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# Phenolic Contents and Antioxidants Activity from Aerial Parts of *phlomis herba-venti L. subsp. Kopetdaghensis*

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## ABSTRACT

Antioxidants are important in reducing heart disease and could prevent damage to DNA in human. Many synthetic antioxidant components have toxic and/or mutagenic effects, which have attracted most of the attention on the natural source of antioxidants. Plants are sources of natural antioxidant because they contain Phenolic compounds such as Phenolic acid, flavonoids, tannins and anthocyanins. This study aimed to evaluate Phenolic content and antioxidant activity from aerial parts of *phlomis herba-venti L. subsp. Kopetdaghensis*. Total Phenolic content of methanol, ethyl acetate and dichloromethane extracts was determined by Folin-Ciocalteu method and antioxidant activity using 2, 2'-diphenyl-1- picrylhydrazyl radical scavenging assay. The amount of total Phenolic contents of the methanol, ethyl acetate and dichloromethane extracts were 157.65, 15.25 and 13.61 mg gallic acid equivalent in 100gr dry weight of plant. In this study, none of extracts showed antioxidant activity as high as positive controls (BHT and vitamin C). The methanol extract of *phlomis herba-venti* was better to all the extracts tested and it had the lowest IC50, while the dichloromethane extract had the highest IC50. The results showed that the methanol extract of *phlomis herba-venti* has a potential source of natural antioxidants and could use in medicine and food production.

**KEY WORDS:** extraction, Phenolic content, antioxidant activity, free radical scavenging, *phlomis herba-venti L. subsp. Kopetdaghensis*

## INTRODUCTION

Lipids are one of the most chemically unstable food components and will easily undergo free-radical chain reactions that not only deteriorate the lipids but also generate oxidative fragments, some of which are volatile and are known as the off-flavors of rancidity. Also oxidation may cause degradation of proteins, vitamins and pigments and cross-linking of lipids and other macromolecules into non-nutritive polymers. On the other hand, free radicals are known to cause aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer. Antioxidants are added to food to reduce the rate of oxidation and make them last longer and prevent them from rancidity [1]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG) and tert-butylhydroquinone (TBHQ), are commercially available and typically used. However, there are concerns about their safety and toxicity in food industry [2]. Therefore, research on alternative antioxidants from natural sources has been considered. The phenolic compounds (flavonoids, phenolic acids and tannins), nitrogenous compounds (alkaloids, amino acids, peptides, amines and chlorophyll byproducts), carotenoids, tocopherol and ascorbic acid are the most important natural antioxidants. [3]. Plants are rich sources of natural antioxidants because they contain phenolic compounds such as Phenolic acid, flavonoids, tannins, and Phenolic diterpenes. In recent years, much attention has been focused on Phenolic antioxidants from different types of plant materials [4].

The genus Phlomis, perennial herbs of the family *Lamiaceace*, consists of more than 100 species distributed in Africa, Asia and Europe. In Iran, 17 species have already been recorded. *Phlomis* species are used for treatment of various diseases such as diabetes, gastric ulcer, hemorrhoids, inflammation, and wounds. The essential oil of *phlomis*is composed of four chemo types dominated by monoterpenes (α-pinene, limonene and linalool), sesquiterpenes (germacrene D and β-caryophyllene), aliphalic compounds (9, 12, 15-octadecatrienoic acid methyl ester), fatty acids (hexadecanoic acid) and other components (trans-phytol, 9, 12, 15-octadecatrien-1-ol). Flavonoids, iridoids and phenylethyl alcohol constitute the main compounds isolated from *phlomis* extracts. Antidiabetic, antinociceptive, antiulcerogenic, protection of the vascular system, anti-inflammatory, antiallergic, anticancer, antimicrobial and antioxidant properties of some phlomis species have been reported [5].

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The chemical composition of plants, differ due to various factors e.g. plant variety, climate, and soil, cultivation practices, harvesting time, and processing method. Some plant extracts of the family Labiatae have already been studied, but sometimes they showed adverse antioxidant activity when used under different conditions [6]. Therefore, and also for the exploitation of native sources, this research was aimed to study total phenolic content and antioxidant activity of the different extracts of *phlomis herba-venti L.subsp. Kopetdaghensis*.

