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AMYLASE GLUCOSIDASE INHIBITORY AND ANTIOXIDANT ACTIVITIES OF PHASEOLUS VULGARIS SEED EXTRACTS BY INVITRO

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ABSTRACT

The main objective of the present study is to evaluate the hypoglycaemic activity of *Phaseolus Vulgaris*. seeds using various *in vitro* models like α-amylase and α-glucosidase inhibitory effects and antioxidant activity. Phytochemical screening of fruit and seed extracts revealed the presence of flavonoids and phenolics, accounting for its antidiabetic and antioxidant properties. Flavonoids are a group of polyphenolic compounds which exhibit several biological effects such as anti- inflammatory, antihepatotoxic, antiulcer activities, antidiabetic, antithrombotic etc. They also inhibit enzymes such as aldose reductase and xanthine oxidase. The *in vitro* antidiabetic activity of PVSP has been evaluated by measuring its α amylase and α glucosidase inhibitory activities and was compared with the standard drug acarbose, which is specific inhibitor of α glucosidase. Acarbose also possess inhibitory action of α amylase. The amount of glucose produced by the action of α glucosidase is estimated by using the enzyme glucosidase and peroxidase *in vitro.* However, the α glucosidase inhibition of the extract was much lesser than the inhibitory action shownby acarbose.

KEYWORDS: Glucosidase Inhibitory, Antioxidant Activities, Phaseolus Vulgaris, Seed Extracts.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of protein, fat, and carbohydrate metabolism attributed to low production of insulin or resistance to its action leading to hyperglycemia.[1–3] All over the world, one of the leading causes of morbidity as well as mortality is DM. Globally, the number of people with DM has increased from 108 million in 1980 to 422 million in 2014.[4] In 2017, around 5 million deaths were reported due to DM in the 20–99 years age range.[5] The increasing prevalence of DM and adverse effects related to modern medications are also important points of apprehension.[6] Acarbose is a medication clinically used to inhibit α-glucosidase and α-amylase. Unfortunately, its long-term administration resulted in side effects including abdominal distention and diarrhea.[7] Alternative plant-derived products with better safety potential may also be used for the management of diabetes mellitus.[7] Oxidative stress is a condition arising due to excessive production of free radicals inside the body that leads to oxidation of biologically important molecules.[8] Chronic hyperglycemia increases the synthesis of nonmitochondrial and mitochondrial reactive oxygen species, leading to the stimulation of polyol pathway flux, hexosamine pathway flux, protein kinase C (PKC) isoforms, and advanced glycation end-products (AGE) associated with hyperglycemia-induced oxidative damage.[9,10] Similarly, the amplified production of reactive oxygen species has harmful regulation of insulin signaling cascade leading to β-cell dysfunction, mitochondrial dysfunction, decreased insulin gene expression, impaired glucose tolerance, and insulin resistance.[9, 11] Natural plant-derived drugs are believed to be safe, effective, and economical.[3] Plant-derived products play a significant role in the development of new therapeutic agents and serve as sources of bioactive substances including antioxidants.[12–14] The antioxidant activity of plant extracts has valuable effects on the conservation of β-cell function in DM.[15–17] The main objective of the present study is to evaluate the hypoglycaemic activity of *Phaseolus Vulgaris*. seeds using various *in vitro* models like α-amylase and α-glucosidase inhibitory effects and antioxidant activity.

The scope of the present study is attributed in exploring the potential of the bioactive compounds from the medicinal plants and in revealing their safety & efficacy, there by realizing the promising ethno botanical herbs, towards the development of phytomedicine.

