

***Salvia virgata* L. Leaves Extract: Qualitative and Quantitative Phenolic Compounds and Antioxidant Activity**

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Abstract: Plants' materials are a rich source of phenolic compounds (flavonoids, tannins and anthocyanin) that are the most important natural antioxidants. Antioxidants in the diet are very important for health and protected the body for oxidative stress. This research has been done for measurement of quantitative and qualitative of phenolic compounds and antioxidant activity of *Salvia virgata* L. as an alternative to synthesis antioxidants. In this work, the phytochemical study was performed on *Salvia virgata* L., and the amount of phenol and flavonoids were measured from three extract using spectrophotometric UV method. The antioxidant activity of the different extract was evaluated in different concentrations using (DPPH). Data analysis and variance analysis were done with SPSS software 16th version. The results showed that total phenolic compounds of aqueous, ethanol and methanol extracts were 35.5, 34, 32.5 GAE/g dry samples respectively, and the total flavonoids content of aqueous, ethanol and methanol extracts were 28.12, 23.40, 26.50 mgQE/g dry sample. The concentration inhibition of 50% ethereal extract (IC₅₀) = 5.4±0.6 and phytochemical results indicate the presence of flavonoids, tannins and anthocyanin in the extract. The results showed that the different extracts are rich with phenolic compounds and also present a high level of antioxidant properties, therefore, *Salvia virgata* L. could be used as a source of antioxidant compounds in food and as medicinal plant.

Key words: *Salvia virgata* L, Flavonoid, Spectrophotometry UV, Phenol antioxidant

1. Introduction

Phenolic compounds are large group of natural plant materials include Flavonoids, tannins and anthocyanin that are commonly seen in fruits, vegetables, leaves, nuts, seeds, roots and in other

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parts of the plant, these materials are usable attention to food, chemistry, pharmacy and medicine considering a wide range of beneficial and biological effects including antioxidant properties (Kumaran and Karunakaran., 2006 ; Raghavendra *et al.*, 2015). Flavonoids and other phenolic compounds have a widely released in plants and a variety of their biological activities including antioxidant, anti-microbial, anti-inflammatory reported in many studies (Noguchi and Niki, 2000; Jamshidi *et al.*, 2010).

Phenolic compounds with having antioxidant and anti-radical properties can play an important role in preserving food products and maintaining human health. Labiatae family are also in that group, it contains many types of flavonoids, tannins, and anthocyanin that these compounds have antioxidant properties. *Salvia virgata* L. is of the Labiatae family, this plant family including the plants which has been used in traditional medicine for many years in Iran. This plant is used to treat anti-flatulence, anti-diarrhea, anti-inflammatory, and digestive diseases (Shariatifar, 2017) Proven in recent years the free radicals are the most important oxidizing agent's food which with a destructive process, causes the loss of valuable food and change in their chemical compounds. (Kris-Ethertonm *et al.*, 2002; Henry and Heppell, 2013; Robards and Kerr, 1988).

In addition to undesirable organoleptic effects in food products by eliminating vitamins and essential fatty acids that can produce toxic compounds and leads to undesirable effects, such as inflammatory diseases, diabetes, Mellitu, cardiovascular ischemia, cancer, immune deficiency in the human body (Dastmalchi *et al.*, 2017; Robards and Kerr, 1988 ; Antolovich *et al.*, 2002) . So, the use of antioxidants to slow down oxidation in food seems necessary, which if it correct and suitable use, can prolong life food products during their use. (Ames, 1983; Mathew and Abraham, 2006). Plants with phenolic compounds and many other compounds have antioxidant potential since the amount of antioxidant activity of compounds and extracts by a wide range of methods have been identified, that is have been raised which of these natural antioxidants has more efficacy. (Eidi *et al.*, 2019 ; Howard *et al.*, 2000 ; Kamkar *et al.*, 2016).

