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ANALYSIS OF PHASEOLUS VULGARIS FOR ITS VALUABLE COMPONENTS

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ABSTRACT

This study present a new approach to evaluate the antioxidant potential and free radical scavenging capacity of the food plant i.e., Phaseolus vulgaris. The crude methanolic extract of the plant was considered for the investigation. Qualitative examination of phytochemical constituents was performed by different protocols as suggested in the literature. At the concentration of 3mg/ml, aqueous fraction of Phaseolus vulgaris (68.5±0.66) showed significant nitric oxide radical scavenging activity. Similarly aqueous fraction of Phaseolus vulgaris (143.0 ± 1.25) revealed greatest total phenolic contents among all other fractions. After the ascorbic acid, aqueous fraction of Phaseolus vulgaris (76.3±0.49) showed highest percent inhibition of Linoleic acid emulsion assay. The chemical composition of the plant was analyzed by HPLC to confirm that Ellagic acid and Caryophyllene oxide are the major composition causing the antioxidant capabilities and their ubiquity in a large range of mainly used foods of plant origin.

Keywords: Phaseolus vulgaris, phytochemical analysis, Antioxidant property, High Performance Liquid Chromatography.

1. INTRODUCTION

Traditional nutrition focuses on consuming nutritionally balanced food according to the recommended dietary guidelines, is now changed into optimal nutrition. Optimal nutrition, as old Chinese quotation let food be the medicine and medicine be the food recommends the functional foods intake for good health and reducing the risk of chronic diseases. An extensive scientific research has been conducted on understanding the medicinal potential of plants as well as to know the basis for the progress and expansion of functional foods and nutraceuticals with physiological benefits of their constituents. (Lattanzio *et al*., 2006; Russell and Duthie, 2011; Banarjee *et al*., 2011). The genus Phaseolus vulgaris includes all species of legume seeds normally known as common beans. (Gepts & Dpbouk, 1991). The seed color of beans is determined by the presence and concentration of flavonol glycosides, anthocyanins, and tannins (Beninger & Hosfield, 2003; Aparicio-Fernandez et al., 2005). Recently, Phaseolus vulgaris is gaining increasing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals with potential health benefits such as proteins, amino acids, complex carbohydrates, dietary fibers, oligosaccharides, phenols, saponins, flavonoids, alkaloids, tannins, among others (Geil & Anderson, 1994; Mishra et al., 2010). Important biological activities have been described for fibers, phenolic compounds, lectins, trypsin inhibitors, and phytic acid from common beans like enhancement of the bifidogenic effect (Queiroz-Monici et al., 2005); antioxidant (Heimler et al., 2005); anticarcinogenic (Hangen & Bennink, 2002) effects. Phaseolus vulgaris seeds have a notable place in the folklore throughout the world and in the traditions of many cultures such as pharmacotherapeutic effects (Hangen & Bennink, 2002; Mishra et al., 2010). Preclinical investigations have unanimously reported how the acute, repeated administration of extracts of Phaseolus vulgaris, as well as some of their isolated ingredient reduced food intake, body weight, and lipid accumulation in lean, diabetes and obese laboratory animals have been carried out on this plant. Thereby, the mode action of the Phaseolus vulgaris seeds producing the therapeutic effect can also be better investigated if the bioactive ingredients are characterized. Hence, the aim of this study is to determine the active phytochemical constituents in Phaseolus vulgaris and their role related with a substantial decrease in chronically-degenerative diseases.

1.2 OBJECTIVE

The aim of this study was to investigate the active phytochemical constituents, antioxidant potential and free radical scavenging activities of the food plant utilized in our daily diet. The plants extract was extracted in the different solvents.

2. REVIEW OF LITERATURE

Geil *et al*., (1994) suggested that the nutrient structure of dry beans makes them ideal related to good health. Protein, essential vitamins complex, carbohydrate, minerals and fiber are widely present in dry beans and they are low in sodium, fat and cholesterol. In this way they are important to minimize the effect of chronic diseases as cancerdiabetes, heart diseases and obesity.

Beninger *et al.,* (2003) showed the antioxidant activity of the seed coat of Phaseolous vulgaris having methanol extracts, tannin fractions, and pure flavonoids in a fluorescence-based liposome assay. They estimated relatively high activity of the condensed tannin (Proanthocyanidin) fractions. The Petunidin 3-O-glucoside, pure anthocyanins, the flavonol quercetin 3-O-glucoside, delphinidin 3-O-glucoside and Malvidin 3-O-glucoside were extracted from seed coats and they also had higher antioxidant activity than the Fe^{2+} control. The activity of kaempferol 3-O-glucoside was not different from that of the Fe^{2+} control. In this way colored dry beans are good source of dietary antioxidants.

