# Characterization and evaluation of antioxidant activity of *Cajanus cajan* and *Pisum sativum*

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## ABSTRACT

The present study was designed to investigate the anti-oxidant activity of the ethanolic extract of *Cajanus cajan and Pisum sativum.* The ethanolic extract was evaluated by UV Spectrophotometer. Anti-oxidant activity of ethanolic extract was determined by FRAP method. The FRAP means the Ferric ion reducing Antioxidant Power. At low pH, reduction of ferric tri-pyridyl triazine ($Fe^{3+}$ TPTZ) complex to ferrous form (which has intense blue colour) can be monitored by measuring the change in adsorption at 593 nm. The reduction is non-specific, is that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction will derive the ferrous ($Fe^{3+}$ to $Fe^{2+}$) in formation. The change in absorbance is therefore directly related to the combined or total reducing power of the electron donating antioxidant present in the reaction mixture.

**Key words:** *Cajanus cajan, Pisum sativum,* Anti-oxidant, UV, FRAP, TPTZ

## Introduction

In prehistoric days, plants are used for shelter, food and medicine. The use of plants for medicinal purposes is as old as our civilization. [1]The first known written record of curative plants was of Sumerian herbal of 2200B.C.Herbs have been used for uncounted time for various purposes like healing the stick and infirm. Most of the people still continue to use herbs to benefit their bodies. People thought that herbs keep the body in tune with nature as nature intended and maintain proper balance. [2]

Ayurvedic medicine was practiced in India as far as back as 5000 years ago and one of the oldest known Indian books on plants, Vedas, records the medicinal and religious use of herbs and plants. [3] When herbs are taken, the body starts to get cleansed, it gets purifying itself. Unlike chemically synthesized, highly concentrated drugs, that may produce many side effects, herbs can efficiently realign the body's defenses.

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Herbs do not produce instant cures, but rather offers a way to put the body in proper tune with nature. [4]

Herbs are generally defined as non woody plants which die after blooming. This definition has been expanded to any of the plants of which part or whole can be used in medicinal treatments, culinary preparations. [5] From the germ theory of diseases and the advent of antibiotics to combat various infections, it appeared as if infectious diseases were a thing of past. With the realization that chemical medicines are making comeback. [6] There are several ways to prepare herbs for consumption and use in medicinal remedies. When herbs are prepared by steeping in boiling water to be drunk as a tea they are known as infusions. If these dried herbs get simmered in hot water they are called as decoction. If gets incorporated in with other ingredients and made into cream, they are viewed as herbal ointment. [7]Sometimes used a herbal compress where piece of cloths is soaked in an herbal compress where piece of cloth is soaked in an infusions or decoction and bin wrapped and applied externally. If herbs are used to cleanse and heal externally, they are called herbal wash. Herbal infusions and decoction can be used as herbal wash for relaxation and heeling.

[8, 9]An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers

electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Antioxidants can cancel out the cell-damaging effects of free radicals. Furthermore, people who eat fruits and vegetables, which happen to be good sources of antioxidants, have a lower risk of heart disease and some neurological diseases [7], and there is evidence that some types of vegetables, and fruits in general, protect against a number of cancers. These observations suggested the idea that antioxidants might help prevent these conditions. There is some evidence that antioxidants might help prevent other diseases such as macular degeneration, suppressed immunity due to poor nutrition, and neurodegeneration. Since antioxidant supplements have no clear effect on the risk of chronic diseases such as cancer and heart disease. This suggests that other substances in fruit and vegetables (possibly flavonoids), or a complex mix of substances, may contribute to the better cardiovascular health of those who consume more fruit and vegetables. Antioxidants that are reducing agents can also act as pro-oxidants. For example, vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide; however, it will also reduce metal ions that generate free radicals through the Fenton reaction.

$2Fe^{3+}+Ascorbate \rightarrow 2Fe^{2+}+Dehydroascorbate$

$2\ Fe^{2+} + 2\ H_2O_2 \rightarrow 2\ Fe^{3+} + 2\ OH\cdot + 2\ OH^-$

The relative importance of the antioxidant and pro-oxidant activities of antioxidants are an area of current research, but vitamin C, for example, appears to have a mostly antioxidant action in the body. However, less data is available for other dietary antioxidants, such as vitamin E, or the polyphenols.

