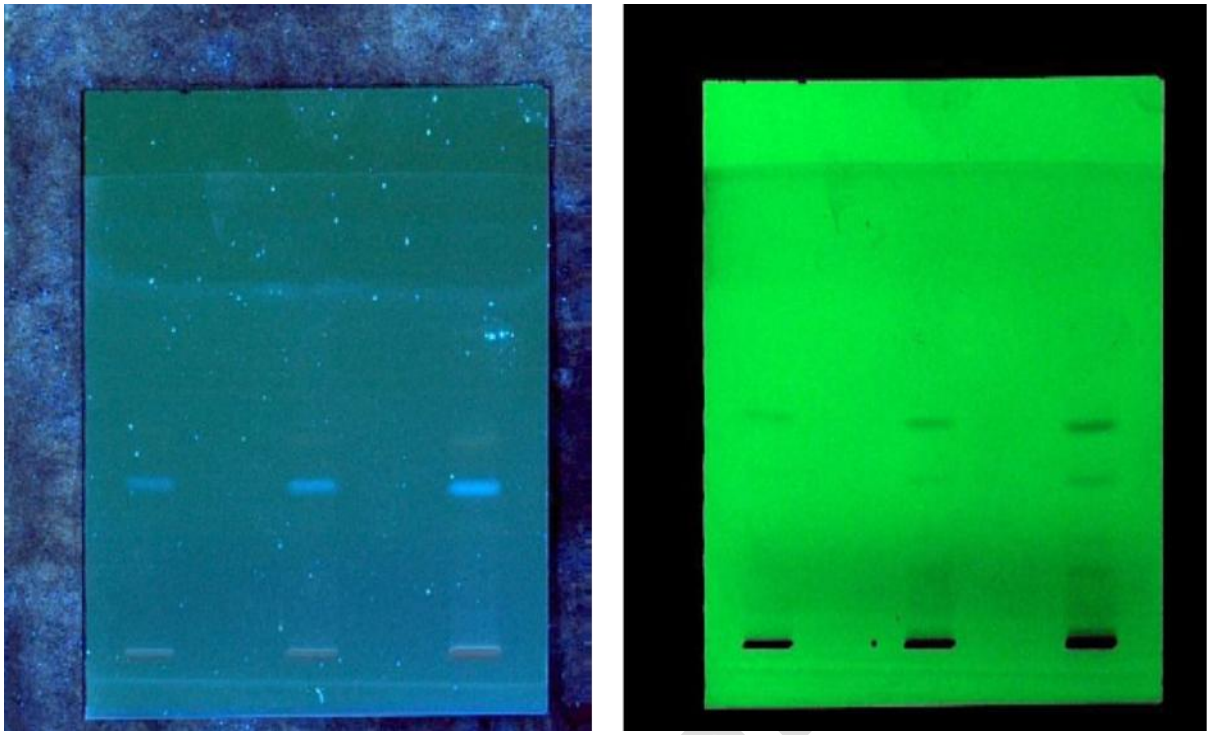

Figure 5: HPTLC of active phytoconstituents in *Phaseolus vulgaris* seed coat

4.7. *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 Figure 6.

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_{50} value	**Concentration of Vitamin C (µg/ml)**	**Concentration of seed coat (µg/ml)**
	354.93 ± 7.37	429 ± 4.07

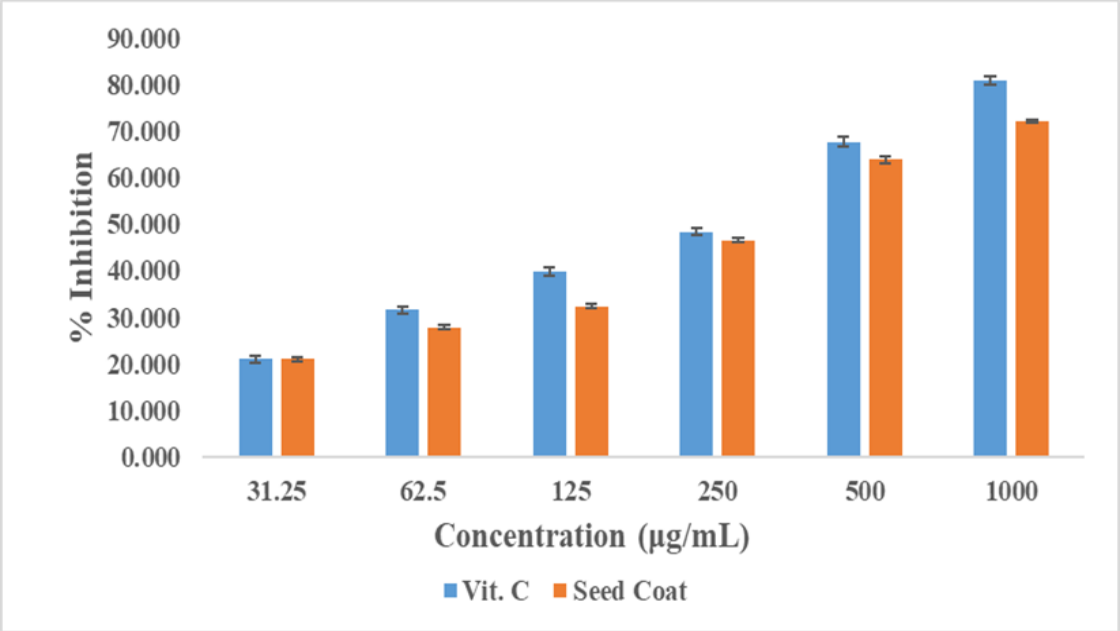

Figure 6: DPPH assay of *Phaseolus vulgaris* seed coat

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 pharmacognostic 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.

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.

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