MATERIALS AND METHODS

DRUGS AND CHEMICALS

Acarbose (Biocon Ltd), α-amylase & α-glucosidase (Sisco Research Laborotaries Ltd Mumbai), Glucose assay kits (Agappe diagnostics, Kerala), 2,2-diphenyl-1-

picryl hydrazyl were purchased from HiMedia Laboratories, Mumbai, ascorbic acid, 2-deoxy-2-ribose, xanthine oxidase, quercetin, kaempferol, hesperidine, rutin, xanthine oxidase, hypoxanthine, pyrocatechol were purchased from Sisco Research Lab, Mumbai and butylated hydroxy toluene from Loba Cheme. Thiobarbituric acid, trichloroacetic acid, and potato starch were purchased from SD Fine Chemicals Ltd. All other chemicals used in the study were of analytical grade purchased from respective suppliers.

PLANT MATERIAL

Collection and authentication

The fruits of *Phaseolus Vulgaris* were collected from the local market during the month of June and July 2022.

Preparation of the *Phaseolus Vulgaris* Seed Extract

The seeds were dried in shade and finely powdered. The powdered seeds were macerated with water and stirred continuously in a mechanical shaker for 4 hours. The preparation was kept aside for 24 h. Itwas again stirred in the mechanical shaker for 4 h and kept aside for 12 h. These contents were taken and filtered through a muslin cloth and the filtrate was distilled to get a dark gummy material. This is then dried, to obtain the *Phaseolus Vulgaris* seed powder (PVSP) andstored in an airtight container.

PVSP were subjected to qualitative phytochemical tests to determine the presence of various phytoconstituents (Trease and Evans, 2002; Sanni *et al*., 2008) like tannins, phenolics, saponins, flavonoids, terpenoids, alkaloids, proteins and glycosides.

a. Test for tannins and phenolics

To the solution of the extract, a few drops of 0.1% ferric chloride, 1% gelatin solution, 10% lead acetate was added and observed for brownish green or a blue-black color.

b. Test for saponins

About 10 ml of the extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent-froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and thenobserved for the formation of emulsion. When mixed with dilutesulphuric acid and boiled with 90% ethanol, if the initial frothing disappears it confirms the presence of higher concentration of saponins.

c. Test for flavonoids

1. To a portion of the extract concentrated H_2SO_4 was added. A yellow colouration indicates the presence of flavonoids. The yellow colour disappears on standing.
2. Few drops of 1% $AlCl_3$ solution was added to a portion of extract. A yellow colouration indicates the presence of flavonoids.
3. A portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates a positive test for flavonoids.

d. Test for terpenoids

About 5 ml of the extract was treated with 2 ml of chloroform and about 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

e. Test for alkaloids

A small portion of the extract was stirred with few drops of dil. HCl and filtered.

1. To the filtrate, Dragendorff's reagent (potassium bismuth iodide solution) was added and an orange brown precipitate indicates the presence of alkaloids.
2. To the filtrate, Mayer's reagent was added and a cream precipitate indicates the presence of alkaloids.

f. Test for proteins

A portion of the extract was mixed with few drops of water and added Millon's test and Biuret reagents. A yellowish-brown precipitate indicates the presence of proteins.

g. Test for glycosides

A portion of the extract was mixed with few drops of Fehling's solution A & B and heated gently. A brick red precipitate indicates the presence of glycosides.

***IN VITRO* HYPOGLYCAEMIC STUDIES**

Glucose can be readily absorbed from the G.I.T. by the presence of enzyme α-amylase and α-glucosidases. Inhibition of these enzymes reduces the postprandial blood glucose levels. Hence *in vitro* α-amylase & α-glucosidase inhibition models were carried out to screen PVSP and evaluate its potential hypoglycaemic activity.

Inhibition of α amylase in vitro

PROCEDURE

A 1% starch solution was prepared in 25 ml of 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65°C for 15 minutes. The α amylase enzyme was obtained from porcine pancreas andits solution was prepared by mixing 1 mg of α amylase in 250 ml of cold deionised water. The calorimetric reagent was prepared by mixing sodium potassium tartarate (12 g in 8 ml of 2 mM sodium hydroxide and 96 mM of3, 5-dinitrosalicylic acid solution. PVSP extracts were dissolved in5% DMSO (Dimethylsulphoxide) to give a final concentration of 1 mg/ml.