The pigeon pea (*Cajanus cajan*, syn. *Cajanus indicus*) is a perennial member of the family Fabaceae. *Cajanus cajan* is the oldest food crop of the world and ranked 5$^{th}$ among edible legumes in world wide production. *Cajanus cajan* is known to produce more nitrogen per unit of plants biomass than the most other legumes and can modulate in most soils. It is also considered to be tolerant to low and high temperature. Seeds of pigeon pea are known to be rich sources of proteins, carbohydrates, and minerals with protein content generally varying from 18 to 25% and as high as 32%. Pigeon pea seeds are rich in sulfur containing amino acids, methionine and cystine.

Raw pigeon pea contains 26.25% crude protein, 7-10% crude fiber. Boiling and soaking of raw pigeon seeds in water have been reported to improve its crude protein content and reduce the antinutritional contents, while toasting the seeds reduced the crude protein and crude extract contents due to volization during dry heat application.

Five isoenergetic and isonitrogenous diets were formulated with raw, boiled toasted, soaked pigeon pea seed meal (PSM) and control (0%). Each of the raw or processed PSM was induced at 20% of the whole diet. The diet had: 15.5% crude protein, 4.27% crude fibre, 1.03% calcium, 0.51% phosphorus, 0.69% lysine, 0.26% methionine

*Cajanus cajan* is useful in the treatment of internal organ swelling. Some herbal practitioners/ researchers are of the opinion that it diminishes the swelling of internal organs like stomach, liver, intestines etc. In case of wound or cancer of these organs it is helpful in reducing them. Its recommended usage is: Green leaves of Pigeon peas around 10 grams along with 7 black peppers should be finely ground and mixed in water and then taken as a drink. Green leaves of Pigeon peas ground in water and added to half boiled water should be applied externally on the affected body part.

A pea is most commonly the small spherical seed or the seed-pod of the legume *Pisum sativum* is a perennial member of the family Fabaceae. Each pod contains several peas. Although it is botanically a fruit, it is treated as a vegetable in cooking. In some parts of the world, dried peas are consumed split as dal, roasted, parched or boiled. Based on protein digestibillity of peas and faba beans is almost entirely digested in the small intestine and the impaired performance in literature was attributed to an increase secretion of endogenous protein.

The protein concentration of peas range from 15.5%-39.7%. An average amino acid composition reported in terms of gram per 100 gram of proteins: 6.9-8.2 lysine, 1.4-2.7 methionine +cystine, 3.9 threonine, 0.9 tryptophan, 0.8-1.7 cystine. Methionine and cystine are the main limiting amino acids. Largest chemical component which constitutes about 55.6% of seed weight. The most abundant pea carbohydrate is starch 36.9-48.6% while amylase is about 34%of seed weight in peas.

*Pisum sativum* shows antioxidant activity due to the presence of tannins. Tannins are astrigent bitter polyphenols that either bind or ppt. Or shrink protein. The term Tannin is widely employed to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups such as carboxyl to form strong bonds with protein and

macromolecules .Tannins are found in leaf tissues, seed tissues, root tissues and stem tissues.

**Material and methods**

**Samples:** Total 500 gram of both *Cajanus cajan* and *Pisum sativum* were purchased from local market of Paonta Sahib District Simour, Himachal Pradesh, India. The seeds were identified by the Botanical Survey of India (B.S.I), Dehradun (U.K.). Both the seeds were cleaned and shade-dried for a week, powdered mechanically (sieve no: 10/44) and stored in air tight containers.

**Chemicals**

The entire chemicals used in this investigation were of analytical grade and were purchased from Sigma, Merck etc. Deinoised was used for the complete study.

**Reagents**: [10]

FRAP reagent

- Acetate buffer 300mM pH 3.6 - 3.1g sodium acetate trihydrate was mixed with 16ml glacial acetic acid of volume was made up to 1 L with distilled water.
- TPTZ (2, 4, 6-tripyridyl-s-triazine) 100Mm IN 40mM HCL
- Ferric chloride 20mM.

