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Studing the Antioxidant Effects of Stachys Lavandulifolia in Chaloos

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Stachys lavandulifolia is from mint family and Labiatae species and grows in different parts of our country sporadically. The purpose of this study is to investigate the chemical properties of *Stachys lavandulifolia* Ethyl acetate extract. In this study, the Ethyl acetate extract was provided using maceration method. The extract then was defatted and solvent removal performed in a subsequent process. Some tests such as Testing the ability of neutralizing free radicals, Measuring the total phenolic compounds using the Folin-Ciocalteu method were used. The Ethyl acetate extract was consequently $IC_{50}\%$ = 53.60, using Testing the ability of neutralizing free radicals method. The reducing percent was calculated in terms of Ethyl acetate concentration and compared with natural and synthetic antioxidant. The results showed that with increasing concentration, antioxidant activity will be increased.

Keywords: Stachys lavandulifolia, Labiatae, Percolation, Antioxidant

INTRODUCTION

Flowers and plants are silent presences; they nourish every sense except the ear, they produce no sound but they are so expressive, as they are a glorious manifestation of the power and majesty of God. Since the medicinal herbs are highly effective nutritional supplement and of particular importance in treating diseases, it has been interested by researchers to identify the compositions of such plants namely the medicinal and aromatic species, especially those native to the land [1]. The *Stachys*, with over 270 species, are widely used in traditional medicine in Iran and has the honor of being one of the largest family of Labiatae around the world, especially in Mediterranean area with over 4000 species and 200 genera [6]. Iran is rich in species of these plants that grow as wild plants. Of the approximately 3000 species of medicinal herbs known to occur in the wild in the world, about 140 species are native or have occurred in Iran [2]. Since there are some plants recorded in Pharmacopoeia as having the antioxidant effects, it is possible to consider the *Stachys lavandulifolia* as having the same effects as those of recorded to prevent cancer and various diseases by evaluating its antioxidant properties [4]. Free radicals are molecules possessing unpaired electrons and thus are reactive and short-lived in a biological setting. Dependent upon the site of and rate of production (concentration), free radicals can mediate both detrimental modifications to biomolecules and/or participate in beneficial cellular signal transduction. Reactive oxygen species (ROS) comprise both free radical and non-free radical oxygen intermediates, so the free radicals and reactive oxygen species as well as their effects on biological systems assumed to be issues that are much importance to medicine and clinical care. These can irreversibly damage vital molecules including nucleic acids, proteins, lipids and lipoproteins. Antioxidants are compounds that can protect biological systems against the potentially harmful effects of these free radicals. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing

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agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so, they are chemicals that interact with and neutralize free radicals, thus preventing them from causing damage. They are also known as "free radical scavengers. Antioxidants, especially the fat-soluble ones, prevent the oxidation of unsaturated fats to epoxides and can sometimes reduce these harmful compounds according to new researches [3]. The most common chemical antioxidants in food industry including BHT, BHA, TBHQ and Propyl gallate have been confirmed to be carcinogenic and having negative effect on human health [5]. The use of natural antioxidants has largely increased due to the toxic effects of synthetic antioxidants on one hand and the consumer demand for natural food additives on the other [6]. Some features should be taken into account when evaluating the antioxidant properties of a plant extract. It is also necessary to use the multiple chemical and biological tests. If the tests are passed successfully, the plant will be introduced as an antioxidant. Hence, an attempt has been made in this paper to study the antioxidants properties of ethyl acetate extract of *stachys lavandulifolia* located in west part of Mazandaran namely Chaloos.

METHODS AND MATERIALS

The corollas of *stachys lavandulifolia vahl* were collected ,during full flowering, from Chaloos in June 2012. The flowers were let to dry in the shade and then were crushed into fine pieces almost to the point where they become too fine of a powder. The *stachys lavandulifolia* was then extracted using maceration method and dissolved into ethyl acetate. The term 'maceration' is softening or breaking into pieces using a liquid. Some herbal preparations call for maceration, as it is one way to extract delicate or highly volatile herbal essences "cold" and thus preserve their signature more accurately. To this end, the plant is brought into powder, added into a flask and placed on a shaker for two or three days (depending on type of plant). Then the resulting powders were screened. The -total phenolic content and DPPH radical scavenging activity methods were used in this study to measure the antioxidant effect of the plant.