Ocho – Anin Atchibri *et al*., (2010) inspected some bioactive components such as alkaloids, saponins, carbohydrate, catechin, fibers, anthocyanin, flavonoids, phytic acid, quercetin, steroids, phasine, tannins, terpenoids and trypsin by phytochemical screening of Phaseolus vulgaris seeds. The bioactive compounds present in Phaseolus vulgaris seeds help in substantial decrease in metabolic diseases such as diabetes, cancer and cardiovascular diseases.

Chao *et al*., (2015) evaluated the antioxidant and anti-inflammatory activities of red beans which is a leguminous seed and mainly used in oriental desserts. Thio- barbituric acid reactive substance method was done to find out the antioxidant activity which showed the presence of Phenolic compounds and cyanidin-3-O-glucoside in red beans. The anti-inflammatory activity was done by 50g/kg of red beans ethanolic extract by calculating the lipopolysaccharide induced expressions of nitric oxide.

Qiong *et al.,* (2020) evaluated antioxidant, anti-proliferative activities, and phytochemical profiles of common bean extracts were measured and compared in 10 common bean cultivars. Antioxidant activity was significantly associated with total phenolics, total flavonoids, and total proanthocyanidins, while anti-proliferative activity of extracts had no correlation with total phenolic contents and *in vitro* antioxidant activity.

3. MATERIALS AND METHODS

3.1 General experimental conditions

Solvents like Methanol, ascorbic acid, acetonitrile, chloroform, dichloromethane, n-butanol, glacial acetic acid, ammoniun thiocyanate, Ethanol, bismuth nitrate, ferric chloride, Iodine, mercuric chloride, potassium dihydrogen phosphate, potassium iodide, sodium nitroprusside, sodium dihydrogen phosphate, sodium carbonate, sodium chloride, sodium hydroxide, sulphanilic acid, sulphuric acid, ammonia solution and chemical reagents like Folin Ciacalteau reagent, Gallic acid, Linoleic acid and 1-Napthyl Ethylene diamine dihydrogen chloride, U.V. Visible Spectrophotometer, Rotary evaporator, Weight balance, Whatmann filter paper, Thin Layer Chromatography sheet, High Performance Liquid Chromatography were used.

3.2 Extraction of the plant material

Beans of *Phaseolus vulgaris* were purchased, air dried, chopped and grinded. They were soaked in 1 liter of 80% methanol and 20% distilled water for 7 days. After filtration, the filtrate was concentrated. Equal amount of the crude extract of the plants were separately run for fractionation using solvents. The pH was maintained by using acetic acid and ammonia.