**FRAP working solutions**

25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3.6H_2O$ solution i.e. all the solutions are mixed in the quantity of 10:1:1.

The working solution must be always freshly prepared.

Aqueous solution of known $FeSO_4$ $7H_2O$ was used for calibration.

**Spectrophotometric Measurements**

Spectrophotometric measurements were performed by UV-VIS Double Beam Spectrophotometer **(Shimadzu 1800).**

**Preparation of extracts**

Both the legume seeds were dried in shade. The dried masses were blended into fine powder by frequent sieving and powders were extracted by soxhlet process with ethanol. 10 gm of the seeds powdered was taken and in a 500 ml Round Bottom Flask and 2000 ml of the Ethanol was added into the same. Soxhlet extraction was carried out in a Heating Mentle for a period of 48 hrs. After extraction process was complete the solvent was evaporated to dryness. The extract was calculated per gram of dried material.

**ASSAY: Blank: FRAP Reagent +DDW**

Monitoring up to 15 sec at 593 nm, 1 cm light path and $37^0$ C, Fe(ll) standard solution tested in parallel, calculation using the calibration curve.

**Preparation of Standards**

*Ferrous sulphate std. solution:* Prepare 1mM solution: 0.278g $FeSo_4.7H_2O$ in 1 litre distilled water.

**Results and discussion**

0.01 ml of ferrous sulphate was mixed with 1.5 ml of FRAP and 3.49ml of distilled water and other concentration as follows:

| $FeSO_4.7H_2O$ Solution (µl) | FRAP Reagent | Distilled Water (ml) | Total Volume (ml) |
|---|---|---|---|
| 10 | 1.5 | 3.49 | 5.0 |
| 20 | 1.5 | 3.48 | 5.0 |
| 30 | 1.5 | 3.47 | 5.0 |
| 40 | 1.5 | 3.46 | 5.0 |
| 50 | 1.5 | 3.45 | 5.0 |
| 60 | 1.5 | 3.44 | 5.0 |
| 70 | 1.5 | 3.43 | 5.0 |
| 80 | 1.5 | 3.42 | 5.0 |
| 90 | 1.5 | 3.41 | 5.0 |
| 100 | 1.5 | 3.40 | 5.0 |

Aqueous solution of $FeSo_4$ was used for calibration (1mM)

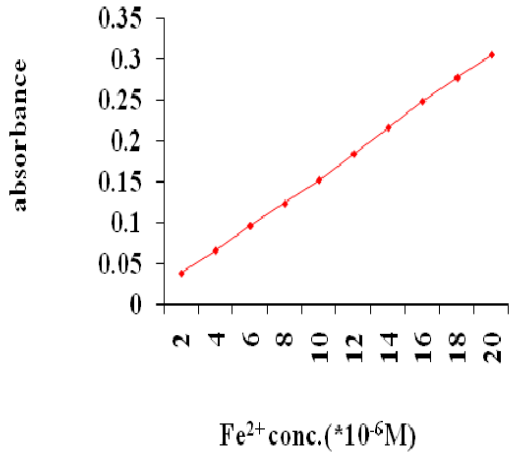
Monitoring up to 5mM at 593nm, 1cm path length and at 37°C, absorbance was recorded and standard curve plotted for absorbance.

**Data for the standarad curve**

| S.NO. | Conc. of $FeSO_4$ (µl) | Absorbance |
|---|---|---|
| 1 | $2*10^{-6}$ | 0.038 |
| 2 | $4*10^{-6}$ | 0.066 |
| 3 | $6*10^{-6}$ | 0.096 |
| 4 | $8*10^{-6}$ | 0.123 |
| 5 | $10*10^{-6}$ | 0.152 |
| 6 | $12*10^{-6}$ | 0.184 |
| 7 | $14*10^{-6}$ | 0.216 |
| 8 | $16*10^{-6}$ | 0.248 |
| 9 | $18*10^{-6}$ | 0.277 |
| 10 | $20*10^{-6}$ | 0.305 |

Freeze @ -20 °c in 0.2 ml aliquots in ependorfs.