One ml of starch solution was mixed with 1 ml of increasing concentration of the PVSP (100-1000 µg/ml) and mixed by swirling and equilibrated to 20°C. Then added one ml of α amylase solution and incubated at 20°C for 5 minutes to undergo the reaction with the starch. To the above solution add 1 ml of the colorimetric

reagent solution and heated in a water bath for 15 minutes. The reduction of 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid corresponds to the maltose generation with the colour change from yellowish orange to winered. Then it is cooled and added 9 ml of deionised water to make a final volume of 13 ml and then the absorbance was recorded at 540 nm for both test and blank using a suitable spectrophotometer (Sigma Aldrich, 1997; Thalapaneni *et al*., 2008).

Assay condition
T = 37°C, pH = 6.9, A540nm, Light path =1 cm, Calorimetric method

Unit definition
One unit will liberate 1mg of maltose from starch in 5 minutes at Ph 6.9 at 20°C and pH 6.9 under specified conditions. The α amylase inhibition was expressed as percentage of inhibitionand the IC50 values determined by linear regression of plots with varying concentration PVSP against the percentage inhibition from three separate tests.

4.1.1. **Inhibition of α glucosidase *in vitro***
α glucosidase enzyme obtained from yeast as lyophilised powderwas used as the target protein source for the study of the enzyme inhibition using maltose as the substrate. Acarbose is used as positive control and the plant extract is prepared at the concentration of 1 mg/ml with 5% v/v DMSO (Dimethyl sulphoxide). The enzyme and the substrate were dissolved in 0.2 M Tris buffer at pH 8.

PROCEDURE
The enzymatic assay mixture consists of 1 ml of glucosidase enzyme (1U/ml), 1 ml of 37 mM of maltose substrate, 1 ml each of PVSP & acarbose at varying concentration (10µg - 100µg) in 5% v/v DMSO (Dimethyl sulphoxide) which is incubated at 37ºC for 30 min. After incubating for 30 min, 0.2 ml of the assay mixture is mixed with 1 ml of the kit reagent. Glucose released in the assay mixture is quantified with commercial glucose oxidase assay kit (GOD-POD Kit, Agappe Diagnostics, Kerala). The enzymatic activity was measured by the amount of glucose released, which was detected spectrophotometrically at 505 nm. The rate of carbohydrate breakdown was determined by calculating the amount of glucose obtained when carbohydrate was completely digested. The enzyme inhibitory activity was determined as the percentage inhibition and the assays were carried out in triplicate & the rate of prevention was calculated. The IC50 of the PVSP required to inhibit the activity of the enzyme by 50% was determined by linear regression of the plots with varying concentration of PVSP Vs percentage inhibition from the three separate tests (Subramanian *et al*., 2008; Thalapaneni *et al*., 2008)

Assay condition
T = 37°C, pH = 6.9, A540nm, Light path =1 cm, Calorimetric method

Unit definition
One unit will liberate 1mg of maltose from starch in 5 minutes at pH 6.9 at 20°C and pH 6.9 under specified conditions.

The α amylase inhibition was expressed as percentage of inhibition and the IC50 values determined by linear regression of plots with varying concentration of PVSP against the percentage inhibition from three separate tests.

STATISTICAL ANALYSIS:
P values are expressed as mean±standard error mean (SEM)and analyzed using GraphPad InStat software.

IN VITRO
DPPH radical scavenging assay (hydrogen donating ability)
The hydrogen donating ability of PVSP was examined in the presence of DPPH stable radical. One millilitre of 0.3 mM DPPH ethanolsolution was added to 2.5 ml of PVSP solutions of different concentrations in ethanol and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm. 1.0 ml ethanol plus 2.5 ml of PVSP solutions were used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus ethanol (2.5 ml) was used as negative control. The positive controls were those using the standard (Ascorbic acid) solutions. Percentage inhibition of DPPH scavenging effect was calculated and the IC50 values were determined by linear regression of plots with varying concentration of PVSP against the percentage inhibition from three separate tests (Mensor *et al.,* 2001).