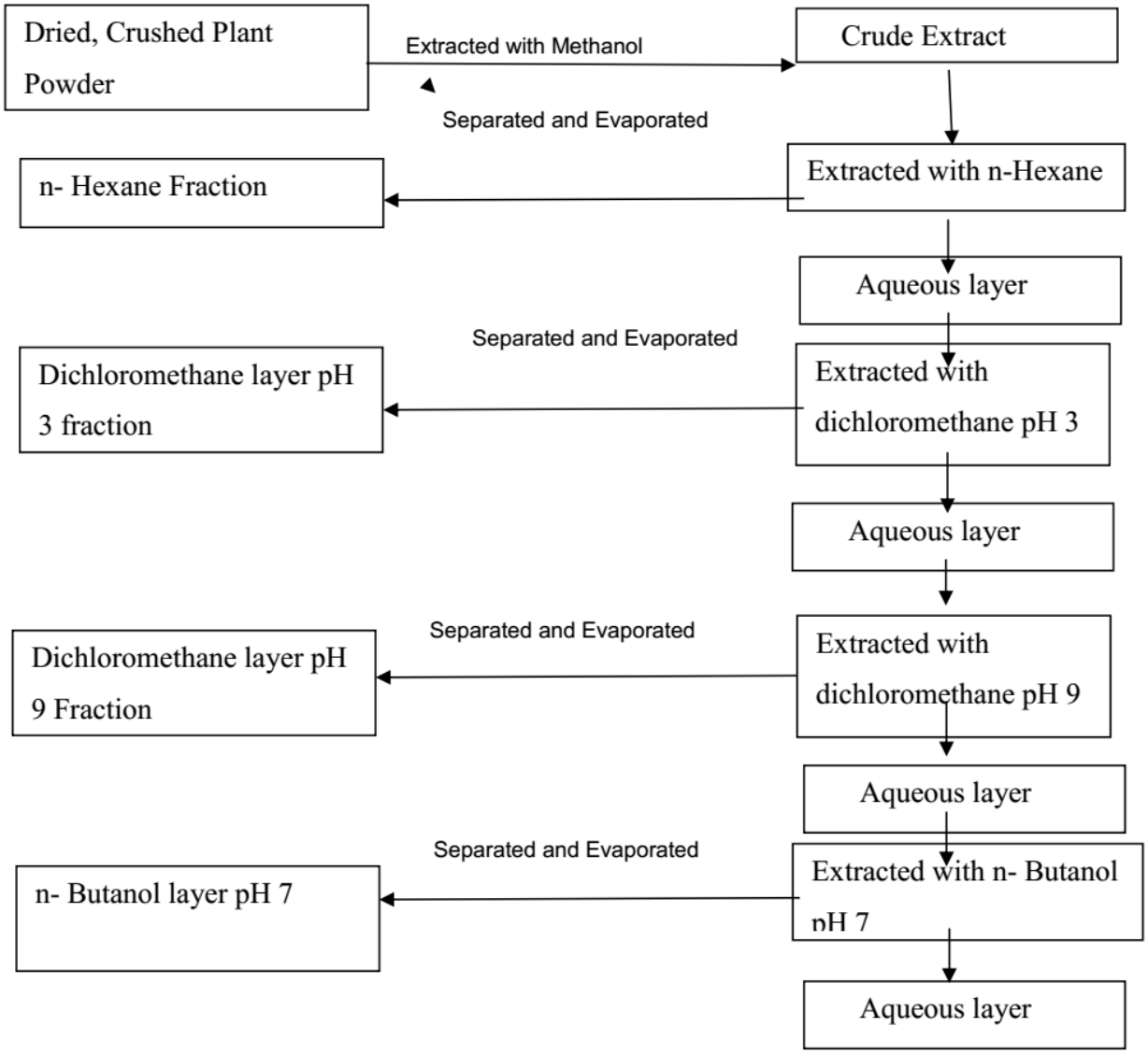

Fig.1 Scheme for partitioning the crude extract in various solvents

3.3 DETERMINATION OF TOTAL PHENOLIC CONTENT

0.2 ml of each extract was mixed with 0.05 ml of Folin Ciocalteu Reagent and 0.2 ml of Sodium carbonate (10 %) and made volume up to 3 ml with distilled water. Absorbance at 760 nm was determined after incubation for 30 min. using U.V. Visible Spectrophotometer. The blank was prepared using the same protocol except sample extract.

3.4 Lipid Peroxidation Value in Linoleic Acid Emulsion System. (Anti-oxidant activity)

4 mg of extract and 4 mg of standard were mixed with 4 ml absolute ethanol, 4 ml of 2.52 % linoleic acid in absolute ethanol, 8 ml of 0.02 M Phosphate buffer (pH7) and 3.9 ml distilled water. The above mixture was placed at

40 ^{0}C. From this 0.1 ml was then mixed with 9.7 ml of 75 % (v/v) ethanol and 0.1 ml of 30 % Ammonium thiocyanate, after adding ferrous chloride (0.1 ml), red color complex has maximum absorbance at 500nm. The procedure was repeated after every 24 hours until standard reaches its maximum. A 5 ml solution consisting of equal quantities of linoleic acid and potassium phosphate buffer was used as a blank. Ascorbic acid was used as a standard and same protocol was followed. Inhibition of lipid peroxidation was measured by using this formula:

$$\%\text{age inhibition} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Sample}}{\text{Absorbance of Blank}} \times 100$$

3.5 Nitric Oxide Free Radical Scavenging Assay

Stock solution of test samples were prepared by dissolving 3 mg of test samples in 1 ml of methanol. Different dilutions i.e., 3, 1.5, 0.75, 0.33 and 0.15 mg/ ml were made from stock solution. 1 ml of each dilution was introduced into separate test tubes along with 1 ml of SNP to make reaction mixture. The mixture was incubated for 90 min. at 27 ^{o}C. After it, 0.5 ml of reaction mixture was added to 1 ml of Sulphanilic acid and incubated at 27 ^{o}C for 5 minutes. 1 ml of NED was added to it and again incubated for 30 minutes at 27 ^{o}C. Results obtained by absorbance at 546 nm. Methanol and Vitamin C was used as a blank and reference respectively. The %age inhibition was measured.

3.6 High performance liquid chromatography

3.6.1 Preparation of plant extract

Plant extract sample was prepared by dissolving 10µL of the extract in 990µL of the mobile phase. The dissolved material was filtered twice and then it was sonicated.

