Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 6, p. 190-197, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Comparative evaluation of the antioxidant potential and phenolic compounds of the cultivars and different genotypes of *Vigna radiata* L.

Ghasemi Mahboobeh[1*], Majd.ahmad[2], Siyahpoosh Amir[3], Nejadsattari Taher[4], Rajabi Memary Hamid[5]

[1]*Science and Research Branch, Islamic Azad University, Biology department, Tehran, Iran*

[2]*North Tehran Branch, Islamic Azad University, Biology Department, Tehran, Iran*

[3]*Jondishapoor medical science vniversity, College of pharmacy, Pharmacogenosy department, Ahwaz, Iran*

[4]*Science and Research Branch, Islamic Azad University, Biology department, Tehran, Iran*

[5]*Agronomy department,Shahid Chamran University, Ahwaz, Iran*

Article published on June 12, 2014

Key words: antioxidant potential, phenolic compounds, seed, *Vigna radiata*

Abstract

This study was conducted in order to compare the antioxidant potential and phenolic compounds of five variety and 10 genotypes of *Vigna radiata L.* in which DPPH method and the calculation of the IC_{50} coefficient were used to check for antioxidant potential and Folin-Ceocalteu method to measure phenolic compounds.Flowers and legumes were harvested kept in the FAA and then in the alcohol 70%. After the preparation and formation in the paraffin, samples were cut up by microtome. Staining was done with eosin – hematoxylin and the structure of grain shell was seen. Results showed a strong correlation (R^2 >0.9) between the percentage of inhibition of DPPH and concentrations of each extract; so that lower IC_{50} represents more inhibition of DPPH (genotype CO3) and vice versa. The existence of phenolic compounds in the extracts is a factor to increase the antioxidant potential .The shell color is also effective in increased antioxidant potential. Also to see macrosclereids with the fat infrastructure in the seed shell structure can be a reason for the existence of phenolic compounds in the shell. Finally, it can be said that the antioxidant potential and phenolic compounds in the cultivars and varieties of a genus and family are different.

***Corresponding Author:** Ghasemi Mahboobeh ✉ ghasemi_mahboobeh2009@yahoo.com

Introduction

Antioxidants are compounds that are used to reduce oxidative damage resulting from natural and non-natural cellular metabolism(Imaida *et al.*, 1983). The oxidative stress causes the production of reactive oxygen species (ROS). The accumulation of ROS that arises from the different environment stresses is one of the reasons for the reduction of the product throughout the world.Recently researchers have conducted interesting studies of phenolic compounds and antioxidant capacity of the medicinal plants (Djeridane *et al.*,2006). It has been reported a lot of medicinal plants in terms of natural antioxidants such as phenolic acids, flavonoids and tannins have a lot of diversity. The effect of health improvements by taking plant antioxidants is due neutralization of the active oxygen species (Wong *et al.*, 2006). Dietary antioxidants protect the body against free radicals; oxidative damage plays the important pathological role in humans. Cancer, flatulence, cirrhosis, arthritis, and arteriosclerosis are associated with oxidative damage (Ramesh *et al.*,2011). These compounds have beneficial effects on heart disease, cancer of the rectum, colon, breast and pancreas(Cadenas and Packer,2002).

Vigna radiata is the family legumes (Fabaceae) rich in protein(%25),minerals and vitamins. Human consumes it in the form of seeds and seedlings. Researchers have reported the use of buds in some plants is more than dry seeds. In the process of germination, vitamins, minerals and proteins are increased but the calories and carbohydrate value are reduced (Chavan and Kadam, 1989).The importance and benefits of grain Fabaceae consumption in preventing chronic disease, such as cancers and heart diseases have been studied. On the other hand, phenolic compounds found in the seeds also have antioxidant properties. Phenols counteract with oxidative damage in the tissue; they are hydrogen donor to the free radicals and by neutralizing them, they often prevent formation of peroxide. In legumes ,the most important antioxidants vitamin C , E and phenolic compounds (Rice-Evans *et al.*,1996). The antioxidant activity of the cultivars and different genotypes of *Vigna radiata* has not been studied, yet; therefore, the test was done in order to compare the antioxidant capacity and polyphenolic compounds of methanolic extracts of 15 varieties and genotype of this plant.