Chemicals: from Merck, Germany Device Specifications: Spectro UV-VIS Double Beam Research Spectrophotometer Cintra, 20, GBS Australia.

Testing the Ability of Neutralizing Free Radicals (DPPH)

DPPH is a common abbreviation for an organic chemical compound (2,2-diphenyl-1-picrylhydrazyl). It is a dark-colored crystalline powder composed of stable free-radical molecules. (DPPH) has two major applications, both in laboratory research: one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay, as we see here, and another is a standard of the position and intensity of electron paramagnetic resonance signals.

In order to test the ability of neutralizing free radicals, these followings are required:

1. Using the stable free radicals (DPPH) by Blois method
2. Preparing (DPPH) solution (100 micromoles per liter)
3. Preparing sample : 50 mg of *stachys lavandulifolia* extract to 50 ml with methanol

Using three test tubes: (sample) extract + (DPPH) and (blank): extract + (methanol) and (negative control): (methanol) + (DPPH)

The Ultraviolet-Visible (UV-Vis) spectrophotometer was zeroed with blank methanol and the absorbance of these three tubes was read at 570 nm. The percentage of cell revival was then calculated and compared with synthetic antioxidants.

Measuring the Total Phenolic Compounds Using the Folin-Ciocalteu Method

The content of phenolic compounds was determined by Folin-Ciocalteu method.

- Providing solutions: 6% solution of sodium bicarbonate, Folin-Ciocalteu reagent.
- Providing standard: Standard stock solution: 2.5 mg/ml of Standard Gallic Acid with appropriate concentrations.
- Preparing sample: 0.075 mg of *stachys lavandulifolia* extract with 25 ml

To this end, 0.4 ml of extract and 0.3 ml of folin reagent

were placed in a test tube and heated on water bath for 5 min ,and to it were then added with 3 ml of sodium bicarbonate followed by heating the sample for 90 min in water bath at 22 °C . The sample was then added with 3 ml of sodium bicarbonate followed by heating the sample for 90 min in water bath at 22 °C. The same titration was made for the blank sample except that, instead of using 0.4 ml extract, the ethanol of 70% was used in this way. The absorbance of sample and standard was measured against reagent blank at 725 nm wave length. The calibration curve of Gallic acid was plotted and the content of total phenolic compounds was determined in 100 g of plant.
(Microsoft excel was used for all statistical analyses)

RESULTS

The results of antioxidant activity of *stachys lavandulifolia* extract using (DPPH) method. The results derived from antioxidant activity of extract using total phenolic compounds.

Table 1. Data Derived from Using DPPH Method Related to Ethyl Acetate Extract Stachys Lavandulifolia

IC_{50}BHT	IC_{50} ethyl acetate extract	Percent of reduction with BHT	Percent of reduction with ethyl acetate extract	Concentration (ppm)
16.61	53.60	0	0	0
		30.57	12.06	10
		58.86	20.65	20
		88.35	34.73	30
		124.93	45.88	40

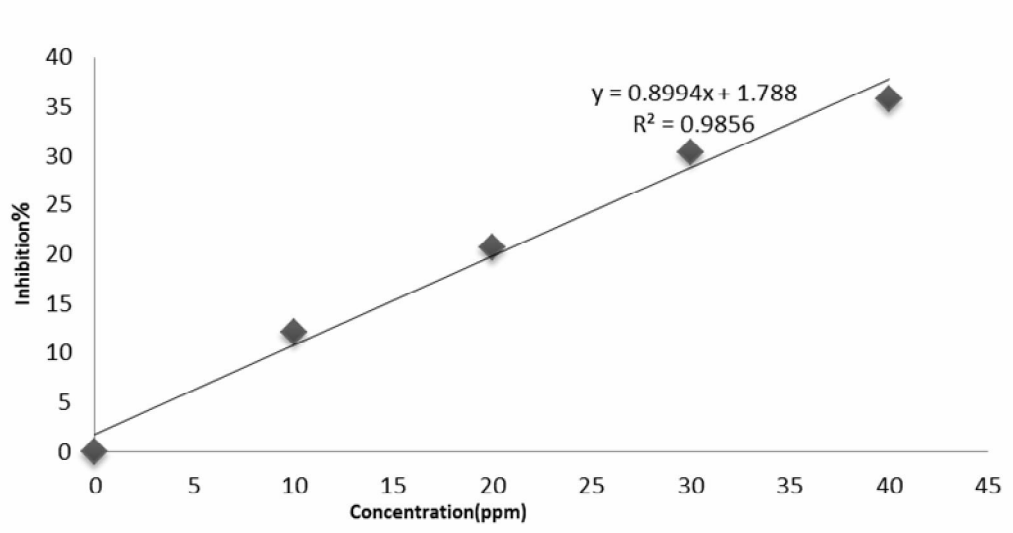

Chart 1. Of antioxidant activity of ethyl acetate extract of *Stachys lavandulifolia* using DPPH method.