3.6.2 Preparation of Mobile phase

Mobile phase was prepared by dissolving 75ml acetonitrile and 0.625g of 0.025M Sodium dihydrogen phosphate buffer into 175ml deionized water. This was 250ml mobile phase.

3.6.3 Preparation of Stationary phase

C 18 reverse phase column with the composition of octasilane was used in HPLC.

3.6.4 Flow Rate

The flow rate was kept at 1ml/min. with the run time of 35-40 minutes.

4. RESULTS AND DISCUSSION

4.1 Phytochemical analysis of *Phaseolus vulgaris* extract

Table.1 Detection of phytochemical constituents of plant extract *Phaseolus vulgaris*

Sr. No.	Phytochemicals	Red beans
01	Alkaloids	+
02	Tannins	+
03	Terpenoids	+
04	Polyphenols	+

4.2 Percent Yield of food plant fractions

In order to determine the percent yield of various fractions of *Phaseolus vulgaris,* the 250 g each of crude methanolic extract fraction was extracted by solvent extraction method with different solvents such as n-hexane, dichloromethane at pH 3, dichloromethane at pH 9 and n-butanol at pH 7 and the remaining liquid was referred as aqueous fraction.

Table 2 Percent yield of *Phaseolus vulgaris* fractions

Sr. No.	Fractions	Percent Yield (%)
1	n-Hexane	13.77
2	Dichloromethane pH 3	26.07
3	Dichloromethane pH 9	34.69
4	n-Butanol pH 7	11.18
5	Aqueous	14.21

4.3 Total Phenolic content Assay

The standard curve of Gallic acid was made in order to determine the total phenolic content of various fractions of plant extracts. Results are given as mg/g of Gallic acid Equivalents (GAE).

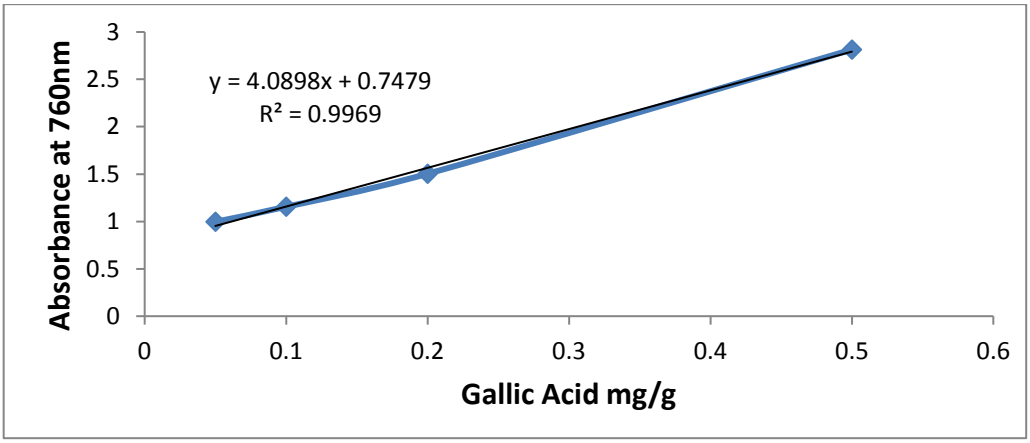

Fig 2 Standard calibration curve of Gallic acid for determination of total phenolic content

Fig 2 shows the graph of Gallic acid in mg/g with respect to absorbance taken at 760nm from the U. V. Visible Spectrophotometer which shows that with the increase in concentration of Gallic acid, the absorbance also increases. Hence a straight line is obtained.

4.4 Total phenolic contents of food plant fractions

Table 3 Total phenolic content of *Phaseolus vulgaris* fractions expressed as mg/g of Gallic acid equivalent.

Serial Number	Fractions	*Phaseolus vulgaris*	
		Dry weight of food plant fraction (g)	mg/g of Gallic acid Equivalent (GAE)
01	Crude methanol extract	285	114.6 ± 1.67
02	n-Hexane	39.262	36.8 ± 1.05
03	Dichloromethane pH 3	74.307	60.6 ± 0.62
04	Dichloromethane pH 9	97.132	116.4 ± 1.12
05	n-Butanol pH 7	31.861	121.8 ± 2.41
06	Aqueous	40.52	143.0 ± 1.25