## MATERIALS AND METHODS

- **Preparation of the extracts**

The aerial part of phlomis herba-venti L. subsp. Kopetdaghensis was collected from Yamandagh Mountain, north khorasan province of Iran. *Phlomis herba-venti L .subsp. Kopetdaghensis* was identified by Research Center of Natural products and Medicinal plants of North Khorasan University of Medical Sciences and Health Services. The aerial parts of plant were dried at room temperature under shade and then powdered. Three types of solvents (methanol, ethyl acetate and dichloromethane) were used for extraction. About 100gr of sample was soaked in 1L of methanol, ethyl acetate and dichloromethane (Merck, Co, Germany) at room temperature for 48h, separately. After filtration of each solvent, extracts were evaporated to dryness using a rotary evaporator (Buchi, Switzerland) and then weighed. The resulting extracts were stored at $+4^{\circ c}$ for further analysis.

- **Determination of total phenolic compounds**

The total phenolic compounds of the extracts of *phlomis herba-venti L.subsp. Kopetdaghensis* were measured by previously reported methods involving Folin-Ciocalteu reagent and Gallic acid as a standard [7]. In this method, 100µl of each extract (at a concentration of 1mg/ml) were transferred in test tubes and then 2.8 ml of distilled water were added. After that, 2ml of saturated solution of sodium carbonate 2% and 100µl of Folin-ciocalteu reagent were mixed. This mixture was shaken and remained for 30 min at room temperature, and then absorbance was measured at 720 nm. The same method was repeated for all the standard Gallic acid solutions and a standard curve was drawn. Results reported in milligrams of Gallic acid equivalents (GAE) per gram of extract (mg/g).

- **Antioxidant assay**

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging of *phlomis herba-venti L. subsp. Kopetdaghensis* aerial extracts was determined using the method described by Choi et al. [8]. In this test, purple DPPH reduced to a yellow colored diphenyl picrylhydrazine. The disappearance of free radicals was determined by spectrophotometry [9].The remaining DPPH which showed maximum absorption at 518 nm. Each extract solution (1.0 mg/ml) was diluted to final concentrations of (0.6 .0.5, 0.4, 0.3, 0.2, 0.1 and 0.05) mg/ml, in methanol. One ml of a DPPH solution (0.3 mM) was mixed to 2.5 ml of extract solution of different concentrations. One ml DPPH solution plus 2.5 ml of methanol was used as a negative control. Methanol was used as a blank. These solutions were allowed to stand at room temperature for 30 minutes. Light should be removed, because DPPH is sensitive.
The absorbance values were read at 518 nm and converted into the percentage antioxidant capacity using the following equation:
Scavenging capacity (%) = 100 – [(absorbance of sample – absorbance of blank) × 100/absorbance of control]
The tests were done in triplicate. The results were presented as IC50 values which mean the concentration of extract required to scavenge 50% of DPPH (IC50).

- **Statistical analysis**

Each experiment was repeated three times and data analyzed by SPSS software (version 19). One-Way analysis of variance (ANOVA) followed Duncan´s Multiple Rang test used to determine significance differences between groups and $p<0.05$ was considered significant.

## RESULTS AND DISCUSSION

Extraction process is widely used to obtain a crude extract of phytochemicals from the plant materials. There are few factors would affect the rate of extraction and quality of extracted bioactive compounds, including type of solvent, particle size of plant materials, temperature and PH of extraction and extraction time [10]. In this study, three types of solvents (methanol, ethyl acetate and dichloromethane) with different polarity were used to extract plant metabolites. The yield percentages of different extracts of phlomis herbaventi are shown in table 1. The yield of methanol extract was high in compared to other extracts.

- **Total Phenolic Content**

Table 2 shows the results of determination of total Phenolic contents in the *phlomis herba-venti L.subsp. Kopetdaghensis* aerial parts extract. The highest ($p<0.05$) amount of Phenolic content was found in the methanol extract (18.90 mgGAE/g), followed by ethyl acetate extract (11.38 mg GAE/g) and dichloromethane (10.16 mg GAE/g) extract. The difference between total Phenolic content of ethyl acetate and dichloromethane extracts of *phlomis herba-venti* was no significant ($p>0.05$), but methanol extract showed high content of total Phenolic content in comparison with other extracts ($p<0.05$).

The extraction of phenolic compounds from natural products is strongly influenced by the solvent used. It is proved that with increasing polarity of the extraction solvent, the greater amount of Phenolic compounds will be extracted [3]. According to Jamshidi et al. (2009) polyphenol compounds, such as Phenolic acid, flavonoids and tannins are the major contributors to the antioxidant activity of plants [11]. Phenolic compounds are especially important antioxidant because their redox potentials that cause they work as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [10].