Materials and methods

Seeds of 10 genotypes and five variety of *Vigna radiata* plant(VC6153-b , NM92 , CO3 , VC6173b-11 , VC3960-88 , SLM154 , PUSA , ML2017 , VC6388 , VC63070 , Indian , Parto , mung bean , Mahalli , Gohar) were collected from the research center of Safi-Abad, Dezful (Khuzestan of Iran).

The extract preparation

To prepare extracts from each sample, 250 cc of methanol was added to 100 g of powdered seed for soaking for 48 hours at room temperature. After smoothing, it was concentrated by Rotary.

The DPPH method to measure the antioxidant capacity(Nimba et al.,2008)

We added 100 µL of the diluted extract to 3.9 mL of DPPH solution stocks (13mg DPPH+500ccMetanole) and absorption in the UV wavelength of 515 nm was read by UV-visible spectrophotometer (X-M3200PC, HUMAN company, Korea). Doing for each instance was along with triplications. For each plant, IC_{50} was calculated to determine the percentage of inhibition of DPPH in accordance with the following formula. Then comparisons were done between samples.

$\%IC_{50}$=[(Control absorbance – Extract absorbance) / Control absorbance)]×100

Folin-Ciocalteu method(FC)

This method was used to determine the total amount of phenolic compounds. The tannic acid was used as standard(Grezegorzky *et al.*,2007).

To 0.5 ml of the sample (extract or standard), 2.5 ml of the **FC** reactive diluted with distilled water in a ratio of 1 to 9.2ml Na2CO3 7.5% (7.5 grams per 100

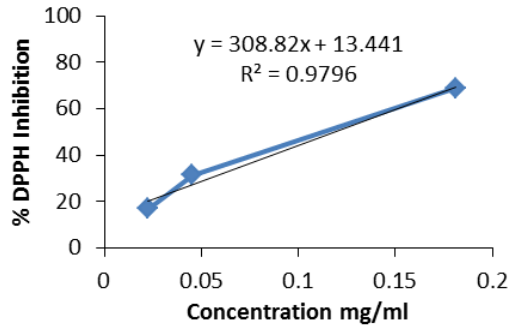
ml of distilled water) was added to the resulting mixture after to be merged and stay for five minutes at room temperature (37 ° c). And it was kept in the dark for two hours at room temperature; Absorption in the UV wavelength of 765 nm was read by UV-visible spectrophotometer and recorded. This test was repeated three times for each extract.Then using the standard curve of tannic acid, the total amounts of phenolic compounds for each extract was reported equivalent to mg of tannic acid in one gram of dry extract of the plant.

Statistical analysis

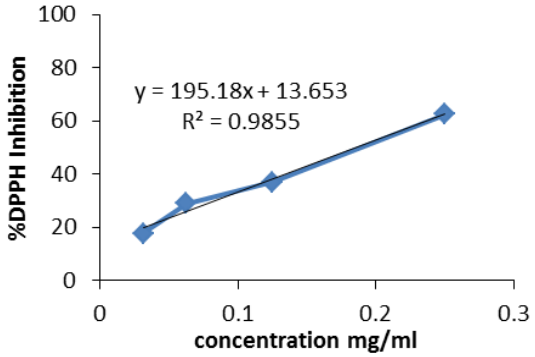
Software of SAS 9.2 and LSD test was used at a level of 0.05 for statistical analysis. The figures also were drawn using Excel software.

Results

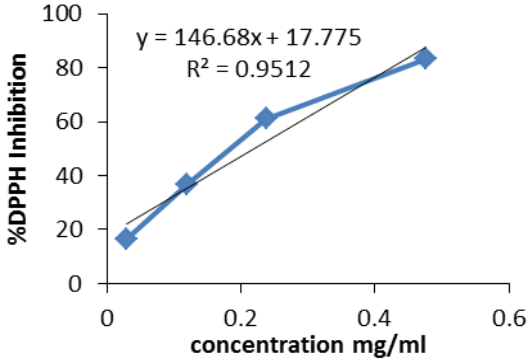
Comparison of the antioxidant capacity of different cultivars and genotypes was done by DPPH method to determine the antioxidant potential. The assessment is based on reduction of DPPH radicals that shows the absorption in the wavelength of 515 nm. In the study, the antioxidant activity of different cultivars and genotypes was determined based on calculation of coefficient of IC_{50} (a concentration of the sample, which inhibits 50% of free radicals). As the figures and the histogram 1 show, black gram (IC_{50}=0.119mg/ml) with least value have most IC_{50}, and genotype VC6173b-11 with a maximum value of IC_{50} (15.97mg/ml) have least antioxidant potential .As well as the coefficient of determination of r^2>0.9 represents a linear relationship between the IC_{50} and concentration of each extract. Graphs show percentage of Inhibition of DPPH for each concentration and value of IC_{50} (mg/ml).