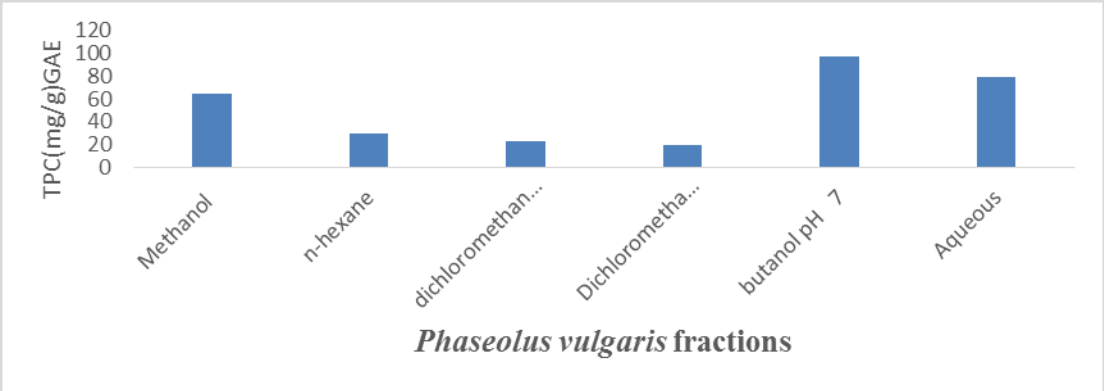

Fig.3 Food plant extracts versus total phenolic contents

Fig 3 depicts the total phenolic contents of *Phaseolus vulgaris.* The total phenolic contents of the fractions of this food plant are in the order: Aqueous > n-Butanol pH 7 > Dichloromethane pH 9 > crude methanol extract> Dichloromethane pH 3 > n-Hexane

4.5 Lipid Peroxidation Value in Linoleic Acid Emulsion System

The lipid peroxidation value of different fractions of food plants was observed in linoleic acid emulsion system. The absorbance of the samples was determined after every 24 hours for 4 days at 500nm. Increase in absorbance indicates decrease in lipid peroxidation value.

Table 4 Percent inhibition values of *Phaseolus vulgaris* fractions

Sr. No	Hours	Crude Methanol	n-Hexane	CH_2Cl_2 pH 3	CH_2Cl_2 pH 9	n-Butanol	Aqueous fraction	Ascorbic Acid
1	12	43±2.61	31.8±1.20	39.2±1.1	43.2±1.0	23.4±1.01	48.7±1.05	73.3±1.15
2	24	54.2±1.06	36.3±1.01	42.9±0.69	50.7±1.0	31.8±1.03	59.5±0.59	77.5±0.75
3	48	65.2±1.7	41.9±0.35	46.3±0.11	54.2±0.61	42.6±0.55	67.4±0.55	82.5±0.98
4	72	73.6±0.96	53.4±0.62	56.9±0.47	66.4±0.77	53.9±0.63	76.3±0.49	91.3±0.90

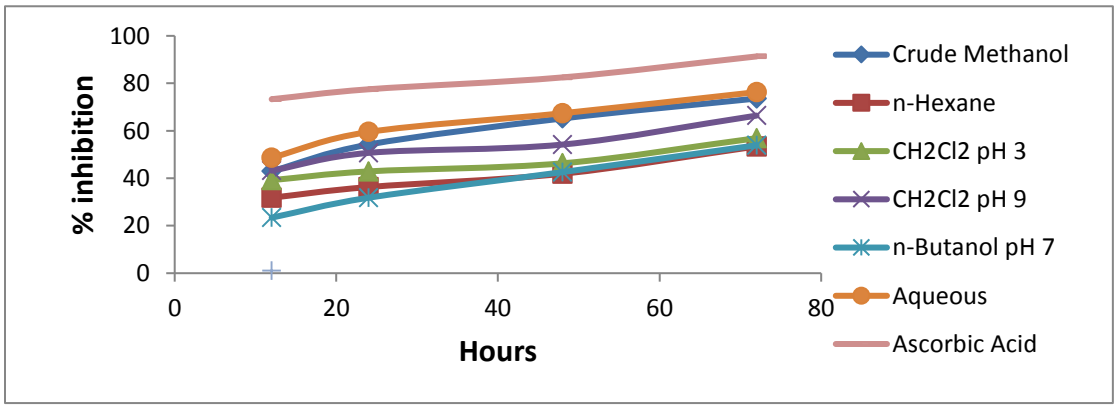

Fig 4 Percent inhibition of food plant versus hour

Fig 4 shows the percent inhibition of ***Phaseolus vulgaris*** with respect to hours. Result of lipid peroxidation assay indicates that from 12 hours to 72 hours, the inhibition power of the different fractions of food plants is increased which depicts its good antioxidant potential and it is correlated with the standard i.e. ascorbic acid. The trend of percent inhibition of different fractions are as follows

Ascorbic acid > Aqueous > crude methanol > dichloromethane at pH 9> dichloromethane at pH 3 > n-butanol > n-hexane

4.6 Free Radical Scavenging Assay

The free radical scavenging assay of different fractions of food plants was observed by the help of Nitric oxide assay and then percent inhibition of these plants fractions with respect to their different concentrations was calculated.