**Standard curve**




Acc to the Beer-Lamberts Law **A=ε.C.T**
Whereas, A=Absorbance
ε=Molar Absorptivity
C=Concentration, T=path Length(1 cm)
Upon calculation ε comes out to be $1.5*10^4$ M

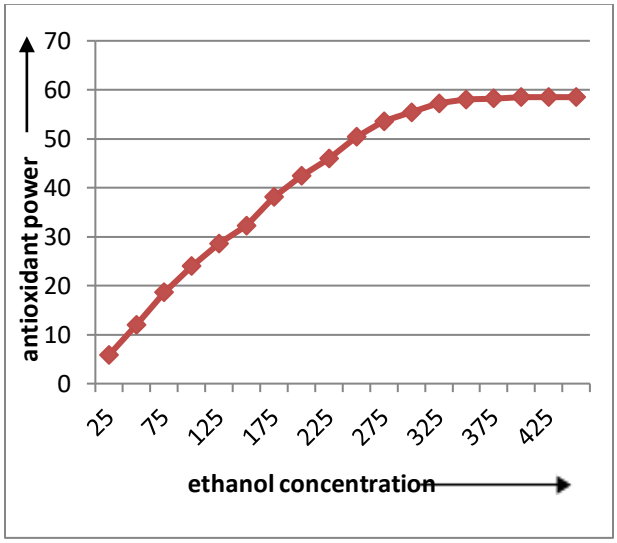
**To calculate the antioxidant power of Pisum Sativum and Cajanus Cajan, we used the formula**

**Antioxidant power = Absorbance / Molar absorptivity**

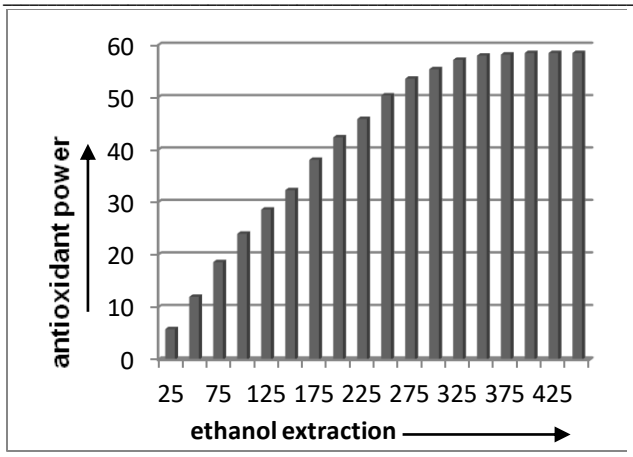
**Tabular reading of Antioxidant power of *Pisum sativum***

| S.No | Ethanol extraction concentration (µg) | Absorbance | Antioxidant power (µM) |
|---|---|---|---|
| 1 | 25 | 0.088 | 5.86 |
| 2 | 50 | 0.180 | 12.0 |
| 3 | 75 | 0.280 | 18.6 |
| 4 | 100 | 0.360 | 24.0 |
| 5 | 125 | 0.427 | 28.6 |
| 6 | 150 | 0.485 | 32.3 |
| 7 | 175 | 0.572 | 38.1 |
| 8 | 200 | 0.637 | 42.4 |
| 9 | 225 | 0.689 | 45.9 |
| 10 | 250 | 0.757 | 50.4 |
| 11 | 275 | 0.804 | 53.6 |
| 12 | 300 | 0.831 | 55.4 |
| 13 | 325 | 0.858 | 57.2 |
| 14 | 350 | 0.870 | 58.0 |
| 15 | 375 | 0.873 | 58.2 |
| 16 | 400 | 0.877 | 58.5 |
| 17 | 425 | 0.878 | 58.5 |
| 18 | 450 | 0.878 | 58.5 |

