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*RESEARCH ARTICLE*

# DETERMINATION OF ANTIOXIDANT ACTIVITY OF *PHASEOLUS VULGARIS* PLANT EXTRACTS BY *IN-VITRO* ANALYSIS

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## *Abstract*

Free radicals produce in body by various environmental and lifestyle changes these days. These free radicals have known to be very unstable species and when formed in high amount can be very dangerous to body and produce condition called Oxidative stress. Oxidative stress has known to be underlying cause for many chronic diseases like cardiovascular disease, Arthritis, Diabetes, cataract, and neurodegenerative diseases. Antioxidants from plant based origin have gained much interest in recent years as they can reduce the harmful effects of oxidative stress without the undesirable side effects. In this approach the plant *Phaseolus vulgaris* has been selected for determining their antioxidant abilities, crude extract of pet ether, chloroform and methanol were tested. The plant's antioxidant potential were determined using DPPH radical Scavenging assay and found that Methanol extract shows the $IC_{50}$ value of 85.84±0.08 µg/mL, which shows maximum radical scavenging power, followed by Chloroform and Pet ether. Superoxide radical scavenging assay showed the value of Methanol 46.79±0.09 µg/mL to be close to standard Ascorbic acid 30.66±0.312 µg/mL. Pet ether has lowest Superoxide scavenging power than Chloroform. The reducing power assay was determined by making different concentrations of plant extracts and found that as the concentration increases the reducing power of all the extract increases as higher value of absorbance indicates high reducing power. Reducing power follows the order ascorbic acid > methanol > chloroform > Pet ether. The given studies prove the antioxidant capacity of *Phaseolus vulgaris* plant extracts and further studies can be conducted to explore its application in Therapeutics.



## Introduction:-

During the process of metabolism and respiration body produces free radicals. These free radicals are unpaired and unstable species having one less electron in their outer shell. To fulfill their need of electron they abstract it from nearby cell and end up generating chain reaction and destroy cells, this cause major damage to cellular structures and affects DNA, enzymes, protein and various cell membranes. Excessive free radical can result in developing many harmful diseases. Antioxidants are body's natural mechanism to deal with free radicals, they interact with free radicals and helps in terminating chain reaction by providing an extra electron. Free radical generates at frequent

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rate with the advanced age, body's natural defense mechanism fails to compensate the formation of free radical and natural antioxidants, a condition arises called Oxidative stress. Oxidative stress is known as a major factor in initiation of many harmful diseases. In many cases providing external synthetic antioxidants can work to help body against harmful radicals (Shang et al., 2017). However it was observed that prolonged usage of synthetic antioxidants can cause adverse effect on body, causing side-effects.

Many studies have conducted in search of safer alternative of antioxidants and have led towards plant based antioxidants. Plants possess many bioactive components which act as radical scavengers and can be used as therapeutics (Paulsamy and Kartika, 2012). Legumes are known for their nutritive values as they are essential source of micro and macro nutrients, these are rich source of plant phytochemicals such as phenolics, flavonoids, alkaloids, tannnis, steroids, amino acid, protein etc. Polyphenols present in legumes such as phenolic compounds can act as radical quenchers (Zhao et al., 2014). One such legume is *Phaseolus vulgaris*, belongs to Fabaceae family also called as Common bean, French bean, Snap bean (Mahamune and Kothekar, 2011). *Phaseolus vulgaris* is consumed widely all around the world, known as source of nutrition in African countries, initially originated in American continents (Atchibri et al., 2010). The plant is rich in various phytoconstituents, polyphenols helps giving the plant various properties like Antioxidant, Anti-inflammatory, Anti-diabetics, Anti-obesity, Osteoprotective, Neuroprotective etc. *Phaseolus vulgaris* is rich in various types of flavonoids and phenolic acids. Health benefits of the plant are largely attributed by its high content of proteins, minerals, phytochemicals, vitamin etc (Ganesan and Xu, 2017).

*Phaseolus vulgaris* has acquired its color due to the presence of flavonols glycosides and condensed tannins. Consumption of common bean has known to decrease the risk of various degenerative diseases due to the presence of polyphenolic compounds (Reynosos-camacho et al., 2006). In this study we examine *Phaseolus vulgaris* pods. Three extracts (Pet ether, Chlororform, Methanol) were evaluated for the presence of antioxidant activity through different assays.

## Material & Methods:-

**Plant material:**

*Phaseolus vulgaris* pods were collected from local market of Bhopal (M.P), in the month of October. The selected plant then verified by Dr Zia-ul-Hasan HOD (Botany), Saifia College, Bhopal. The Vocucher Specimen number given was 448/Bot/Saifia/17

**Extraction:**

The collected pods of French beans were shade dried for 7-10 days before use, than they were grinded in coarsed powdered form. The technique used for the extraction is Maceration. The grinded plant was kept in jar of solvents with various polarities for the separation of phytoconstituents. Three extracts namely Pet ether, Chloroform, Methanol were prepared.