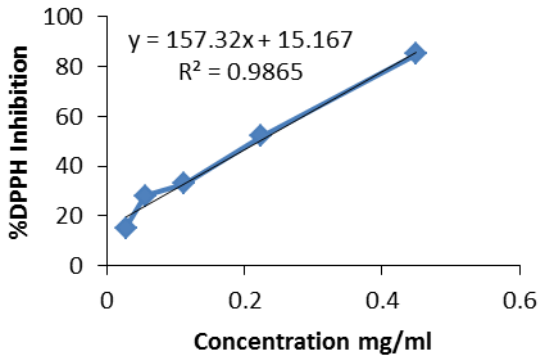
Black gram:IC_{50}=0.119mg/ml



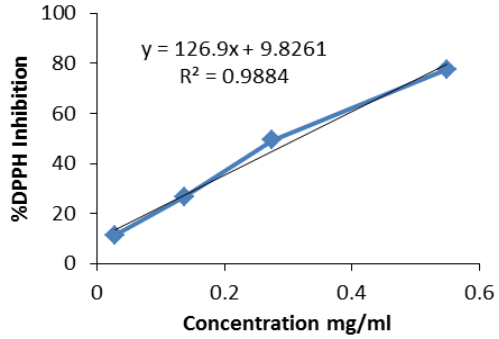
Indian:IC_{50}=0.186mg/ml



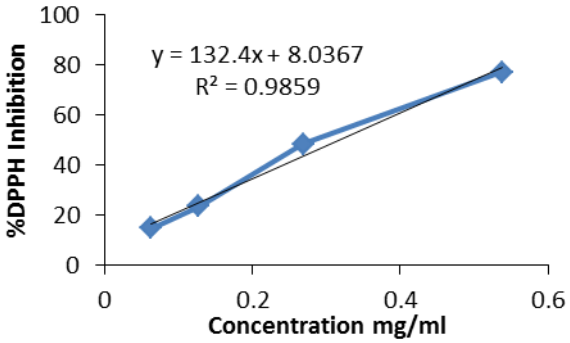
NM92:IC_{50}=0.22mg/ml



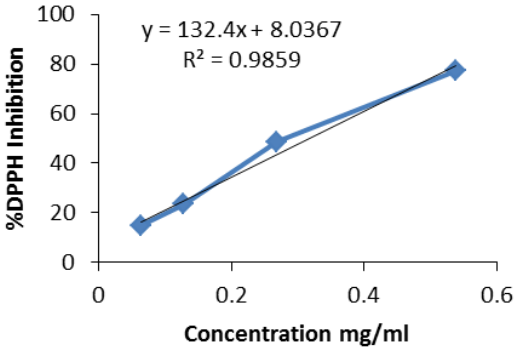
VC6153-b:IC_{50}=0.221mg/ml



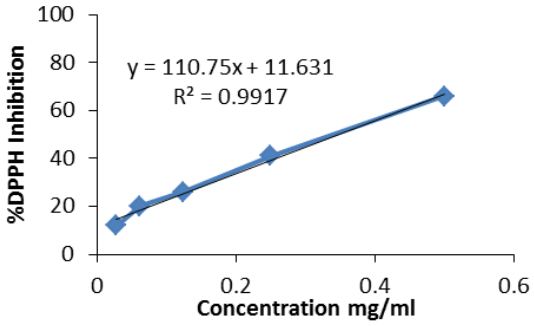
Parto:IC_{50}=0.32mg/ml



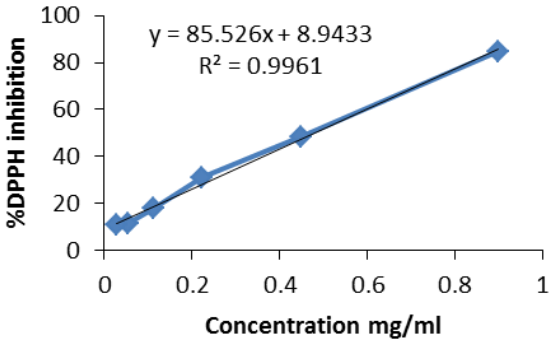
VC63070:IC_{50}=0.316mg/ml



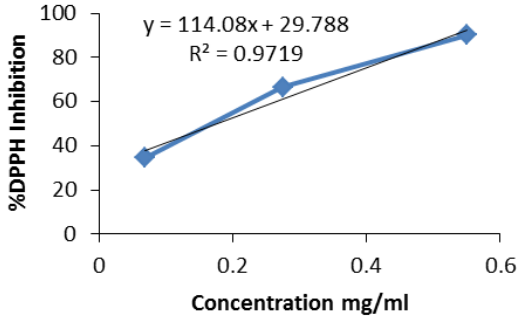
CO3:IC_{50}=0.317mg/ml



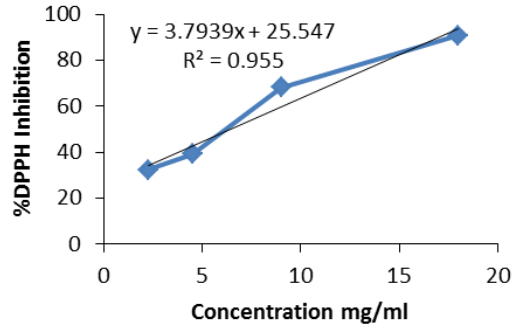
VC6388:IC_{50}=0.347mg/ml



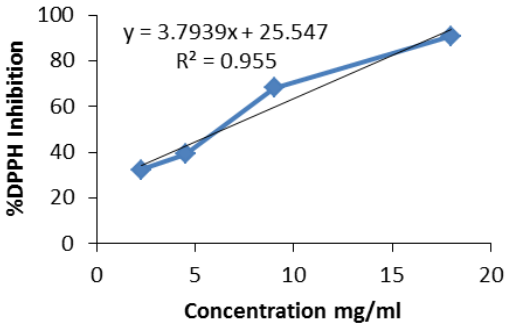
Mahalli:IC_{50}=0.48mg/ml



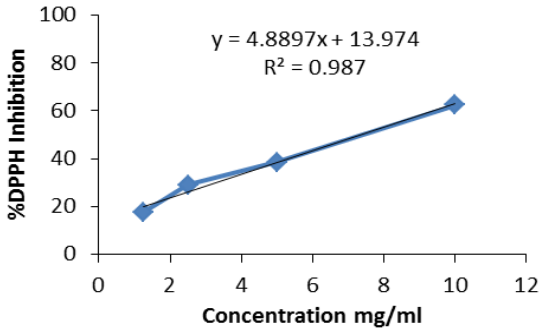
ML2017:IC_{50}=1.773mg/ml



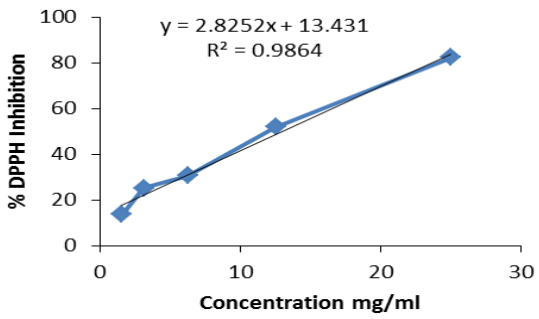
Gohar:IC_{50}=6.528 mg/ml



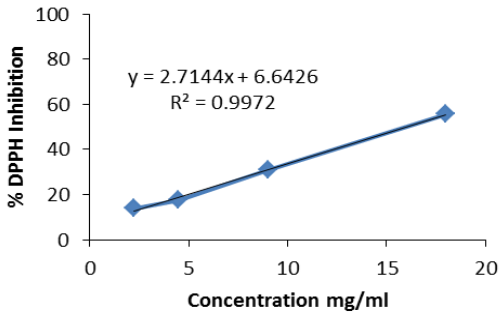