Deoxyribose degradation assay (Hydroxyl radical scavengingactivity)
The decomposing effect of PVSP on hydroxyl radicals was determined by the assay of malondialdehyde chromogen formation due to 2-deoxy 2-ribose degradation. The assay mixture contained in a final volume of 1ml: 100 µl of 28 mM 2-deoxy 2-ribose dissolved in phosphate buffer, pH 7.4, 500 µl of the PVSP of various concentrations in buffer, 200 µl of 200 mM ferric chloride (1:1 v/v) and 1.04 mM EDTA and 100 µl of 1.0 mM hydrogen peroxide and 100 µl of 1.0 µM ascorbic acid. After incubation of the test sample at 37ºC for 60 min (Fentons system- generation of hydroxyl radicals). The extent of deoxy ribose degradation by the formed hydroxyl radicals was measured directly using thiobarbituric acid (TBA) test; 1.0 ml TBA (1%) in 0.05M NaoH) and 1.0 ml 2.8% (w/v) trichloroacetic acid were added to the test tubes and heated at 100 ºC for 15 min, cooled and the absorbance was measured at 532 nm against the blank containing deoxyribose and buffer solution. The positive controls were those using the standard (Quercetin) solutions. Percentage inhibition of deoxyribose degradation was calculated and the IC50 values were determined by

linear regression of plots with varying concentration of PVSP against the percentage inhibition from three separate tests(Gomes *et al.*, 2001).

NBT reduction assay (Superoxide radical scavenging activity)

The capacity of the plant extracts to scavenge the superoxide anion was assayed by using NBT reduction assay. The superoxide anion radical was generated *in vitro* with hypoxanthine and xanthine oxidase. A reaction mixture with a final volume of 3 ml per tube was prepared with 1.4 ml of 50 mM KH_2PO_4-KOH pH 7.4 containing 1 mM EDTA, 0.5 ml of 100 m hypoxanthine, 0.5 ml of 100 µM nitro blue tetrazolium (NBT). The reaction was started by adding 0.066 units per tube of xanthine oxidase freshly diluted in 100 ml of phosphate buffer and 0.5 ml of PVSP in aline. The xanthine oxidase was added last. The subsequent rate of NBT reduction (measure of superoxide scavenging activity) was determined on the basis of spectrophotometric determinations of absorbance at 560 nm. Ascorbic acid was used as standard. The results are expressed as the percentage inhibition of NBT reduction rate with respect to the reaction mixture without test compound (Guzman *et al.*, 2001; Gulcin., 2003).

Reducing power ability

Reducing power ability was measured by mixing 1.0 ml PVSP of varying concentrations (50, 100, 200, 400, 800 µg/ml) in 1 ml of distilled water to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50°C for 30 min. Later 2.5 ml of trichloroacetic acid (10%) were added to the mixture and centrifuged at 3000 rpm for 10 min. Finally 2.5 ml from the supernatant were mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power. Allexperiments were done in triplicate using butylated hydroxyltoluene (BHT) as positive control (Yildrim *et al.*, 2001).

Determination of % Inhibition

Percentage antioxidant activity (%AA) was calculated using the formula,

$$\%\ \text{Antioxidant Activity (\%AA)} = 100 - \left[\frac{A_0 - A_1}{A_0} \times 100\right]$$

Where, A0 is the absorbance of the control and A1 is the absorbance ofthe sample.

Determination of 50% Inhibitory Concentration (IC50)

The concentration (mg/ml) of the plant extracts required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extract. The IC50 values were calculated using GraphPad Instat statistical software.

Statistical analysis

All determinations were carried out in triplicate and the values are expressed as the mean ± SEM.

RESULTS

Percentage yield

The percentage yield of the powder of the seeds of *Phaseolus Vulgarisn* seed powder was 17%.