**In vitro Antioxidant Assays:**

DPPH (1,1 diphenyl-2-picryl hydrazyl) (Gulcin et al., 2006)

In this analysis we use 0.1 mM DPPH solution, the plant extracts of concentration range (20, 40, 60, 80, 100 µg/mL) were prepared in solvent methanol. 2 ml of test sample is mixed with 1ml of DPPH solution and incubated for 10 minutes. Absorbance of the solutions was taken at 515 nm keeping methanol as blank.

% Inhibition can be calculated by the given formula

$$\% \text{ Inhibition} = \frac{(A_{\text{Control}} - A_{\text{Sample}}) \times 100}{A_{\text{Control}}}$$

Where, $A_{\text{control}}$ = (test reagents without extract), $A_{\text{Sample}}$ = (test reagents with extract)

Calculation of $IC_{50}$ value is done by plotting graph between % inhibition and concentration with the help of line of regression equation. Where $IC_{50}$ is extract concentration which is needed to inhibit 50% DPPH solution.

Superoxide Radical Scavenging Assay (Arumugam et al., 2015)

0.1 mL NBT (Nitro blue tetrazolium) having reaction mixture added to 0.3 ml of extract, to this 1 mL of alkaline DMSO (Dimethyl sulfoxide) is added. Final volume becomes 1.4 mL. Absorbance is taken at 560 nm, keeping DMSO as blank.

**Superoxide radical scavenging capacity formula:**

$$\text{Superoxide radical scavenging capacity (\%)} = \frac{(A_{control} - A_{Sample})}{A_{control}} \times 100$$

Where, $A_{control}$ = Absorbance of Control without extract, $A_{Sample}$ = Absorbance of extract

% inhibition is calculated using graph and $IC_{50}$ is found, taking ascorbic acid as standard

Reducing power Assay (Jain and Jain, 2011)
In this methodology test samples of various concentration ranges were prepared. To 0.5 ml of extract sample add 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and add 0.5 ml of 1% potassium ferricyanide. The test samples were kept for incubation at 50$^{o}$C for 20 minutes. After the reaction mixture is cooled, add 1.5 ml of 10% trichloroacetic acid, this helps terminating the reaction, 0.5 ml of ferric chloride 0.1% is added at the end. The absorbance is measured at 700 nm. Graph between absorbance and concentration was plotted, and reducing power of test samples was determined.

**Statistical Analysis:**
The results were taken in triplicates and expressed as mean values ± standard deviation

## Results and Discussion:-

The *In-vitro* Antioxidant assays were performed on pet ether, chloroform and methanol plant extract of *Phaseolus vulgaris.*

DPPH Radical Scavenging Assay (1,1 diphenyl-2-picryl hydrazyl)

DPPH radical scavenging assay is used to determine quenching potential of antioxidants. DPPH (1,1 diphenyl-2-picryl hydrazyl) is an unstable radical, purple in color which gets reduced in the presence of antioxidant to non-radical stable form. The purple color on reduction changes to yellow. During the process of reduction the absorbance of the solution containing DPPH decreases. The absorbance is measured at 517 nm by UV spectrophotometer (Gangwar et al., 2014). Ascorbic acid is taken as standard and results were obtained in triplicates, as the radical scavenging capacity of antioxidant increase the absorbance decreases. $IC_{50}$ value was calculated with the help of graph and radical scavenging is calculated by $IC_{50}$ value, lower $IC_{50}$ gives better radical scavenging potential (Shekhar and Anju, 2014).

The studies conducted on plant extracts reveals that *Phaseolus vulgaris* shows DPPH radical scavenging ability in concentration dependent manner. In this assay standard ascorbic acid shows the maximum radical scavenging, which was tested against various plant extracts. In these extracts closest $IC_{50}$ value was found to be of methanol followed by chloroform and pet ether. With increased concentration % inhibition increases and radical scavenging increases as well. Results were expressed as ascorbic acid equivalent (AAE).