PUSA:IC_{50}=6.538 mg/ml



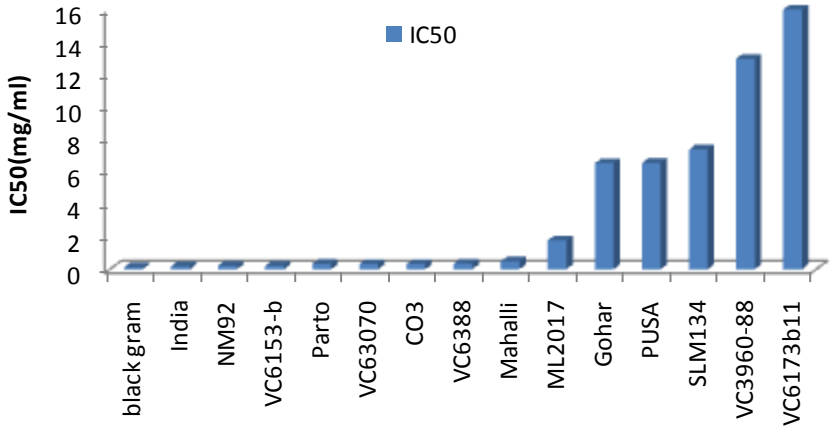
SLM134:IC_{50}=7.37mg/ml



VC3960-88:IC_{50}=12.94mg/ml



VC6173b-11:IC_{50}=15.975mg/ml



Histogram 1. the value of IC_{50} (mg/ml) of each sample.

Determination of total phenolic compounds

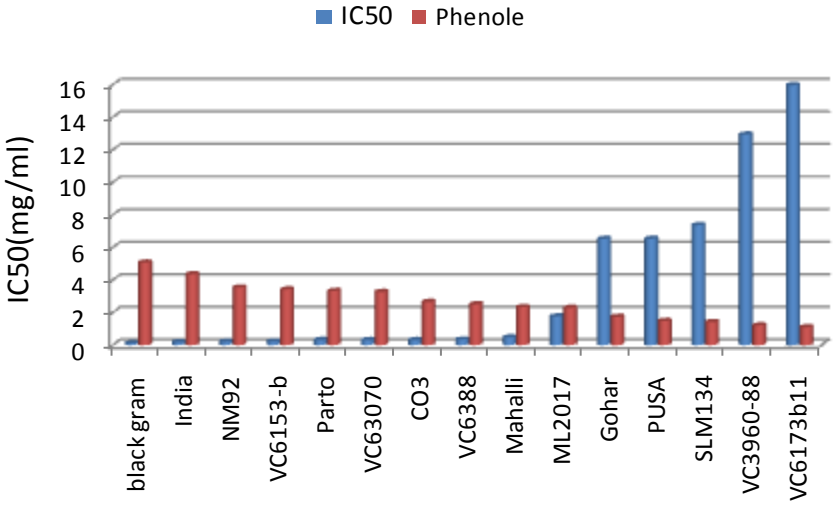
A significant difference was seen between genotypes and cultivars studied in terms of the level of 0.001. The amount of phenolic compounds about 1.1-5.08mg per 100 g of extract. Comparison of averages revealed that all samples are statistically not in a group. The maximum amount of phenol was related to CO3 with genotype 5.08 mg/ml and then Indian cultivar with an amount of 4.36 mg/ml and the lowest amount was related to genotype Vc6173b -11 with an amount of 1.1 mg/ml and the other genotypes are between these values. In this case, the letters has been used for the genotypes; it does not show any significant differences between the genotype with the similar

letters (table 1).Histogram 2 also shows that in the genotypes and cultivars in which value of IC50 is low, phenolic compounds are increased and this is the reason for the higher antioxidant potential .Microtomic cuttings of flowers and seeds revealed substances with fat infrastructure can be found in the ovule integuments of the samples studied (Fig. A).When the transformation of the ovule to seed , ovule integuments create the seed coat. Cells of macrosclereids type are observed in the seed coat (Fig. A: 1-6). The origin of these cells is the same substances found in the ovules that are likely polyphenolic compounds.