- **Antioxidant assay**

The antioxidant capacity of different extracts was determined using a DPPH method. As shown in Fig1 (A), the absorbance of the extracts at 518 nm decreased with the increase in concentration of extracts. The DPPH free radical scavenging ability increased with an increase in concentration of the *Phlomis herba-venti* extracts and positive controls (BHT and Vit C) (Fig 1(B)). In this study none of extracts showed activity as high as BHT and Vit C ($p<0.05$). With regard to the three extracts of *Phlomis herba-venti* (methanol, ethyl acetate and dichloromethane), methanol extracts had the highest radical scavenging activity, followed by ethyl acetate and dichloromethane extract.

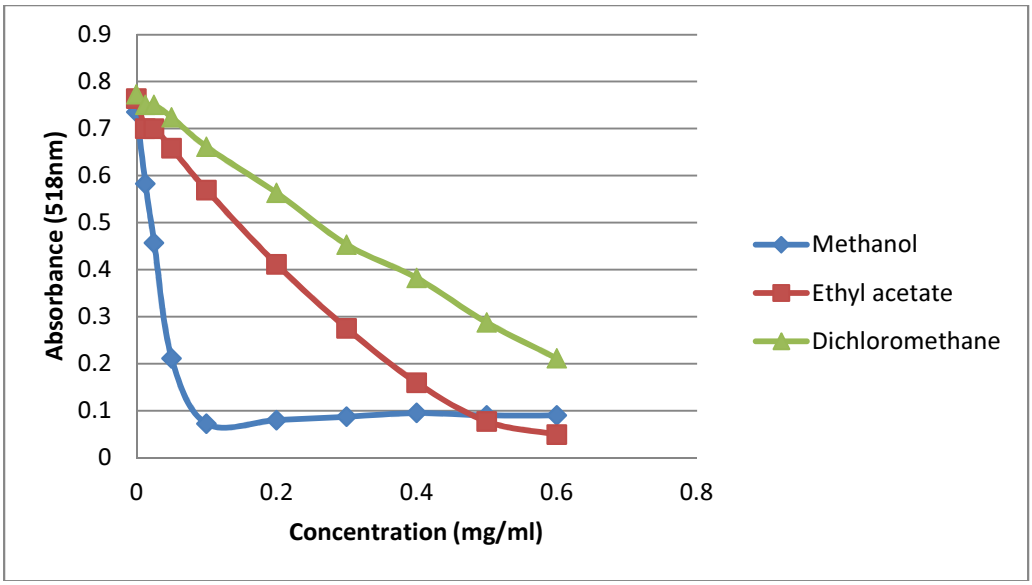
The corresponding $IC_{50}$ values for methanol, ethyl acetate and dichloromethane extracts were 0.033mg/ml, 0.178mg/ml and 0.347 mg/ml, respectively (Table 3). The methanol's extracts $IC_{50}$ value was lower than other extracts ($p<0.05$), but higher than butylated hydroxytoluene (BHT) and vitamin C which had an $IC_{50}$ value of 0.005 mg/ml and 0.004 mg/ml, respectively (Table 3). The difference between $IC_{50}$ values of methanol and ethyl acetate extracts of *phlomis herba-venti L.subsp. Kopetdaghensis* was no significant ($p>0.05$). The dichloromethane extract $IC_{50}$ value was higher than other extracts and did not work effectively. The type and polarity of extraction solvent, the isolation procedures, purity and identity of antioxidant active components from the raw materials could affect antioxidant activity of extracts. [6].The high level of Phenolic compounds in the methanol extract might explain its stronger antioxidant activity in comparison with other extracts. There is evidence of a good correlation between Phenolic contents of the different extracts and their $IC_{50}$ values. These findings were similar to the results of many researchers who reported such correlation between total Phenolic composition and $IC_{50}$ antioxidant activity [12].
In 2003, the in vitro antioxidant activity of the ethanol extracts obtained from 21 aromatic plants belonging to the *Lamiaceace* family was studied, the extracts of *S. spruneri* and *P. lanata* showed the same activity as alpha-tocopherol [13]. Zhang and Wang in 2009 have been investigeted Phenolic content and antioxidant abilities of two phlomis species (*phlomis umbrosaTurcz*. and *phlomis megalanta* as well as five pure Phenolic compounds (protocatechic, chlorogenic, benzoic, rosmarinic acid arutin) in acetone and methanol extracts from leaves. Antioxidant activities of pure compounds and correlation analysis indicated that protocatechic and rosmarinic acids were the most relevant to the antioxidant activities of the investigated *phlomis* extracts [4]. Morteza-Semnani et al. in 2006, tested antioxidant capacity of the methanol extracts of some species of *Phlomis* and *Stachys* on sunflower oil that stored at 70°c. They found that methanol extracts of *P.bruguieri* and *S.laxa* are most effective in stabilizing sunflower oil [6].