**Table 1:-** $IC_{50}$ value of DPPH radical scavenging activity of *Phaseolus vulgaris.*

| ***Phaseolus vulgaris*** | **IC50 (µg/mL AAE)** |
|---|---|
| Ascorbic acid | 27.08± 0.190 |
| Methanol | 85.84±0.08 |
| Chloroform | 125.67±0.26 |
| Pet ether | 157.89±0.33 |

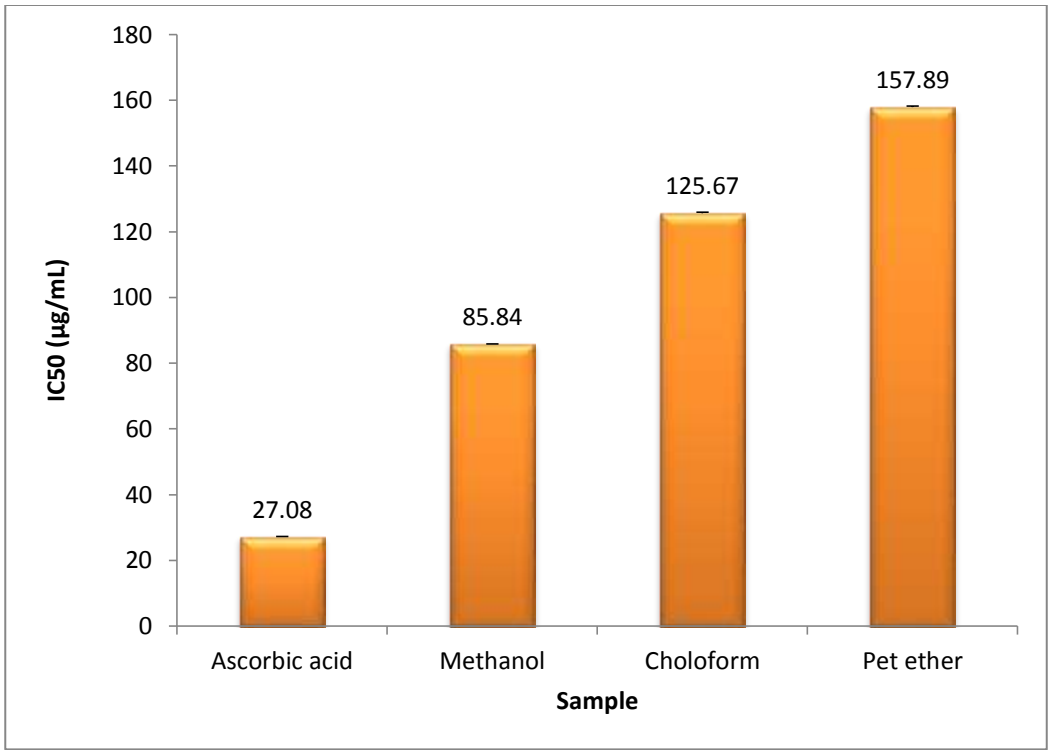


**Figure 1:-** DPPH radical Scavenging activity of *Phaseolus vulgaris* extracts

**Superoxide Scavenging Assay:**

In this methodology Superoxide radical Scavenging ability is determined by generating Superoxide radical by adding sodium hydroxide to air saturated DMSO. This superoxide formed becomes stable in solution and reduces Nitroblue tetrazolium (NBT) into formazan dye at room temperature (Arumugam et al., 2015). The sample extracts radical scavenging activity is measured by decreased absorbance of reaction mixture and taken at 560 nm by UV spectrophotometer. Presence of antioxidants in plant extract decreases the reduction of NBT. Ascorbic acid is taken as standard (Shareef et al., 2014).

The extracts were subjected to the superoxide radical scavenging assay and results were compared with standard ascorbic acid. The ascorbic acid showed highest scavenging potential, % inhibition increases as concentration increases in all the tested extracts, depending on % inhibition and concentration, graph is plotted and $IC_{50}$ values were calculated. Methanol showed the lowest $IC_{50}$ value amongst all extracts, indicating the highest scavenging potential, chloroform and pet ether followed methanol. Results of Scavenging potential were expressed as AAE (ascorbic acid equivalent).

**Table 2:-** $IC_{50}$ value, of Superoxide Scavenging activity of Phaseolus vulgaris.

| ***Phaseolus vulgaris*** | **IC50 (µg/mL AAE)** |
|---|---|
| ascorbic acid | 30.66± 0.312 |
| Methanol | 46.79±0.09 |
| Chloroform | 98.46±2.2 |
| pet ether | 122.07±1.32 |

