ANTIOXIDANT ACTIVITIES OF IRANIAN BITTER VETCH IN DIFFERENT METHODS

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ABSTRACT

Introduction: Bitter vetch is an ancient legume crop of the Mediterranean region. It has been used mostly for grain, hay production and feed ruminant for increase of milk production. This study was managed for determination of antioxidant activity and phenol contents.

Material and Methods: Vicia ervilia (Bitter vetch) aerial plant and seeds were prepared in Yasuj Iran. Extraction was carried out in distilled water with maceration methods. For determination of antioxidant activities used diphenyl-2-picrylhydrazyl (DPPH), Trolox equivalent antioxidant capacity (TEAC) radical scavenging assays and ferric reducing antioxidant power (FRAP) assay for reducing power as an antioxidant potenti. Total phenol and flavonoids were estimated as antioxidant component. Analysis of data was carried out by one way analysis of variance ANOVA. Results: Total phenol and flavonoids content ranged from 62.7-105 mg Gallic acid and 22.1-43 mg Rutin /g samples respectively .The aerial parts had the highest antioxidant activity by DPPH and FRAP methods. The antioxidant activity of the samples in FRAP and DPPH method can be attributed to their total phenolic and flavonoid content. Conclusion: aerial part was reported with high total phenol, flavonoid and antioxidant activity by different systems. The highest antioxidant activity revealed by TEAC assay .it is could be suppose the aerial part of bitter vetch contain both hydrophilic and lipophilic substances with chain-breaking ability of free radicals.

Key words: Antioxidant Activities, Bitter vetch, DPPH, TEAC.
INTRODUCTION

Bitter vetch (Vicia ervilia(L) Willd.) is family of Leguminosae belongs to the tribe Vicieae of the sub-family Papilionaceae. The genus Vicia has 180-200 species which are mostly temperature annual end perennial, auto and allogamous erect or climbing plants \(^1\). Bitter vetch is an ancient legume crop of the Mediterranean, Turkey, Cyprus, Spainand Greece region that has been used mostly for grain and hay production. It is traditionally cultivated for ruminant feed to increase milk production. The crushed grain is also useful for bovine draught animals and reinforcement of poor ruminant animals \(^1,2\). Bitter vetch is rich in crude protein, energy and nutritional values. It is an important economical source due to resistance to insects and drought, nitrogen fixation potential, grow in poor and alkaline soils, easy cultivate and harvest \(^2,3\).

According to some research study, raw bitter vetch due to presence of certain antinutritional substances such as L-canavanine and trypsin inhibitor is toxic for animals mainly chickens. Consumption of raw bitter vetch after 15 days in broilers and rats induce weight loss and growth retardation and produce egg production disturbance in laying hens \(^4,5\).

Plant collection and extraction

Vicia ervilia plant and seeds were prepared in Boyerahmad Yasuj, Iran. Samples were recognized and a voucher specimen was placed in the biochemistry laboratory, Yasuj University of Medical Sciences, Yasuj, Iran. The samples were washed, shade-dried, and aqueous extraction was done by maceration techniques in 24 h. at room temperature with agitation.

Samples contain seed, skin (external layer of seed) seed ground (crushed seed), aerial parts (leaves and shoot) were prepared. Extraction was carried out by distilled water. All samples soaked in distilled water at room temperature for 24 h, and then extracted filtered with filter paper whatman no. 1. The extracts were prepared at the concentration normally used in Iran.

Determination of total flavonoid content

The total flavonoid content was determined with aluminium chloride (AlCl3) according to method (Kosalec et al., 2004). The total flavonoid values were determined in terms of rutin equivalents/g extract \(^6\).
Determination of total phenolic compounds
The total phenolic contents of extracts were determined using Folin-Ciocalteau method with some modifications. Total phenol was expressed as Gallic acid equivalent (GAE) / g extract [7].

Antioxidant activity of Dipheny-picrylhydrazyl (DPPH).
The antioxidant activity of extract assessed with little modification. Percent of inhibition was calculated as follow: % Inhibition = [(A0 - A1)/A0] ×100 [8].

A0 is the absorbance of control and A1 is the absorbance of the plant extracts. IC50 or Inhibition concentration in 50% also was calculated. IC50 is the maximal concentration of extract to cause 50% inhibition of free radicals activity or damages.

Trolox equivalent antioxidant activity (TEAC)
The antioxidant activity was measured using TEAC based on Re method with some modification. Percent of inhibition same DPPH method was calculated. IC50 or Inhibition concentration in 50% also was determined [9].

