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# Evaluation of Antioxidant Activity of Ethanolic Extracts of *Lens Culinaris, Vigna Unguiculata, Dolichos Biflorus* and *Phalaseous Vulgaris* Seeds

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## Abstract

The present investigation has been carried out to determine the antioxidant activity of the ethanolic extracts obtained from plant seeds belonging to family Leguminosae i.e. Lens culinaris, Vigna unguiculata, Dolichos biflorus and Phalaseous. Phenolic compounds present in the extracts showed the antioxidant and antiradical properties when investigated using a ferrous ion chelating ability and reducing power assay. The results indicated that ethanolic extracts of all four plant seeds resembled in the aforementioned activities. Phenolic constituents contained in above mentioned plant seeds may have a future role as ingredients in the development of functional foods.

Key-Words: *Lens culinaris*, *Vigna unguiculata*, *Dolichos biflorus* and *Phalaseous vulgaris*, *ferrous ion chelating ability, reducing power assay.*

## Introduction

Antioxidant compound in food play an important role as a healthy protecting factor evidence suggest that antioxidant reduce the risk for chronic disease including cancer and heart disease the main characteristics of an antioxidant is its ability to trap free radicals. Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule. Antioxidants prevent cell and tissue damage as they act as scavenger. Antioxidants can terminate or retard the oxidation process by scavenging free radicals. Overproduction of the free radicals can be responsible for tissue injury. Cell membranes are made of unsaturated lipids and these unsaturated lipid molecules of cell membranes are particularly susceptible to free radicals. [1] Oxidative damage can direct to a breakdown or even hardening of lipids. Anti-oxidants are substances capable to mop up free radicals and prevent them from causing cell damage. Free radicals are responsible for causing a wide number of health problems which include cancer, aging, heart diseases, gastric problems etc. Antioxidants cause protective effects by neutralizing free radicals which are toxic byproducts of natural cell metabolism. [2]

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There is a growing interest in the antioxidant activity of phenolics and condensed tannin contents of plant extracts due to their potential role in disease prevention and health promotion. [3]

Plants have been the major source of therapeutic agents for curing the human diseases.. Leguminosae is the third largest family of flowering plants, which is commonly known as the legume family, pea family, bean family or pulse family. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems. *Lens culinaris* which claimed to have blood purifying property, to get rid of old skin marks, treats various kidney and gastric ailments and exhibit antifungal properties.[4] The seeds of *Dolichus biflorus* are anthelmintic, astringent, diaphoretic, diuretic, emmenagogue, expectorant, febrifuge, ophthalmic and tonic in activity.[5] *Vigna unguiculata* seeds possess nematicidal and antifungal properties. [6] The seed is diuretic and after eating af is considered to destroy worms in the stomach. Seed oil exhibit antidiabetic properties [7] and *Phalaseous vulgaris* seeds have a notable place in the folklore throughout the world and in the traditions of many cultures such as pharmacotherapeutic effects, diabetes and obesity.[8].

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## Material and Methods

**Preparation of plant seed extract:**

The samples were collected and authenticated and were coded as follows:

| S No. | Plants studied on | Code |
|---|---|---|
| 1 | *Lens culinaris* | *Lc* |
| 2 | *Vigna unguiculata* | Vu |
| 3 | *Dolichus biflorus* | Db |
| 4 | *Phalaseous vulgaris* | Pv |

The collected plant material was washed and dried under room temperature and processed for extraction. With the help of soxhlet apparatus extraction of dried plant material was carried out using ethanol for 72 hours or till the decolourisation of the solvent in the siphon tube whichever is earlier.

**Reagent Used:**

**Preparation of Ferrozine**

0.7 gm of ferrous sulphate was dissolved in 1.5 gm of 1, 10 –phenantroline hydrochloride in 70 ml of water was added. Phosphate buffer pH 6.8 was mixed. 13.872 gm of potassium dihydrogen phosphate was dissolved and 35.084 gm of disodium hydrogen phosphate was added in sufficient amount of water to produce 1000ml.

**Preparation of 2mm $FeCl_2$**

19.87 mg of $Fecl_2$ was dissolved in 50 ml of 2MHCl.

**Preparation of 2MHCl**

73 ml HCL was taken in 1000 ml of water.

**METHOD:**

**Preparation of standard curve of ascorbic acid**

1gm of ascorbic acid was taken and dissolved with 100 gm of distilled water and to taken the 2ml, 4ml, 6ml, 8ml, and 10ml respectively and make up the volume 10ml with distilled water.