Table 5 Percent inhibition values of food plant fractions

Sr. No	Conc.	Crude Methanol	n-Hexane	CH_2Cl_2 pH 3	CH_2Cl_2 pH 9	n-Butanol	Aqueous fraction
1	3	67.44 ±0.46	37.11 ±0.9	28.53 ±0.66	21.48 ±0.7	62.76 ±0.88	68.5 ±0.66
2	1.5	42.71 ±1.05	34.86±0.89	27.96 ±0.49	16.55 ±0.78	59.19 ±1.01	59.3 ±1.24
3	0.75	37.8±0.96	29.08±0.75	20.65 ±1.05	14.99 ±0.65	50.79 ±0.95	44.9 ±1.10
4	0.33	26.72 ±0.85	18.91±1.26	14.99 ±0.87	9.83±0.7	42.97 ±0.58	43.2 ±0.81
5	0.15	22.56 ±0.92	15.82±0.87	2.99 ±1.04	6.16 ±1.00	30.07 ±0.57	31.9 ±0.72

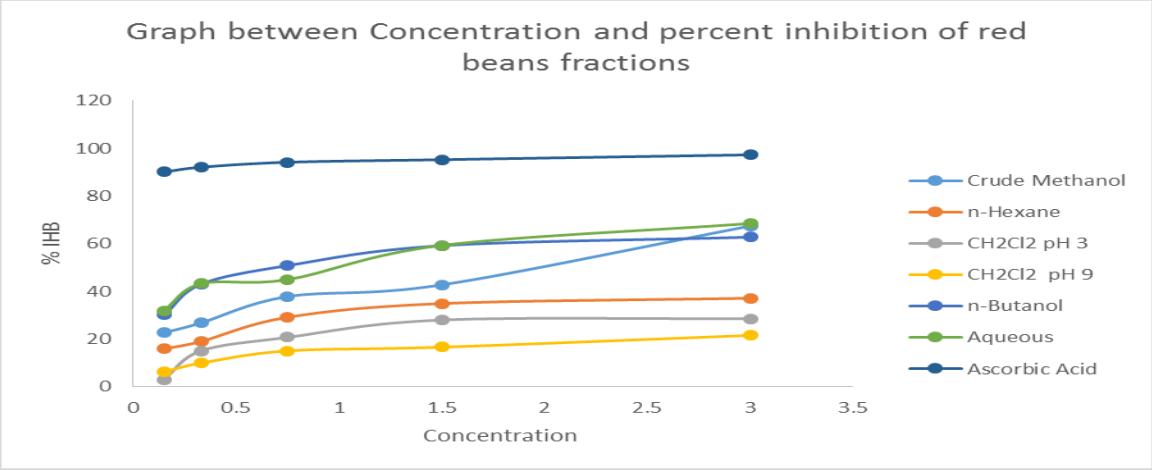

Fig 5 Nitric oxide assay of various fractions of food plants

Fig 5 shows the percent inhibition of food plant with respect to concentration. This result indicates that with the increase in the concentration, the inhibition power of the different fractions of plants is increased which depicts its good antioxidant potential and it is correlated with the standard i.e. ascorbic acid. The trend of the inhibition potential of plants was observed which is as follows:
Ascorbic acid > Aqueous > crude methanol > n-butanol > n-hexane > dichloromethane pH 3 > dichloromethane pH 9

4.7 Comparison of Assays i.e. Linoleic acid emulsion assay and Nitric oxide free radical scavenging assay of different fractions of *Phaseolus vulgaris*