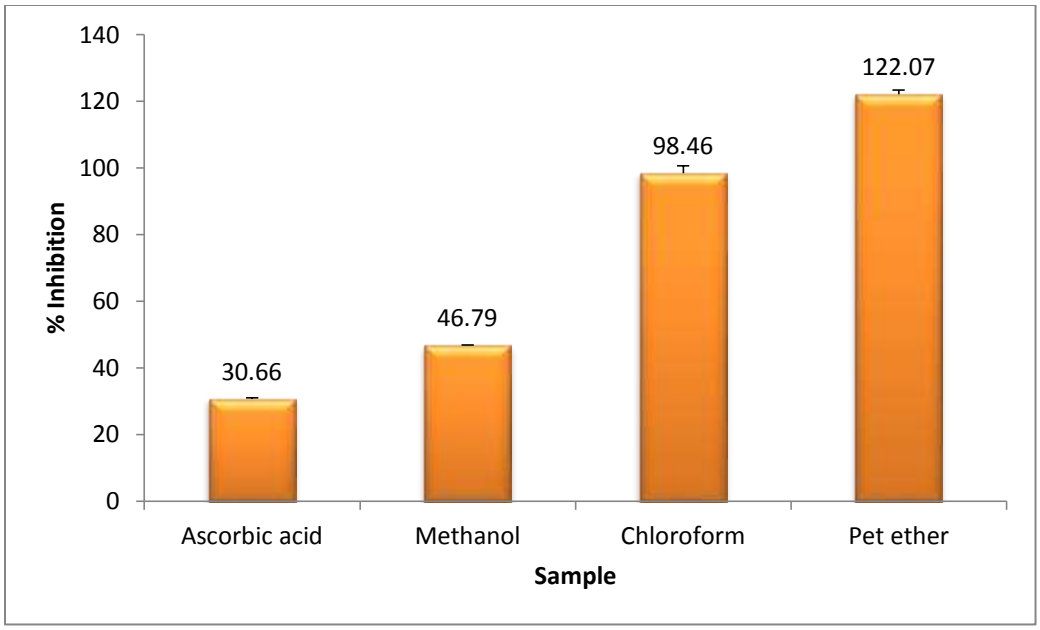


**Figure 2:-** Superoxide scavenging activity of *Phaseolus vulgaris* extracts.

**Reducing Power Assay**

Plants possess various phytochemicals and are known to be responsible for their antioxidant potentials, and calculating reducing power is linked to their antioxidant potential (yan zhao et al., 2014). The presence of antioxidant in plant extract is determined by its potential to reduce $Fe^{+3}$/ferricyanide to $Fe^{+2}$/ferrocyanide complex by donating free electron. The results were expressed by the formation of Prussian blue color complex and it is measured with the help of UV spectrophotometer at 700 nm. The color changes from yellow to various shades of blue and green, depending upon the reducing power of test solutions with concentration. The graph is plotted between concentration and absorbance and reducing power is determined (Ferreira et al 2007).

The reducing power assay of various plant extracts of *Phaseolus vulgaris* indicates that methanol have higher reducing power than the chloroform and pet ether extracts, keeping ascorbic acid as standard, with increased concentration of extracts the absorbance increases.

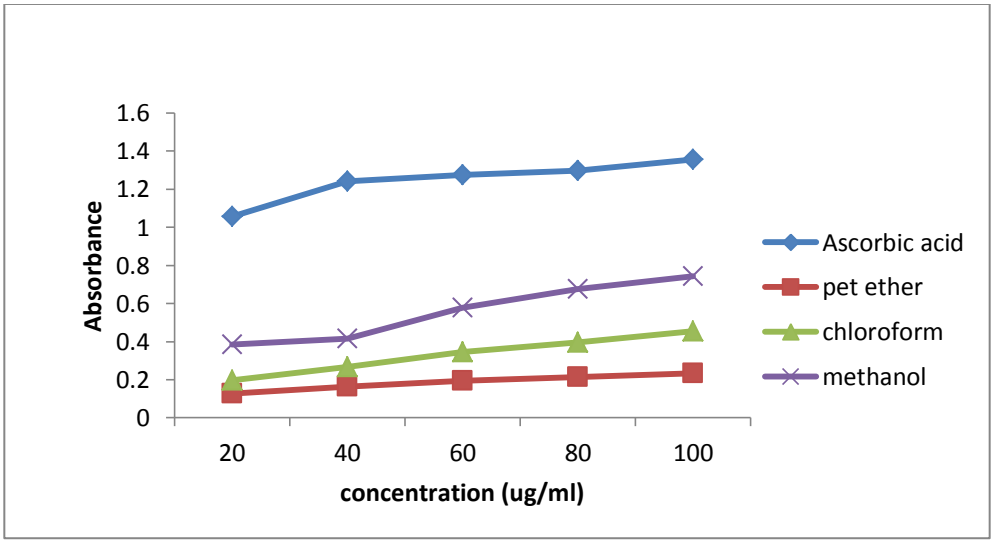


**Figure 3:-** Reducing power of *Phaseolus vulgaris* extracts, Where, STD (standard), PE (Pet ether), CF (Chloroform), ME (Methanol).

## Conclusion:-

The above study concluded that *Phaseolus vulgaris* possess antioxidant potential in all of its tested crude extracts. However methanol extract showed significant activity as compared to other extracts. To investigate the key components involves for antioxidant activity further isolation and characterization of plant extracts must be done. The plant can be used as a potential source of antioxidant derived from natural sources and can prove helpful in slowing or preventing disease arises by Oxidative stress conditions.

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