Table 1. Comparison of the average genotypes based on LSD test

Genotype	IC50 mg/ml	Phenol (mg/ml)	SD
Black gram	0.118	5.08	0.01a
India	0.19	4.36	0.07b
NM92	0.22	3.54	0.14c
VC6153-b	0.221	3.43	0.01c
Parto	0.326	3.33	0.04c
VC63070	0.317	3.27	0.007cd
CO3	0.317	2.66	0.01cd
VC6388	0.347	2.52	0.03c
Mahalli	0.48	2.34	0.004ef
ML2017	1.773	2.3	0.04gh
Cohar	6.528	1.74	0.02fg
PUSA	6.538	1.49	0.001gh
SLM134	7.37	1.42	0.01gh
VC3960-88	12.945	1.22	0.04gh
VC6173b11	15.975	1.1	0.03h

The similar letters indicate the lack of significant differences in the level of 0.001.



Histogram 2. the relationship between IC_{50} and phenolic compounds for each genotype

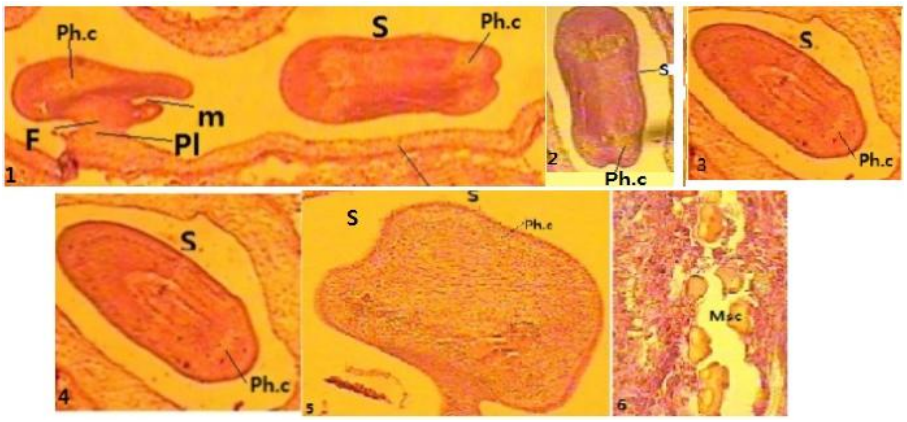


Fig. A. Ovule and seed structure-F:Funicul , m:micropyl , Pl:Placenta , Phc:Phenolic components , MSC:Macrosclerid Cells , S:Seed

Discussion

Antioxidants are materials that delay oxidative processes; they are inhibitors for a chain of polymerization founded by reactive oxygen species(ROS) and other oxidative substances (Halliwell, and Aruoma,1991). Seed coat has a large amount of phenolic compounds to protect the internal nutrients of the plant. The seed coat plays an important role in the chemical protection on the oxidative damage through the inner of anti-oxidants such as phenolic compounds. They act also as a protective barrier for the cotyledons(Duenas *et al*.,2003).The results showed that in the seeds studied, phenolic components content is between 1.22-5.08 mg/ml.Values of this compounds in this test are inagreement with the results reported by many reaserchers that shown in legumes total phenolic compounds from 1.9-5.7mg/ml varies (Madhujith *et al*.,2004).Researches have shown that legumes seed coats are a rich source of anti-oxidants, anti-microbial material and other bio-active compounds (Shahidi and Naczk,1995). The active compounds have been found in the peanut (Yen and Duh,1994) and wheat bud (Watanabe *et al*.,1997). Researchers have reported the phenolic compounds are associated with the antioxidant capacity measured by the DPPH (Tabart *et al*.,2009). A high correlation between this relationship shows the active compounds in the seed extracts are responsible for the antioxidant potential.In the tested genotypes and cultivars also there is $r^2 > 0.9$ and represents the existence of a link between the active compounds and antioxidant potential.Macrosclereids cells can be observed on the seeds coat studied that are made of fat and probably are Polyphenolic compounds. In addition to thickening of the seed, these compounds are of significant importance in increasing the antioxidant activity.The seed color is also effective in increased antioxidant potential. Genotypes with more antioxidant potential have darker seeds because they have more anthocyanin pigments (Barampama and Simard,1995).Also the results of our investigations have shown that black gram have higher antioxidant activity than other genotypes.The phenolic compounds with high molecular weight have greater ability in free radical-scavenging and their impact is related to the molecular weight, the number of aromatic rings and the functional hydroxyl substituted by especial functional groups(Hagerman *et al*.,1998).According to the reports presented by researchers, at the value of phenolic compounds in different parts of a plant and even in a family and variety is different (Amarowicz *et al*.,2004). Our testing also is consistent with these reports even though all genotypes and cultivars are relating to a genus and family , every sample has especial phenolic compounds and antioxidant potential.