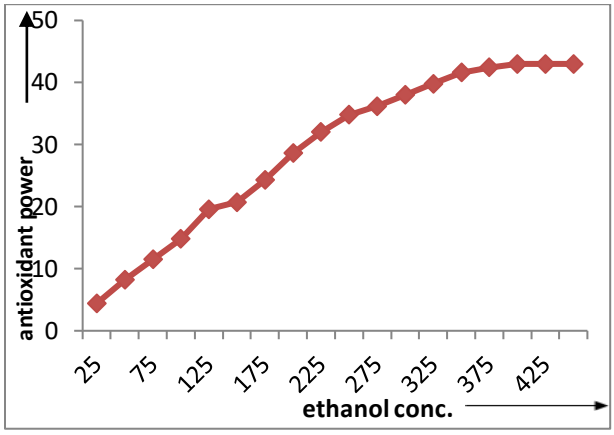
**Graphical representation of antioxidant power of *Pisum sativum***




**Bar graph representation of antioxidant power of *Pisum sativum***



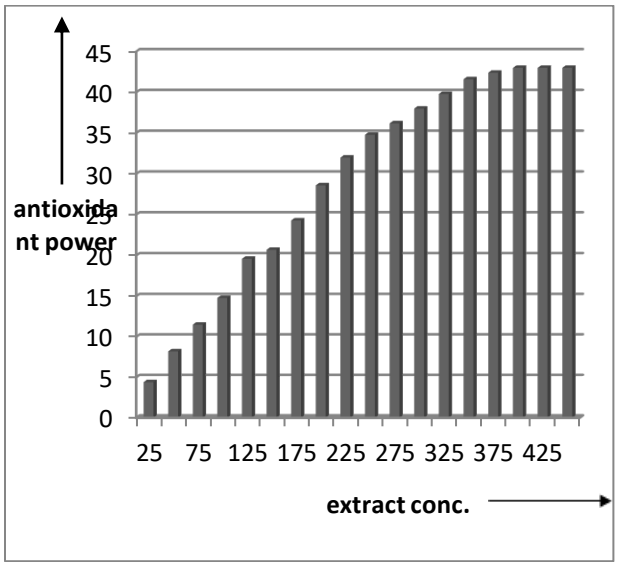

**Graphical representation of antioxidant power of *Cajanus cajan***




**Tabular reading of antioxidant power of *Cajanus cajan***

| S.No | Ethanol extraction concentration (µg) | Absorbance | Antioxidant power ( µM) |
|---|---|---|---|
| 1 | 25 | 0.066 | 4.4 |
| 2 | 50 | 0.123 | 8.2 |
| 3 | 75 | 0.173 | 11.5 |
| 4 | 100 | 0.223 | 14.8 |
| 5 | 125 | 0.295 | 19.6 |
| 6 | 150 | 0.311 | 20.7 |
| 7 | 175 | 0.365 | 24.3 |
| 8 | 200 | 0.430 | 28.6 |
| 9 | 225 | 0.480 | 32.0 |
| 10 | 250 | 0.523 | 34.8 |
| 11 | 275 | 0.543 | 36.2 |
| 12 | 300 | 0.570 | 38.0 |
| 13 | 325 | 0.597 | 39.8 |
| 14 | 350 | 0.624 | 41.6 |
| 15 | 375 | 0.636 | 42.4 |
| 16 | 400 | 0.645 | 43.0 |
| 17 | 425 | 0.646 | 43.0 |
| 18 | 450 | 0.646 | 43.0 |

**Bar graph representation of antioxidant power of *Cajanus cajan***




**Conclusion**

The Ethanol extract of *Pisum sativum* seeds at 25 µg concentration showed its antioxidant power of 5.86 µM which is continuously increases with increase in amount of extract weight till the FRAP value 58.5 µM (at 450 µg concentration) after which the graph assumed at least straight line showing the >=450 µg concentration, the FRAP value assumed the same value i.e. at 400 µg concentration, 425 µg concentration and 450 µg concentration is 58.5 µM. The free radical scavenging activity was found to be good in 58.5 µM.

The Ethanol extract of Cajanus Cajan seeds at 25 µg concentration showed its antioxidant power of 4.4

µM which is continuously increases with increase in amount of extract weight till the FRAP value 43.0 µM (at 450 µg concentration) after which the graph assumed at least straight line showing the >=450 µg concentration, the FRAP value assumed the same value i.e. at 400 µg concentration , 425 µg concentration and 450 µg concentration is 43.0 µM. The free radical scavenging activity was found to be good in 43.0 µM.

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