Phytochemical screening

The preliminary phytochemical screening of PVSP showed the presence offlavonoids, alkaloids, saponins, proteins, tannins and phenolics.

Inhibition of α amylase *in vitro*

Phaseolus Vulgaris seed powder showed α amylase inhibitory activity at the varying concentrations tested (50, 100, 200, 400 and 800µg/ml). There was a dose dependent increase in the percentage inhibition for all the concentrations tested (Table 3).

Phaseolus Vulgaris seed powder (PVSP) showed significant α amylase inhibitory activity at the varying concentrations tested (50, 100, 200, 400 and 800µg/ml). There was a dose dependent increase in the percentage inhibition for all the concentrations tested. PVSP at a concentration of 50 µg/ml showed a percentage inhibition of 16.68, for 100 µg/ml it was 30.70, for 400 µg/ml it was increased to 54.30, 800 µg/ml it was 71.23. The IC_{50} value was found to be 338.66 µg/ml.

Acarbose was used as the standard drug for the determination of α amylase inhibitory activity. The concentration of acarbose is varied (50, 100, 200, 400 and 800µg/ml). Acarbose at a concentration of 100µg/ml exhibited a percentage inhibition of 37.21% and for 800 it was found to be 94.20%. A graded increase inthe percentage inhibition was observed for the increasing concentrations of the drug. The IC_{50} values of acarbose were found to be 170.80. An increase in the IC_{50} value was observed for the plant extract when compared with the standard drug acarbose.

Inhibition of α glucosidase *in vitro*

The study revealed that PVSP had significant α glucosidase inhibitory activity at the varying concentrations tested (50, 100, 200, 400, 800 µg/ml). There was a dose dependent increase in the percentage inhibition for all the concentrations tested (Table 4).

PVSP at a concentration of 50µg/ml showed a percentage inhibition of 15.59%, at 200µg/ml it was found to be 36.48% and at 800 µg/ml it increased to 49.29. The IC_{50} value was found to be 429 µg/ml.

Acarbose was used as the standard drug for the determination of α glucosidase inhibitory activity. The concentration of acarbose is varied from 50, 100, 200, 400, 800 µg/ml. Acarbose at a concentration of 100µg/ml exhibited a percentage inhibition of 40.73%

and for 800 µg/ml it was found to be 72.56%. Agraded increase in the percentage inhibition was observed for the increasing concentrations of the drug. The IC_{50} values of acarbose were found to be 202.15µg/ml. An increase in the IC_{50} values was observed for the plant extract when compared with the standard drug acarbose (Table 4).

DPPH radical scavenging assay (hydrogen donating ability)

The extracts PVSP at various concentrations demonstrated H- donor activity. The radical scavenging activity of PVSP was determined from the reduction in the absorbance at 518 nm due to scavenging ofthe stable DPPH free radical. The DPPH scavenging potential forPVSP varied at varying concentrations (10, 20, 40, 80, 160 µg/ml) and the resultsare shown in Table 5. PVSP showed the graded increase in percentage of inhibition for all the doses tested and the percentage inhibition ranged from 9.57% to 57.91%. The IC_{50} values of PVSP were found to be 130.06 µg/ml.

Ascorbic acid was used as the reference standard and similar increase in the percentage of inhibition was observed for all the concentrations (10, 20, 40, 80, 160 µg/ml) tested. The DPPH scavenging effect for various extracts was less than that of standard compound, ascorbic acid. The IC_{50} value of standard was found to be 53.04µg/ml. DPPH was reduced with the addition of PVSP in a concentration dependent manner**.**

Deoxyribose degradation assay (hydroxyl radical scavenging activity)

Hydroxyl radical scavenging activity was quantified by reaction with thiobarbituric acid and the results are shown in the. The PVSP showed 30.12% of activity at 20µg/ml and it was increased to 54.79 % at 80µg/ml. The IC_{50} value of PVSP were found to be 27 µg/ml respectively. Quercetin was used as the reference standard and similar increase in the percentage of inhibition was observed for all the concentrations (5, 10, 20, 40, 80 µg/ml) tested. The IC_{50} value of quercetin was found to be 18.5 µg/ml. The degradation of deoxyribose by Fe^{3+}-ascorbate-EDTA-H_2O_2 system was markedly decreased by PVSP tested at various concentrations indicating hydroxyl radical scavenging activity.