Conclusion

Oxidant examinations showed lower IC50 represents more inhibition percentages of DPPH that is because of the polyphenolic compounds. Genotypes with more antioxidant potential have darker seeds because they have more anthocyanin pigments(Black gram) .All genotypes and cultivars are relating to a genus and family but every sample has especial phenolic compounds and antioxidant potential.

References

Amarowicz R, Troszynska A, Barylko-Pikielna N, *et al.*, 2004. Polyphenolics extracts from legume seeds: Correlations between total antioxidant activity, total phenolics content, tannins content and astringency.Journal of Food Lipids, **11**, 278–286.

Barampama Z, Simard RE. 1995. Effect of soaking, cooking, and fermentation on composition in vitro starch digestibility and nutritive value of common beans. Plant Food for Human Nutrition, **48**, 349–365

Cadenas E, Packer L. 2002. Handbook of antioxidants. New York: Marcel Dekker, Inc.

Chavan J, Kadam SS. 1989. Nutritional improvement of cereals by sprouting. Critical Reviews in Food Science and Nutrition. 1989; **28**(5), 401-437.

Djeridane A, Yousfi M, Nadjemi B, *et al.*, 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry, **97**, 654–660.

Duenas M, Sun B, Hernandez T, *et al.*, 2003. Roanthocyanidin composition in the seed coat of lentils (*Lens culinaris* L.). Journal of Agricultural and Food Chemistry, **51**, 7999–8004.

Grezegorzyk I, Matkowski A, Wyosokinsa H. 2007. Antioxidant activity of extracts from invitro cultures of *Salive officinalis* L.. *J Food Chem*. **104**, 536-541.

Hagerman AE, Riedl KM, Jones GA, *et al.*, 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry, **46**, 1887–1892.

Halliwell B, Aruoma OI. 1991. DNA damage by oxygen derived species. Its mechanism and measurement in mammalian systems. FEBS Letters, **281**, 9–19.

Imaida K, Fukushima S, Shivai T, *et al.*, 1983. Promoting activities of butylated hydroxyl anisole and butylated hydroxyl toluene on 2-stage urinary bladder caricinogensis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. Carcinogen. 1983; **4**, 885-89.

Madhujith T, Naczk M, Shahidi F. 2004. Antioxidant activity of common beans (*Phaseolus vulgaris* L.). Journal of Food Lipids, **11**, 220–233.

Nimba RY, Kikuzaki Y, Konishi Y. 2008. Antioxidant activity of various extract and fractions of *Chenopodium quina* and *Amarantus spp* seeds. *J Food Chem*. **106**, 760-766

Ramesh CK, Abdul Rehman Prabhakar BT, Vijay Avin BR, *et al.*, 2011. Antioxidant potentials in sprouts vs.seeds of *Vigna radiata* and *Macrotyloma uniflorum*.Journal of applied Pharmacoutical Science. **01**(07), 99-103

Rice-Evans CA, Miller NM, Paganda G. 1996. Structure antioxidant activity relationships of flavonoids and phenolic acids.Free Radical Biology and Medicine, **20**, 933–956.

Shahidi F, Naczk M. 1995. Food phenolics: Sources, chemistry, effects, applications.

Tabart J, Kevers C, Pincemail J, *et al.*, 2009. Comparative antioxidants capacities of phenolic compounds measured by various tests. Food Chemistry, **113**, 1226–1233.

Watanabe M, Ohshita Y, Tsushida T. 1997. Antioxidant compounds from uckwheat (Fagopyram isculentum moench) hulls. Journal of Agricultural and Food Chemistry, **46**, 839–845.

Wong C, Li H, Cheng K, *et al.*, 2006. A systematic survey of antioxidant ctivity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chemistry, **97**, 705–711.

Yen GC, Duh PD. 1994. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. Journal of Agricultural and Food Chemistry, **42**, 629-632