Ferric reducing antioxidant power (FRAP) assay
For reducing determination of extract The Benzie method was used with a little modification. The reducing activity of the extract was calculated according to the calibration curve with aqueous solutions of FeSO4 H2O in the range of 100–1000µM [10].

Statistical Analysis
All data were expressed as means ± standard deviation of (n=3) measurements. For detect of significant differences in samples one-way analysis of variance (ANOVA) was applied which followed by post hoc tests. P-values less than 0.05 were considered significant.

RESULTS
Total phenol content, which expressed as mg Gallic acid /g sample ranged from 62.7-105 mg Gallic acid . The maximum order of total phenol content were reported in aerial parts > external layer > crashed seed > seed (Figure 1).

Total flavonoids content, which expressed as mg rutin / g sample ranged from 22.1-43 mg Rutin /gr (Figure2). The samples are arranged from the lowest to the highest total flavonoids
concentration, which ordered from aerial parts > seed > external layer > crashed seed > (Figure 2).

Amongst all samples aerial parts had the highest DPPH activity (72%), however external layer of seed had the lowest activity (6%) were reported per gram fresh weight.

The order of antioxidant activity were reported in aerial parts > seed > crashed seed > .external layer (Figure 3).

All samples in the TEAC assay demonstrated a potent antioxidant activity compare to DPPH method. The free radical scavenging effect ranged in TEAC 69.7-92% in gram dry weight. The order of antioxidant activity were reported in seed > aerial parts > crashed seed > .external layer (Figure 4).

In FRAP assay aerial parts with highest activity (345) and external layer with lowest activity (163) u mole iron was seen. The order of antioxidant activity were reported in aerial parts > seed > crashed seed > .external layer (Figure 5).

There was a relationship between the DPPH and FRAP assays. The antioxidant activity of the samples in FRAP and DPPH method can be attributed to their total phenolic and flavonoid content.

Figure 1 .Total phenol contents of aqueous extract of *Vicia ervilia* in different parts of seed and aerial part.
Figure: 2. Total flavonoids contents of aqueous extract of *Vicia ervilia* in different parts of seed and aerial part.

Figure: 3. Antioxidant activity of aqueous extract of *Vicia ervilia* by diphenyl-2-picrylhydrazyl (DPPH) in different parts of seed and aerial part.

Figure 4. Antioxidant activity of aqueous extract of *Vicia ervilia* by Trolox Equivalent Antioxidant Capacity (TEAC) in different parts of seed and aerial part.
DISCUSSION

Free radicals are related with numerous degenerative diseases such as cancer, heart diseases, rheumatoid arthritis and others. Antioxidants induced defend against free radical generation.

Phenolic compounds such as total phenols and flavonoids are the important group of antioxidant substances that related to quality and food value. They have naturalized free radicals such as peroxide, hydro peroxide and metal chelating. Therefore, high intake of of phenolic compound revealed inhibitory activity on mutagenesis and carcinogen development \[11, 12\].

According to many research ,antioxidants can neutralized radicals by inhibit of breaking chain propagation or restrain of free radicals by interact to the metal ions, reducing hydrogen peroxide, and quenching radical oxygen containing sources . So they could be an important task in the prevention of degenerative diseases.

It is well known that flavonoids are a big class of phenolic substances with antioxidant potential .It was observed that the antioxidant capacity of the samples was also associated with the flavonoid level .The antioxidant potentials obtained in this study probably due to the presence of total phenols, flavonoids.

Routinely, plant antioxidant activity is determined by DPPH and TEAC free radical scavenging methods. The present research results revealed that aerial part of plant with potent activity by inhibiting free radicals.
The DPPH method revealed the capability of the extract to transfer electrons or hydrogen atoms, although the TEAC assay specified the hydrogen donating and the chain-breaking ability of the extract to free radical \[13\]. Furthermore, it was differences in solubility DPPH and TEAC assays. In general, DPPH could assess hydrophilic substances, whereas TEAC can be used to study both hydrophilic and lipophilic substances \[14\].

CONCLUSION

In the present research aerial part was reported with high total phenol, flavonoid and antioxidant activity by different systems. The highest antioxidant activity revealed by TEAC assay .it is could be suppose the aerial part of Bitter vetch contain both hydrophilic and lipophilic substances with chain-breaking ability of free radicals.

ACKNOWLEDGMENTS

The authors are grateful to the Yasuj Medicinal Plant Research Center for providing grants and supporting this study.

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