Table 6: Comparison of percent inhibition of *Phaseolus vulgaris* fractions

	Linoleic acid emulsion assay				Nitric oxide scavenging assay				
Phaseolus vulgaris	% Inhibition (Hours)				% Inhibition (Concentration mg/ml)				
	01	**02**	**03**	**04**	**3**	**1.5**	**0.75**	**0.33**	**0.15**
Crude methanol	43±2.61	54.2 ±1.06	65.2 ±1.7	73.6 ±0.96	67.44 ±0.46	42.71 ±1.05	37.8 ±0.96	26.72 ±0.85	22.56±0.92
n-Hexane	31.8±1.2	36.3 ±1.01	41.9 ±0.35	53.4 ±0.62	37.11 ±0.9	34.86 ±0.89	29.08 ±0.75	18.91 ±1.26	15.82±0.87
CH_2Cl_2 pH 3	39.2±1.1	42.9 ±0.69	46.3 ±0.11	56.9 ±0.47	28.57 ±0.66	27.96 ±0.49	20.65 ±1.05	14.99 ±0.87	2.99 ±1.04
CH_2Cl_2 pH 9	43.2±1.0	50.7 ±1.0	54.2 ±0.61	66.4 ±0.77	21.48 ±0.7	16.55 ±0.78	14.99 ±0.65	9.83 ±0.7	6.16 ±1.00
n-Butanol	23.0±1.0	31.8 ±1.03	42.6 ±0.55	53.9 ±0.63	62.76 ±0.88	59.19 ±1.01	50.79 ±0.95	42.97 ±0.58	30.07±0.57
Aqueous	48.7±1.1	59.5 ±0.59	67.3 ±0.55	76.3 ±0.49	68.5 ±0.66	59.3 ±1.24	44.9 ±1.10	43.2 ±0.81	31.9 ±0.72

Table 6 shows the percent inhibition of all the fractions of *Phaseolus vulgaris* of linoleic acid emulsion and nitric oxide free radical scavenging assays. This results depict that at 72 hours maximum percent inhibition was shown by aqueous fraction and crude methanol extract of *Phaseolus vulgaris* as compared to others in linoleic acid emulsion assay while at the concentration of 3 mg/ml, the maximum percent inhibition was shown by crude methanol and aqueous fraction by nitric oxide free radical scavenging assay which revealed that the plants show maximum inhibition in the polar solvents as compared to others.

4.8 Identification of Compounds from High Performance Liquid Chromatography (HPLC)

4.8.1 HPLC analysis of *Phaseolus vulgaris*

i. Phaseolus vulgaris extract

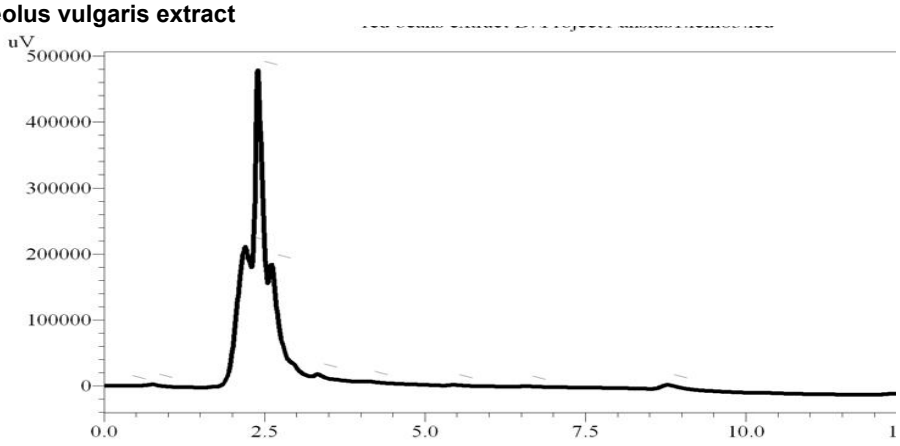

Fig 6 Chromatogram of *Phaseolus vulgaris* Extract

The major organic compounds which were isolated from the *Phaseolus vulgaris* sample from HPLC technique were **Ellagic acid** and **Caryophyllene oxide.** Ellagic acid and Caryophyllene oxide present in the sample and their retention time was 3.324 min.

5. DISCUSSION

Food plants i.e. *Phaseolus vulgaris* has been reported to possess a number of different phytochemical constituents such as alkaloids, poly phenols, terpenoids etc. The different fractions of the food plant in different solvents of varying polarity were analyzed for different biological activities. The yield of the plant is highly dependent on the solvent system with its polarity, extraction time, pH and extraction temperature. The solvent fractions formed from the methanol extracts were n-hexane fraction, dichloromethane pH 3 fraction, dichloromethane pH 9 fraction, n-butanol fraction, and aqueous fraction. In order to find out and compare the presence of the total phenolic contents, antioxidant activity and radical scavenging potential, several tests had been performed with all the plants fractions along with methanolic extracts. Afterwards HPLC of the plant extract had been done for the isolation and identification of chemical compounds present in red beans.