Table1. The yield percentages of different extracts of *phlomis herba-venti L. subsp. Kopetdaghensis*

| | **Methanol extract of *P. herba-venti*** | **Ethyl acetate extract of *P. herba-venti*** | **Dichloromethane extract of *P. herba-venti*** |
|---|---|---|---|
| Extraction yield (%) | 8.34 | 1.34 | 1.34 |

Table2. The total Phenolic contents in the *phlomis herba-venti L. subsp. Kopetdaghensis* aerial parts extract.

| **Extract** | **Total phenolic content mg/g** | **Total phenolic content (mg) in 100g dry weight of plant** |
|---|---|---|
| Methanol extract | 18.90 | 157.65 |
| Ethyl acetate extract | 11.38 | 15.25 |
| Dichloromethane extract | 10.16 | 13.61 |

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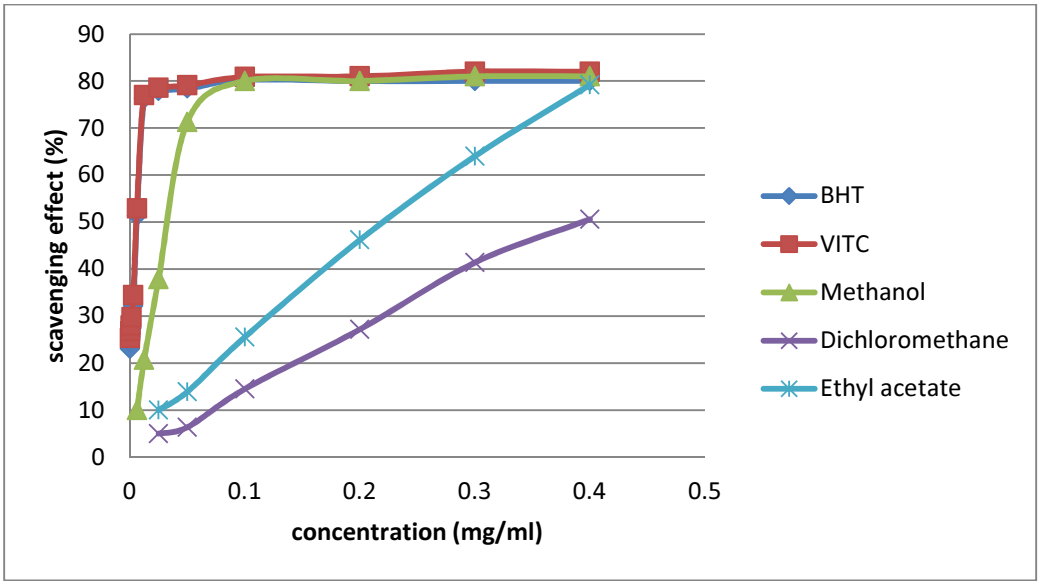


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Fig1. Antioxidant activities of extracts from aerial parts of *Phlomis herba-venti L. subsp. Kopetdaghensis*. (A) The absorbance of extracts in 518nm in DPPH method. (B) The DPPH radical scavenging activity of positive controls (Vit C and BHT) and extracts.

Table3. The DPPH $IC_{50}$ of various *Phlomis herba-venti L. subsp. Kopetdaghensis* extract and positive controls (Vit C and BHT)

| Extract | DPPH $IC_{50}$(mg/ml) |
|---|---|
| Methanol extract | 0.033 |
| Ethyl acetate extract | 0.178 |
| Dichloromethane extract | 0.347 |
| BHT | 0.005 |
| Vitamin C | 0.004 |

## Conclusion

This study is the first research on the phenolic content and antioxidant activities of *Phlomis herba-venti L.subsp. Kopetdaghensis* and the results showed that the methanol extract could be used as a potential natural antioxidant source for medicine and food production. Further studies to separate active compounds and in vivo evaluation of antioxidant activity along with toxicity assay of the extracts from *phlomis herba-venti L.subsp. Kopetdaghensis* are therefore suggested.

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