5.5. **NBT reduction assay (superoxide radical scavenging activity**)

The PVSP at various concentrations were found to be a scavenger of superoxide anion generated in xanthine oxidase-NBT systems *in vitro* and their activity were comparable to that of ascorbic acid and the results were given in (Table 7). All the concentrations of PVSP offered greater percentage of inhibition with increase in the concentrations. Ascorbic acid was used as the positive control. The IC_{50} value for PVSP were 285 and 122 µg/ml respectively and that of standard was 39.26 µg/ml.

5.6. **Reducing power ability**

The reductive capabilities of PVSP when compared to the standard BHT were given in Table 8. The reductive ability of the PVSP serves as a significant indicator of its antioxidant activity. The reducing power of PVSP was dose dependent and found to increase with increasing concentrations. PVSP increased the absorbance up to 0.563 and 0.394 at 800µg/ml respectively, when the absorbance of the standard at 800µg/ml was 1.397. All the concentrations of PVSP offered higher absorbance values than the control.

Table 1: Preliminary phytochemical screening of PVSP.

S.No	Phytochemical compounds	Presenceor Absence
1	*Triterpenoids*	**Absence**
2	*Flavonoids*	**Presence**
3	*Glycosides*	**Absence**
4	*Alkaloids*	**Presence**
5	*Saponins*	**Presence**
6	*Carbohydrates*	**Absence**
7	*Proteins*	**Presence**
8	*Tannins and phenolics*	**Presence**

Table 2: Alpha amylase inhibitory activity of PVSP.

Sample	oncentration(µg/ml)	% Inhibition	IC $_{50}$ µg/ml
PVSP	50	16.68 ± 1.40	
	100	30.70 ± 0.98	
	200	41.12 ± 0.46	338.66 ± 0.881
	400	54.30 ± 0.52	
	800	71.23 ± 0.97	
Acarbose(standard)	50	22.77 ± 0.21	
	100	37.21 ± 0.16	

	200	57.49 ± 0.17	170.80 ± 0.144
	400	77.98 ± 0.16	
	800	87.70 ± 0.16	

All determinations were carried out in triplicate manner and values areexpressed as the mean ± SEM.

Table 3: Alpha glucosidase inhibitory activity of PVSP.

Sample	Concentration(µg/ml)	% Inhibition	IC $_{50}$ µg/ml
PVSP	50	15.59 ± 0.13	
	100	28.83 ± 0.14	
	200	36.48 ± 0.34	429 ± 1.155
	400	49.24 ± 0.35	
	800	61.07 ± 0.34	
Acarbose(standard)	50	22.77 ± 0.21	
	100	40.73 ± 1.39	
	200	49.34 ± 1.04	
	400	63.48 ± 0.91	202.15 ± 1.021
	800	72.56 ± 1.22	

All determinations were carried out in triplicate manner and values areexpressed as the mean ± SEM.

Table 4: Hydrogen donating ability of PVSP using DPPH method.

Sample	Concentration (µg/ml)	% inhibition	IC $_{50}$ µg/ml
PVSP	10	15.54 ± 0.41	
	20	22.24 ± 0.19	
	40	30.63 ± 0.22	130.06 ± 0.096
	80	41.72 ± 0.30	
	160	57.91 ± 0.17	
Ascorbic acid (standard)	10	17.48 ± 0.38	
	20	32.87 ± 1.38	
	40	47.94 ± 0.30	
	80	68.99 ± 0.07	53.04 ± 0.57
	160	84.23 ± 0.21	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Table 5: Scavenging of hydroxyl radical activity by PVSP using deoxyribose degradation method.