Considering the indigenous nature of *Salvia virgata* L. in Iran, easy and inexpensive access, food and medicine use of this plant for distant times in the country, this study can be a prelude to practical use of extracts of this plant (source of phenolic compounds) as an antioxidant in the food and pharmaceutical industries, In this way, it is also possible to use an easy source affordable and both product wasting and damage caused by it and ultimately a step to promote the health and safety of the community. The aim of this study was to evaluate the quantitative

and qualitative properties of the phenolic compound's antioxidant properties of *Salvia virgata* L. extracts and inhibition-free radicals using the free radical method (DPPH).

2. Material and Methods:

This research is a laboratory study that includes the collection, identification, extraction of *Salvia virgata* L. and the evaluation of phenolic, flavonoids and antioxidant properties of this plant.

2.1. Preparation of the plant

Plant material was added in June 2016, from the city of Shahrekord (Ben village) and dried for 10 days in a shade, the desired sample has been approved by botanist Research Institute of Forests and Rangelands in Iran (RIFR).

2.2. Preparation of extracts

50 g of powdered dried leaves of plants mixed with 250 ml methanol, ether, ethanol, and distilled water separately and the extraction process done by soxhlet and percolation method, after straightening and cross with wattermen paper No. 1 and evaporation solvent, the resulting solution was dried at 40 °C and until the test sample was kept in a refrigerator of +4°C, all chemicals and solvents used in this research were from Merck (Germany) and free radical DPPH was from Sigma Aldrich (USA) and they have the highest percentage of purity.

2.3. Diagnosis of alkaloid

To 0.25g of water and alcohol extracts, 5 ml of hydrochloric acid (HCl)1% was added and boiled during 5 minutes, then the volume bring to an initial amount and the acidic solution was filtered, the solution is obtained by the appropriate amount of alkaline ammonia (NH₃)10% with diethyl ether (C₂H₅)₂O, solution evaporated extent to the dry and added it 5 ml of hydrochloric acid (HCl)1 %, then, resulting in acidic solution divided into 3 parts, 1 part considered as a control and 2 other parts introduced by Busharda and Meyer reagent ([Chhabra et al., 1984](#)). The formation of brown deposition with the addition of Busharda reagent and white sediment yellowish with an increase in the Meyer reagent showed the present the alkaloids ([Brain and Turner., 1975](#); [Chhabra et al., 1984](#)).

2.4. Diagnosis of tannin

0.25 g of extract dissolved in 10 ml of distilled water, then 3-4 ml of sodium chloride (NaCl) 10% was added. Then two drops of pure ferric chloride (FeCl_3) 5% were added to 5 ml of distilled water in a test tube. A few drops from the solution of the extracts were added to the test tube creating a blue-green color indicating the presence of tannin (Brain and Turner, 1975; Chhabra et al., 1984).

2.5. Diagnosis of saponin

0.5g of extract poured in the test tube and dissolved into 10 ml of distilled water and was shaken severely for 30 seconds. Staying of foam for a half an hour indicates the presence of saponin (Brain and Turner, 1975; Chhabra et al., 1984).

2.6. Diagnosis of anthocyanin

0.25g of extract dissolved in 10 ml of distilled water then added HCl 1% as obtaining the acidic solution. The presence of red color at the pH =3-4 which changes with the variation of pH indicates the presence of anthocyanin (Brain and Turner, 1975).

2.7. Determination of total phenolic content of extracts

Total phenolic content was measured using Folin-Ciocalteu reagent. To 0.5 ml of each extract (10 mg /ml) added 2.5 ml of Folin-Ciocalteu 0.2 normal, after 5 minutes added it 2 ml of sodium carbonate (Na_2CO_3)75g/l. The mixture presents a read color after 2 hours and the absorbance results were determined at the wavelength of 760 nm using spectrophotometer UV against the blank. The gallic acid ($\text{C}_7\text{H}_6\text{O}_5$) was used as a standard to plot the calibration curve. Total phenolic content based on the equivalent of "mg of gallic acid in, g extract" experiments were repeated three times and the average reported (Ordonez et al., 2016; Wollenweber et al., 1995).

