Comparative study of antioxidant activity, phenol and flavonoid content in *Phaseolus vulgaris* and *Vigna sinensis* in different stages of seed germination

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Abstract

The study of free radicals and antioxidants in biology is producing medical revolution that promises a new age of health and disease management. Therefore, the aim of this study was investigated antioxidant effectsand phenol and flavonoid content in *Phaseolus vulgaris* and *Vigna sinensis* in different stages of seeds germination. Seeds of *Phaseolus vulgaris* and *Vigna sinensis* were prepared from the Pakan bazr center of Isfahan. Seeds were sterilized in 50% sodium hypochlorite solution for 3 min, and then were air-dried. 10 seeds from each plant were separately placed in Petri dishes. The plates were placed into an incubator at 25 ±2°C in darkness for 2 days. Germination percentages of seeds of *Phaseolus vulgaris* and *Vigna sinensis* were recorded after 2, 4, 6, 8 days. Seeds powder (100g) was macerated using methanol at room temperature for 1 days. Then, for phytochemical studies of this plants for evaluation of antioxidant activity and total phenolic and flavonoid contents of methanolic extract of this plants were used from DPPH model system, Folin-Ciocalteu method and aluminum chloride colorimetric method, respectively. Results indicated that the amount of phenol, flavonoid and antioxidant activity were increased in second day of seeds. Therefore, analysis of phenolic compounds in *Phaseolus vulgaris* and *Vigna sinensis* seeds in second day of germination could be useful for explaining the relationships between total antioxidant capacity and total phenolic and flavonoid contents in these plants.

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Introduction

Free radicals contribute to more than one hundred disorders in humans body. Free radicals due to environmental pollutants chemicals, toxins, radiation, etc caves depletion of immune system antioxidants, change in gene expression and induce abnormal proteins (Pourmorad et al., 2006). Free radicals are controlled by enzymes such as medicinal plants, fruits, vegetables and seeds can constitute an important source of antioxidants and they may be used reduce oxidative damage and tissue injury (Amjad and Shafighi, 2013).

Many plants have antioxidative and pharmacological activities. Bioactive phenols, especially bioflavonoids are very interesting as antioxidants because the ability to act as free radical scavenging, inhibition of hydrolytic and oxidative enzymes (Frankel, 1995).

Some researchers suggest that the biogical activity of these compounds are related to their antioxidant action (Gryglewski et al., 1987).

Phaseolus vulgaris L. and Vigna sinensis L. (Leguminosae), are food items in eastern and Asian countries (Venkateswaran and Pari, 2002).

In 1997, Suchmita and Ranjana showed that the phaseolus vulgaris possessed nearly 50 mg of flavonoids per log.

Vigna sinensis (Cowpea) is a member of the Vigna (peas or beans) genus. Insects are a major factor in the low yields of crops and they affect each tissue component and developmental stage of the plant (Jackal and Daoust, 1986). Cowpeas provide a rich source of proteins and calories, as well as minerals and vitamins. A cowpea seed can consist of 25% protein and is low in anti-nutritional factors (Rangel et al., 2003).

Maillard et al. (1996) showed that antioxidant activity of barley is relationship with the content of the three phenolic compounds.

Thus, Lopez-Amoros et al. (2006) indicated that germination modifies in legumes depend on the type of legume and the germination conditions. These changes influence the functional properties of the legumes as consequence of the variation in antioxidant activity. Peas and beans undergo a significant increase in antioxidant activity after germination, whereas lentils show a decrease.

Therefore, the aim of the present study was to examine the total phenol, total flavonoid,-Flavonol and related total antioxidant potential in Vigna sinensis and Phaseolus vulgaris seeds germination.

Materials and methods

Plant material and preparation of extract

Seeds of two plant (Phaseolus vulgaris L. and Vigna sinensis L.) were used for the study. The seeds were obtained from the Pakan bazar center of Isfahan. Seeds were sterilized in 50% sodium hypochlorite solution for 3 min, and then were rinsed with sterilized water and were air-dried. 10 seeds from each plant were separately germinated on two sheets of Whatman No.1 filter paper placed in 9-cm diameter Petri dishes. The plates were placed into an incubator at 25 ±2°C in darkness for 2 days. Germination percentages were recorded after 2, 4,6,8 days. Seeds powder (100g) was macerated using methanol at room temperature for 1 days. The methanol extract was filtered and concentrated under reduced pressure to yield its respective crude extract (Vlietinck et al, 1995).

Determination of antioxidant activity

The antioxidant activity of seeds extract of Phaseolus vulgaris and Vigna sinensis were evaluated by the DPPH method as described in the literature (Shirzad et al., 2011). The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the extracts. Different concentrations of each extracts were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 30 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid were used as standard control.
Determination of total phenolic content
The total phenolic content of seeds extract of *Phaseolus vulgaris* and *Vigna sinensis* were evaluated using Folin-Ciocalteu method following the procedure described in the literature (Sharafati et al., 2011). Briefly, 0.1 ml of each of the diluted test samples was added to 0.5 ml of 10% (v/v) Folin–Ciocalteu reagent and after 3-8 min, 0.4 ml of 7.5% (w/v) sodium carbonate solution was added to the mixture. The absorbance of the reaction mixture was measured at 765 nm after incubation for 30 min at room temperature. A standard curve was plotted using different concentrations of gallic acid. The samples were tested in triplicate and their total phenolic contents were determined by a comparison of the values obtained with the calibration curve of gallic acid. Total phenolic contents of the fractions were expressed as milligrams of gallic acid equivalents per gram of dry fraction (mg g⁻¹).