5.1 Percent yield of fractions

The crude methanol extract of plants was fractionated with different solvents of different polarity. For red beans the highest yield of 51.20 % was obtained in the aqueous fraction followed by 38.13 % in n-butanol, 4.33 % in n-hexane, 4.057 % in dichloromethane pH 9 and 1.59% in dichloromethane pH 3 and for mustard plant the highest yield of 34.69 % was obtained in the dichloromethane pH 9 fraction followed by 26.07 % in dichloromethane pH 3, 14.21 % in aqueous, 13.77 % in n-hexane and 11.18 % in n-butanol. These results showed that red beans has highest yield in aqueous fraction.

5.2 Antioxidant Assays

5.2.1 Total Phenolic Content Assay

The antioxidant activity of the plants was determined by the total phenolic contents because phenol has the ability to stabilize the free radical. As phenol contains the hydroxyl group which are strong hydrogen donors, in this way they stop the chain reactions. The total phenolic content was determined by the Follin Ciocalteu method followed by Akhtar et al., 2015. It is a calorimetry technique which undergoes reduction by the reagents of oxides of tungsten and molybdenum. The blue color shows the reduction of these oxides and the absorbance was checked at 765nm. The Gallic acid is the phenol which was used as a standard and estimation of phenolics in different food plants was done with reference to it.
The highest phenolic contents 143.01 ± 1.25 mg/ml of GAE was found to be present in aqueous fraction of *Phaseolus vulgaris.* The contents of phenolic compounds were higher in polar fractions as compared to non-polar fractions.

5.2.2 Lipid Peroxidation Assay

One of the main reason for the rancidity and degradation of stored or processed food is the peroxidation of lipids which ultimately forms peroxyl radicals. Free radicals attacked on polyunsaturated fatty acids and may cause oxidation. This may cause many diseases as cardiovascular diseases or it may be carcinogenic. Up till now many chemicals are synthesized which act as radical scavengers. These chemicals such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) help in preventing the peroxidation of fats in body and food and the method given by Beninger *et al.* In this method, the peroxides are formed after the oxidation of linoleic acid. These peroxides have the ability to oxidize ferrous ions into ferric ions. Afterwards these ferric ions react with the thiocyanate ions and formed the colored complex which is detected at 500nm. The darker the color is, the greater the peroxide radicals present in the plant sample. Similarly presence of the antioxidant in the plant extract decreases the absorbance i.e. increase in lipid peroxidation ultimately decrease the antioxidant in the food plant extract.
On the last day, the greatest percent inhibition 76.3 ± 0.49% inhibition was shown in aqueous fraction of *Phaseolus vulgaris.*

5.2.3 Nitric oxide Radical Scavenging Assay

This assay is based on the diazotization reaction of napthylethyldiamine dihydrochloride and sulphanilamide under the acidic conditions. The sodium nitroprusside formed the nitric oxide which ultimately reacts with oxygen and formed nitric ions. This reagent is used in order to find out the nitrite in the plant samples. The nitric oxide free radical scavenging assay was measured by the method given by Chakraborthy *et al.,* 2009.

The Ascorbic acid which was taken as a standard has percent inhibition 97.34 ± 0.37 %.The greatest percent inhibition for free radical scavenging capacity was usually shown by aqueous solution of *Phaseolus vulgaris* 68.5 ± 0.66 %.

5.3 Identification of Organic compounds

HPLC technique was done in order to find out the chemical constituents present in the food plant extract. The results revealed that usually phenols, carboxylic acids and their derivatives were present in the samples. The present study investigated the presence of Phytic acid, Saponins, sinapic acid, *p*-coumeric acid, and proanto cyanidins were reported as chemical compounds isolated from *Phaseolus vulgaris* and this study explored Ellagic acid and Caryophyllene oxide from it. In view of all these, this study identified many compounds which are not characterized yet and are good for further studies.

6. CONCLUSION

Phytochemical screening of the methanolic extracts of the food plant *Phaseolus vulgaris* had exposed the presence of polyphenols, tannins, terpenoids and alkaloids with the respective test reagent. The results obtained from the different assays revealed that it was rich in phenolics and it had good antioxidant potential as well as highest radical scavenging capability. It was observed that polar compound fractions of the food plant such as methanol, aqueous and n-butanol showed maximum antioxidant capacity as compared to n-hexane. Similarly the isolation and characterization of the samples from HPLC suggests the presence of many organic compounds including aromatic phenols and hydroxyl carboxylic acids and their derivatives in the samples. In this way, this study suggests that the plant i.e. *Phaseolus vulgaris* is bioactive compound and could be best source of natural antioxidant which has a highest importance in medicines in order to cure the metabolic syndrome as diabetes and cardiovascular diseases.

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