2.8. Determination of total flavonoid content of extracts

The total of flavonoid content was measured using aluminum chloride reagent (AlCl_3). To 0.5ml of each extract (10 mg/ml), 1.5ml methanol (CH_3OH), 0.1 ml aluminum chloride (AlCl_3) 10% in ethanol, 0.1ml potassium acetate ($\text{CH}_3\text{CO}_2\text{K}$) 1mol and 2.8ml of distilled water was added.

After half an hour and storage at room temperature, the absorbance of the mixture was read at the wavelength of 415 nm by spectrophotometer against blank. Quercetin (C₁₅H₁₀O₇) (Merck) was used as a standard for curve drawing calibration. The number of flavonoids based on the amount the equivalent of "mg of quercetin in extract" was reported. Experiments were repeated three times and their average was reported (Chang *et al.*, 2002 Chung *et al.*, 2006).

2.9. Evaluation of Antioxidant activity

The evaluation of an anti-radical property by the DPPH method is the ability to give a hydrogen atom or electrons in various compounds and extracts, in this test the colorless violet solution DPPH in methanol measured, in this method, use of DPPH (Sigma Aldrich., USA) as a reagent. 50 µl of different concentrations of water, ethanol and methanol extracts in the methanol was added to 5 ml of DPPH solution (0.004% in methanol), after 30 minutes of incubation at room temperature, the absorbance of different samples was read by spectrophotometer against blank at 517 nm. The percentage of free radicals inhibition of DPPH was calculated using the following formula.

$$I\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

In this formula, Blank's is the negative absorption spectrum which shows all the materials except for the extracts and A sample represents the absorbance of different plant extracts. After that, the concentration of plant extracts that has 50% of radical (IC₅₀) was calculated by the diagram. Obviously, the smaller the number shows the more antioxidant or inhibition of free radicals. In this test, used the synthetic antioxidants BHT as a positive control and all experiments repeated three times.

2.10. Statistical analysis

Information obtained as a Mean ± SD and data analysis done by using SPSS software version 16 and ANOVA test and significance level considered 5%.

3. Results and Discussion:

In this research, the phytochemical experiments confirmed the existence (+) of anthocyanin, tannin, and flavonoids in aqueous extract, the presence (+) of alkaloids, anthocyanin, tannins, and flavonoids in alcoholic extract and the presence (+) of flavonoids in the ether extract. (Table 1). The results of phenolic compounds and flavonoids test confirmed high levels of these compounds in different extracts (Table 2). The previous study indicated peppermint (*Mentha piperita* L.) extract has high levels of phenolic and flavonoid compounds and it shows high anti-oxidation properties. (Carreon *et al.*, 2002; Swetie *et al.*, 2007) in another study, *Rosmarinus officinalis* extract has high antioxidant activity and this activity is directly related to the phenolic content of the plant (Elmasta *et al.*, 2006; Jamshidi *et al.*, 2010; Saeidniaa *et al.*, 2007) evaluated the methanol extracts of several native plants in terms of flavonoids and phenolic compounds in Iran, in these studies, they showed there is a good relationship between antioxidant activity and polyphenol compounds of the plant.

Table 1: Results of phytochemical experiments of aqueous, alcohol and ether extracts leaves of *Salvia virgata* L.

Extract	Alkaloids	Saponin	Anthocyanin	Tannin	Flavonoids
Aqueous Extract	-	-	+	+	++
Alcoholic extract	+	-	+	+	++
Etheric extract	-	-	-	-	++

Table 2: The amount of phenolic and flavonoid compounds aqueous, alcohol and ether extracts leaves of *Salvia virgata* L.

Extract	Average	
	Phenolic compounds (mg QE/g dry sample)	Flavonoid compounds (mg GAE/g dry sample)
Aqueous Extract	35.5 ±0.25	28.12 ±0.40
Alcoholic extract	34.0 ±0.50	23.40 ±0.75
Etheric extract	32.5 ±0.68	26.5 ±0.33

The power of inhibiting free radicals and also the ability to inhibit oxidation of lipids by the etheric extract of *Salvia virgata* L. was studied by an experimental method and the ability to control free radical evaluated by DPPH test, in this experiment with increasing concentrations of extract, radical control was done with more power. The concentration of the extract, which causes 50% of inhibition (IC₅₀) compared with butylated hydroxyl toluene (BHT), that (IC₅₀) of ether extract was 8.5 ± 0.3 . In these experiments used from BHT as a positive control, (IC₅₀) of BHT was 4.2 ± 0.11 µg/ml. In this test antioxidant power of the etheric extract was weaker from, synthetic antioxidants BHT (Table 3).