Sample	Concentration (µg/ml)	% Inhibition	IC $_{50}$ µg/ml
PVSP	5	16.26 ± 0.09	
	10	29.13 ± 0.93	
	20	45.10 ± 0.07	27 ± 1.60
	40	55.61 ± 0.06	
	80	67.87 ± 0.10	
Quercetin (standard)	5	19.79 ± 0.39	
	10	35.16 ± 0.21	
	20	57.81 ± 0.28	18.5 ± 0.29
	40	73.75 ± 0.23	
	80	92.26 ± 0.21	

All determinations were carried out in triplicate manner and values areexpressed as the mean ± SEM.

Table 6: Superoxide anion scavenging activity of PVSP usingNBT reduction assay.

Sample	Concentration (µg/ml)	% Inhibition	IC $_{50}$ µg/ml
PVSP	25	20.22 ± 0.12	
	50	33.66 ± 0.15	
	100	47.74 ± 0.09	122 ± 0.176
	200	61.67 ± 0.18	
	400	71.07 ± 0.10	

Ascorbic acid (standard)	25	33.33 ± 0.03	39.26 ± 0.54
	50	63.87 ± 0.45	
	100	76.78 ± 0.21	
	200	85.79 ± 0.17	
	400	91.82 ± 0.14	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Table 7: Reductive ability of PVSP.

Sample	Concentration (µg/ml)	Absorbance at 700 nm IC $_{50}$ µg/ml
PVSP	50	0.081 ± 0.0008
	100	0.125 ± 0.0023
	200	0.201 ± 0.0017
	400	0.279 ± 0.0029
	800	0.394 ± 0.0011
BHT (standard)	50	0.1647 ± 0.001
	100	0.3252 ± 0.001
	200	0.5862 ± 0.002
	400	0.7635 ± 0.003
	800	1.3972 ± 0.033

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

DISCUSSION

Diabetes mellitus is the world's largest growing metabolic disorder of carbohydrate, fat & protein metabolism. A relative or absolute deficiency of insulin secretion leads to impaired carbohydrate utilization by tissue, which is the characteristic feature of diabetes.

Alternate strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries.

Phaseolus Vulgaris is used in the folk medicine and presumably it has no side effects reported. Since ancient times, people trusted on plants and herbs as medicine, as dietary supplements and as adjuvant with drugs for better control of diseases and symptoms.

The present study deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant *Phaseolus Vulgaris* which is traditionally used by the local people and tribals in India for the treatment of asthma, cough, diabetes, haemoptysis, hemorrhages from internal organs, epilepsy, fever. The fruits and seeds are useful in diabetes (Indian Medicinal Plants, 1985).

Phytochemical screening of fruit and seed extracts revealed the presence of flavonoids and phenolics, accounting for its antidiabetic and antioxidant properties. Flavonoids are a group of polyphenolic compounds which exhibit several biological effects such as anti- inflammatory, antihepatotoxic, antiulcer activities, antidiabetic, antithrombotic etc. They also inhibit enzymes such as aldose reductase and xanthine oxidase.

The present study was carried out to evaluate the *in vitro* antidiabetic and antioxidant activities of the seeds of *Phaseolus Vulgaris*.

The *in vitro* antidiabetic activity of PVSP has been evaluated by measuring its α amylase and α glucosidase inhibitory activities and was compared with the standard drug acarbose, which is specific inhibitor of α glucosidase. Acarbose also possess inhibitory action of α amylase. The amount of glucose produced by the action of α glucosidase is estimated by using the enzyme glucosidase and peroxidase *in vitro.* However, the α glucosidase inhibition of the extract was much lesser than the inhibitory action shown by acarbose. The possible mechanism of action of α amylase may be due to the blocking of the starch binding site and α glucosidase inhibition may be due to blockade of the oligosaccharide binding site. Alpha amylase catalyses the hydrolysis of the internal α 1,4 glucosidic linkages in starch and other related polysaccharides which has been the targets for the suppression of post prandial hyperglycemia. In the light of the result present study indicates that PVSP possess antidiabetic activity.

