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EVALUATION OF ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS OF *LENS CULINARIS, VIGNA UNGUICULATA, DOLICHOS BIFLORUS* AND *PHALASEOUS VULGARIS* SEEDS

Garima Mishra * and K. K. Chagti

Smt. Vidyawati College of Pharmacy, Gora Macchiya, Kanpur Road, Jhansi- 284128, Uttar Pradesh, India.

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Correspondence to Author:
Garima Mishra

Smt. Vidyawati College of Pharmacy, Gora Macchiya, Kanpur Road, Jhansi- 284128, Uttar Pradesh, India.

E-mail: garimamishra2708@gmail.com

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 activities above. Phenolic constituents contained in plant mentioned above seeds may have a future role as ingredients in the development of functional foods.

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 delays or inhibits oxidative damage to a target molecule. Antioxidants prevent cell and tissue damage as they act as a scavenger. Antioxidants can terminate or retard the oxidation process by scavenging free radicals. Over-production 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].

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Oxidative damage can direct to a breakdown or even hardening of lipids. Anti-oxidants are substances capable of mopping 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] 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 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 *Dolichos 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 of 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].

MATERIAL AND METHODS:

Preparation of Plant Seed Extract: The samples were collected and authenticated and were coded as follows:

TABLE 1: LIST OF PLANTS

S. no.	Plants studied on	Code
1	*Lens culinaris*	Lc
2	*Vigna unguiculata*	Vu
3	*Dolichos 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 h or till the decolorisation of the solvent in the siphon tube whichever is earlier.

Reagent Used:

Preparation of Ferrozine: 0.7 gm of ferrous sulfate was dissolved in 1.5 gm of 1, 10-phenanthroline 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 1000 ml.

Preparation of 2 mm $FeCl_2$: 19.87 mg of $Fecl_2$ was dissolved in 50 ml of 2M HCl.

Preparation of 2M HCl: 73 ml HCl was taken in 1000 ml of water.

Method:

Preparation of Standard Curve of Ascorbic Acid: 1 gm of ascorbic acid was taken and dissolved with 100 gm of distilled water and to take the 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml respectively and make up the volume 10ml with distilled water.

Ferrous ion Chelating Ability: 2 ml of the drug sample was taken. 0.1 ml of 2 mm $FeCl_2$ solution was added, and 0.2 ml of 5 mm 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 than 2.5 ml of 1% potassium ferricyanide hexacyanoferrate was added. The mixture was kept at 50 ºC in a water bath for 20 min. After cooling, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. 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.

TABLE 2: OBSERVATION

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

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 2 and by reducing power assay, the result can be evaluated from **Table 3**.

Ferrous Ion Chelating Ability: Percentage inhibition was calculated using the formula where ferric chloride and ferrozine solution served as control.

Percent inhibition = 1- Absorbance of test / Absorbance of control

Reducing Power Assay:

TABLE 3: 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

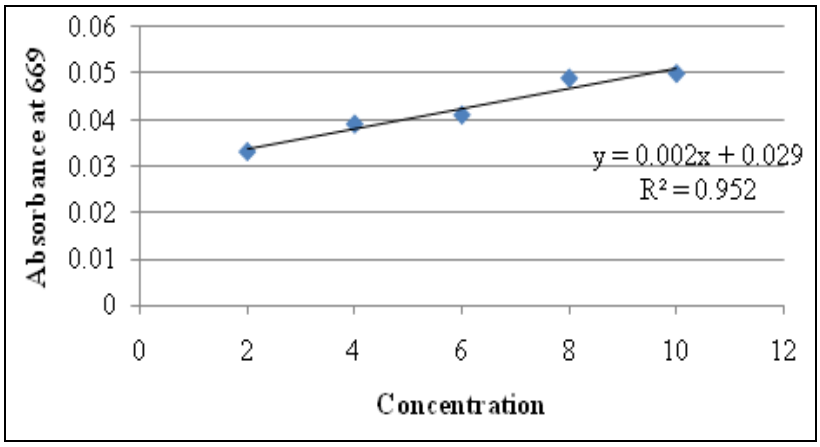

FIG. 1: STANDARD CURVE

TABLE 4: OBSERVATION

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

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

$$y = 0.002x + 0.029$$

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, Dolichos biflorus* and *Phalaseous vulgaris* could contribute significantly in the management and 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 the active principle responsible for the overall antioxidant activity of the extracts.

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CONFLICT OF INTEREST: Nil

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