Stankovic *et al.*, (2011) compared phenolic and flavonoids contents the aqueous and methanol extract of the different parts of the *Teucrium montanum*, their results showed that phenolic and flavonoid content of the aqueous extract more than methanol extract. In evaluating the antioxidant properties of methanol extract of *Mentha longifolia* that reported (Golluce *et al.*, 2007) by the DPPH method, IC₅₀ was 74.4 µg /ml. Kamkar *et al.*, (2016) in their research indicated that concentration inhibits 50% ethanol extract of *Anethum graveolens*, was 340 µg/ml, in association with BHT this amount was 5 µg/ml.

Patterson *et al.*, (2001) studied antioxidant properties of *Avena sativa* by DPPH and beta-carotene colorless methods and determined the total phenolic compounds; the researchers showed that there is a good connection between the number phenolic compounds and antioxidant activity. Briefly, studies show that high phenolic compounds is the main reason the high antioxidant activity of some extracts include polar extracts, because based on the evidence there is a positive relationship between the amount of phenolic compounds and antioxidant activity in the plants, on the other hand, it seems that the phenolic compounds that are widely found in plants and have high antioxidant power, can be extracted through plant extracts (Jahanian *et al.*, 2005; Candan *et al.*, 2003; Muret *et al.*, 2007), also the key role of phenolic compounds as the elimination of free radicals have been reported in several articles (Katalinic *et al.*, 2006; Aeschbach *et al.*, 1994). It should be noted that the phenolic compounds acting as a hydrogen supplier effectively, so they are an effective antioxidant (Golshani *et al.*, 2004; Golluce *et al.*, 2007).

Table 3. Antioxidant activity different extract of *Salvia virgata* L. by (DPPH)

Sample	DPPH IC ₅₀ (µg/ml)
Aqueous Extract	150.2 ± 0.35

Alcoholic extract	235.2 ± 0.19
Etheric extract	8.5 ± 0.3
BHT	4.2 ± 0.11

4. Conclusion

This study provides new information about the phenolic profile and antioxidant capacity of *Salvia virgata* L. Qualitative and quantitative differences in phenolics were noted between the studied leaves extract. Overall, the phenolics quantified, mainly belong to secoiridoid phenolics, phenolic alcohols. The comparative study indicated *Salvia virgata* L can be considered as appropriate sources of bioactive phytochemicals, which play a major role in human health as free radical scavenger and can replace synthetic antioxidant in the food products; for this, it will be interesting to promote the culture of *Salvia virgata* L derived from these cultivars. In order to give more information about the authenticity and quality of the studied *Salvia virgata* L extracts, the determination of aromatic profile should be performed to complete the present investigation.

The correct use of medicinal plants requires precise information and scientific knowledge of the chemical compounds present in them because the chemical compounds have the therapeutic effect in the plants. In this research, experiments confirmed flavonoids and phenolic compounds. In all plants, antioxidant activity has a direct relationship with the amount of phenolic and flavonoids compounds. As is known, the effect of radical inhibition of the etheric extract of the *Salvia virgata* L is stronger than the ethanolic extract of *Anethum graveolens* in the other hand the comparison of between the BHT used in the present study and the study of *Anethum graveolens* ethanol extract, indicate the BHT inhibition is equal in both studies. In this study, it was shown that the water, alcoholic and ethereal extracts of *Salvia virgata* L. has a high level of phenol and flavonoids compounds, in this feature, and in comparison with a similar study that done by other scholars has shown more antioxidants power. So for use, the practical application of these compounds in the industry is recommended and more research is needed on identifying components and evaluating antioxidant extract of this plant.

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