Free radicals are known to play a definite role in the pathological manifestation of diabetes. Antioxidant fights against free radicals byprotecting us from various diseases and scavenges the reactive oxygen radicals or protects the antioxidant defense mechanism. Reactive oxygen species (ROS) are capable of damaging biological macromolecules such as DNA, carbohydrates and proteins. ROS is a collective term, which includes not only oxygen radicals ($O2^{\cdot}$ and $OH^{\cdot}$) but also some non-radical derivatives of oxygen like H2O2, HOCl, and

ozone (O3). In addition, antioxidant activity may be regarded as a fundamental property important for life.
The *in vitro* antioxidant activities of the PVSP have been evaluated by measuring its scavenging activities by various methods suchas DPPH radical scavenging assay, deoxyribose degradation assay, NBT reduction assay to study the superoxide scavenging activity and reducing power ability method to study the total antioxidant capacity.

DPPH is a stable free radical at room temperature, which producesa violet solution in ethanol DPPH is widely used to evaluate the free radical scavenging effect of natural antioxidant. DPPH shows a strong absorptionband at 517 nm in visible spectrum (deep violet colour). As the electron becomes paired in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stochiometrically coincides with the number of electrons taken up (Mensor *et al*., 2001). The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge free radicals independently. DPPH radical scavenging activities increased with increasing concentrations of PVSP. Based on the mechanism of reduction of DPPH molecule described in the literature, it is correlated with the presence of hydroxyl groups on the antioxidant molecule. We can infer that the very good activity of PVSP was probably due to the presence of substance with an available hydroxyl groups.

Hydroxyl radicals are the highly reactive radicals which are produced through the Fenton's reaction in living system. Hydroxyl radicals scavenging activity was quantified by measuring inhibition of the degradation of deoxyribose by free radicals (Guzman *et al.,* 2001). Deoxyribose levels were determined by reaction with thiobarbituric acid. PVSP showed a good hydroxyl radical scavenging activity but lesser than standard, quercetin.

Superoxide anion radicals were generated enzymatically *in vitro* by the hypoxanthine and xanthine oxidase system was determined by using NBT reduction assay. Superoxide reduces NBT to form a blue coloured complex formazone which is measured spectrophotometrically. The decrease of absorbance at 560 nm with PVSP indicates the consumption of superoxide anion in the reaction mixture (Gulcin *et al*., 2003). Determination of the mean rate of increase in absorbance overone minute period provides a measure of the extent to which the test fraction is capable of inhibiting NBT reduction by the superoxide anion radical and thus of superoxide scavenging activity.

The reductive capabilities of PVSP was compared with BHT. For the measurements of the reductive ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of PVSP. The reducingcapacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the antioxidant activity of antioxidants have been attributed to various mechanism, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging antioxidant activity (Gulcin *et al.*, 2004; Amarowiz *et al*., 2004). The reducing power of PVSP increased with increasing amount of sample. Here the color change occurs from yellow to greenish blue depending upon the reducing power of PVSP.

Thus, from the present investigation, it can be said that the *Phaseolus Vulgaris*. exhibited remarkable antioxidant property in various i*n vitro* assay systems.

CONCLUSION

In conclusion, there has been a growing interest in the alternative medicine and the therapeutic properties of the natural products derived from plants in the recent years. Based on the evaluation done using the various *in vitro* assay models it may be concluded that *Phaseolus Vulgaris* seed extract possess more α amylase and α glucosidase enzyme inhibitory actions and thus retards glucose absorption and reduces post prandial hyperglycaemia. The antioxidant activity of the plant was proved using various *in vitro* assay systems. The preliminary chemical tests provided insights into the presence of polyphenolics compounds in the extract. Hence the above stated activities can be attributed due to the presence of these flavonoids. Further studies using *in vivo* models are necessary to confirm these activities and to explore the exact mechanism by which the plant constituents act.

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