**Ferrous Ion Chelating Ability**

2 ml of drug sample was taken. 0.1 ml of 2mm $FeCl_2$ solution was added and 0.2ml of 5mm ferrozine solution was added respectively, it was put for 10 min at room temperature and the absorbance was taken at 562 nm.

**Reducing Power Assay**

2.5 ml of drug sample was taken and 2.5 ml phosphate buffer was added then 2.5 ml of 1% potassium ferricyanide hexacyano ferrate was added .The mixture was kept at 50 ℃ in water bath for 20 min. After cooling 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (2.5 ml) was mixed with DW (2.5 ml) and a freshly prepared 0.01 % ferric chloride (0.5 ml). The absorption was measured at 699 nm.

## Results and Discussion

The prepared ethanolic extracts of the dried plant material were subjected to screening for their possible antioxidant activities. The assessment of antioxidant potential might be a fruitful approach for advocating them as nutraceuticals, in addition to them being potential protein and carbohydrate sources. All four plant seed ethanolic extracts were evaluated by two methods of determining the antioxidant activity, first is Ferrous ion chelating ability method and second is Reducing power assay. The total antioxidant activity for Lens culinaris, Vigna unguiculata, Dolichos biflorus and Phalaseous vulgaris ethanolic extracts by Ferrous ion chelating ability method can be determined by table 1 and by reducing power assay, the result can be evaluated from table 2.

**Ferrous Ion Chelating Ability**

Percentage inhibition was calculated using the formula

$$\text{Percent inhibition} = 1 - \frac{\text{Absorbance of test}}{\text{Absorbance of control}}$$

Where ferric chloride and ferrozine solution served as control.

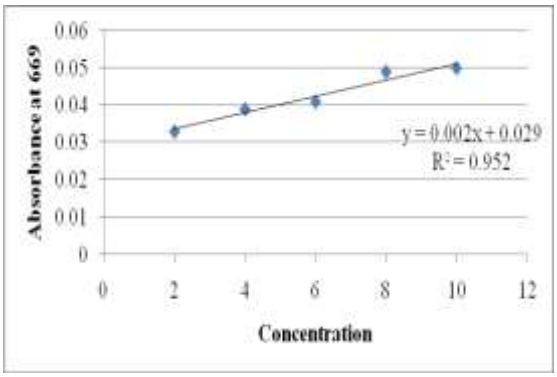
**Observation table 1**

| Sample | Absorbance Of Test | Absorbance Of Control | Percent Inhibition |
|---|---|---|---|
| *Lc* | 0.137 | 0.300 | 1-(0.137/.300)=0.54 |
| Vu | 1.388 | 0.300 | 1-(1.388/0.300)=3.626 |
| Db | 0.520 | 0.300 | 1-(0.520/0.300)=0.733 |
| Pv | 0.728 | 0.300 | 1-(0.728/0.300)=0.733 |

**Reducing Power Assay**

**Observation for absorbance for the standard curve**

| S.No | Ascorbic acid concentration(mg/ml) | Absorbance at 699 nm |
|---|---|---|
| 1 | 2 | 0.033 |
| 2 | 4 | 0.039 |
| 3 | 6 | 0.041 |
| 4 | 8 | 0.041 |
| 5 | 10 | 0.05 |




According to the standard curve, following regression equation was obtained and using the equation the concentration of sample was calculated.

$$y = 0.002x + 0.029$$

**Observation table 2**

| S.No. | Samples | Absorbance at 699 nm | Concentration (ug) |
|---|---|---|---|
| 1. | *Lc* | 0.98 | 475.5 |
| 2. | Vu | 0.76 | 365.5 |
| 3. | Db | 0.84 | 405.5 |
| 4. | Pv | 0.54 | 255.5 |

## Conclusion

In the present study the antioxidant activity of four edible seeds Lens culinaris, Vigna unguiculata, Dolichos biflorus and Phalaseous vulgaris which belong to legume family were evaluated and it was found that Lens culinaris possess higher antioxidant potential. It could therefore be concluded that Lens culinaris, Vigna unguiculta, Dolichs biflorus and Phalaseous vulgaris could contribute significantly in the management and/or prevention of degenerative diseases associated with free radical damage, in addition to their traditional role of preventing protein malnutrition. However further studies are needed to isolate active principle responsible for overall antioxidant activity of the extracts.

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