Determination of total flavonoid content
The total flavonoid content of seeds extract of *Phaseolus vulgaris* and *Vigna sinensis* were evaluated using aluminum chloride colorimetric method following the procedure described in the literature (Sharafati-Chaleshtori et al., 2012). Briefly, 0.5 ml of each diluted test sample was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% (w/v) aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The absorbance of the reaction mixture was measured at 415 nm after incubation for 40 min at room temperature (total flavonoid). The absorbance of the reaction mixture was measured at 440 nm after incubation for 150 min at room temperature (total flavonol). A standard curve was plotted using different concentrations of rutin. The samples were tested in triplicate and their total flavonoid content were determined by a comparison of the values obtained with the calibration curve of rutin. The total flavonoid content of the fractions was expressed as milligrams of rutin equivalents per gram of dry extract (mg g⁻¹).

Statistical analysis
The values were reported as mean±SD. One-way ANOVA tests were used for the analyses.

Results
In the present study, the antioxidant ability of the seeds of *Phaseolus vulgaris* and *Vigna sinensis* were investigated through DPPH model system. Samples at 10mg/mL concentration compared with Ascorbic acid which is well-known commercial antioxidant. The data showed that this two extract had higher antioxidant activity than Ascorbic acid in second day and eighth day of germination (Fig 1).

![Fig. 1. Significant increase of antioxidant (inhibition percent) of *Phaseolus vulgaris* in second day and eighth day of germination in compared with control group. Bars are least significant differences where p < 0.05.](image1)

Total phenolic contents of seeds of *Phaseolus vulgaris* and *Vigna sinensis* were determined as gallic acid equivalents in milligrams per gram of dry sample (mg GAE/g extract) and total flavonoid and flavonol contents were calculated as rutin equivalents in milligrams per gram of dry sample (mg RTN/g extract).
The highest contents of total phenolic and flavonoid of the compounds were detected in *Phaseolus vulgaris* in second day (56±1 mg GAE/g and 44.67±0.02 mg RTN/g, respectively) (Fig 2,3), whereas the highest contents of flavonol were measured in *Vigna sinensis* in second day (1.58±0.4 mg GAE/g) (P<0.05)(Fig 4).

**Fig. 3.** Significant increase of total flavonoid (mg/g RTA) of *Phaseolus vulgaris* in second day of germination in compared with other groups. Bars are least significant differences where p < 0.05.

**Fig. 4.** Significant increase of flavonol (mg/g RTA) of *Vigna sinensis* in second day of germination in compared with other groups. Bars are least significant differences where p < 0.05.

**Discussion**

Almost all organisms are well protected against free radical harm by oxidative enzymes similar superoxide dismutase and catalase or chemical compounds such as flavonoids, ascorbic acid, carotenoids, polyphenol compounds and glutathione (Matkowski *et al.*, 2009). Many fruits and vegetables, herbs, cereals, seeds that contain natural antioxidants can abstract the lone electron from free-radical molecules and help humans to keep control on these harmful species. Most of these antioxidants in plants are highly colored anthocyanines, proanthocyanidins, flavonols, flavonoids, and their glycosides, carotenoids, like β-carotene and lycopene (Matkowski *et al.*, 2009). Isolation of antioxidants from plants depends on the polarity of these compounds. First distribution of antioxidants between a polar (aqueous, hydro ethanol) and a semi-polar solvent (n-butanol, ethyl acetate) can be used to determine the distribution factor of the compounds between phases (Matkowski *et al.*, 2009).

Flavonoids as antioxidant compounds in our study reported in range of 26.96-44.67 mg/g RTA. In our study, polyphenols and flavonoids content were correlated with antioxidant activity in the DPPH assay. Several studies have reported on the relationships between phenolic content and antioxidant activity (Ismail *et al.*, 2004). Velioglu *et al.* (1998) reported a strong relationship between total phenolic content and antioxidant activity in selected fruits and vegetables. In our study, the findings showed relationship between antioxidant activity and total phenolic and total flavonoid contents.
the process, with a significant increase of flavonoids. An increase in the antioxidant activity was also observed as a consequence of the process (Duenas et al., 2009).

**Conclusion**

The results of the present study showed that *Phaseolus vulgaris* seeds in second day of germination are rich in phenolic constituents and demonstrate good antioxidant activity. This plant, rich in flavonoids and phenolic acids could be a good source of natural antioxidant. The present study demonstrated that *Phaseolus vulgaris* seeds extracts containing high amounts of phenolic compounds so that these compounds are efficient free radical scavengers. Therefore, qualitative and quantitative analysis of major individual phenolics in *Phaseolus vulgaris* seeds in second day of germination could be useful for explaining the relationships between total antioxidant capacity and total phenolic contents in this plant. This provides a supplementary prophylactic value for this antioxidant plant and supports its gaining popularity as a botanical food supplement.

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