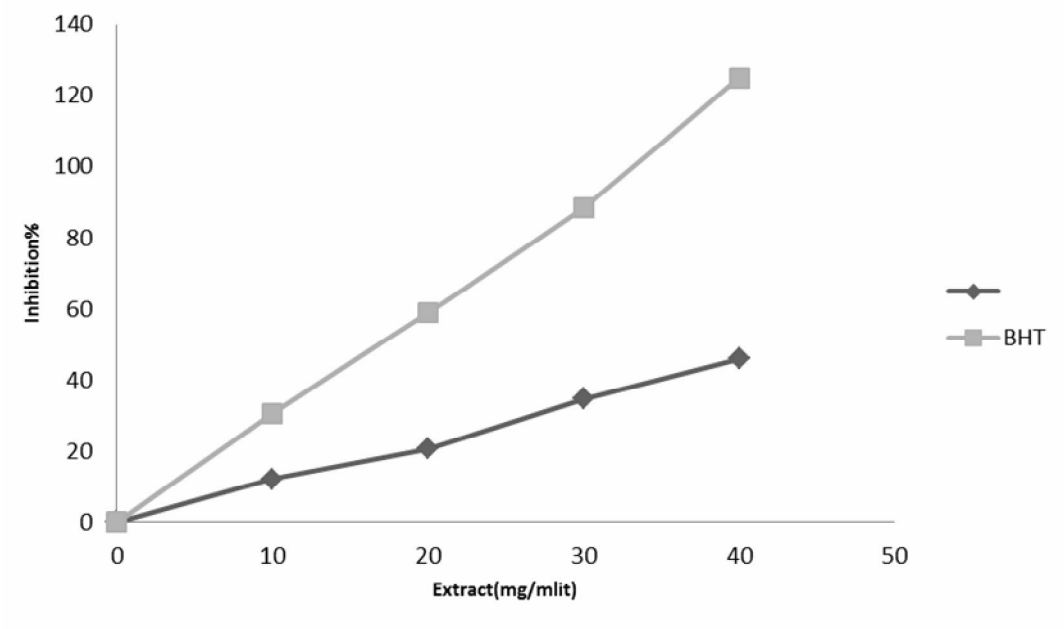

Chart 2. Comparison chart between DPPH and BHT methods concerning antioxidant activity of ethyl acetate extract of *Stachys lavandulifolia*.

Table 2. Absorbance and Concentration Derived from Using Total Pphenolic Compounds

X amount of phenolic compounds for each 100 g of ethyl acetate	X amount of phenolic compounds for each 0.075 g of ethyl acetate	The absorbance rate of 0.075 g of ethyl acetate
60.094	45.071	0.2390

DISCUSSION AND CONCLUSION

(DPPH) is an organic chemical compound of (2,2-diphenyl-1-picrylhydrazyl). It is a dark-colored crystalline powder composed of stable free-radical molecules. It serves as a monitor of chemical reactions involving radicals; most notably it is a common antioxidant assay. The rate reduction of a chemical reaction upon addition of (DPPH) is used as an indicator of the radical nature of that reaction. (DPPH) is commonly used for studying the antioxidant properties of bioactive compounds isolated from the plant. In recent years, it has also been used for studying the antioxidant activities in complex biological systems as well.

The test results (revival percentage) namely (DPPH) free radical scavenging, were evaluated and the antioxidant activity results derived from using (DPPH) were compared with those of (BHT) method. The inhibitory concentration 50 (IC50) of extract was calculated as well as the antioxidant positive control. As it was seen, the ethyl acetate extract had less revival percentage compared to synthetic antioxidants. The plants continued the upside trend namely; the more the concentration, the more the antioxidant activity. The (BHT) method showed more revival percentage compared to the other. The *Stachys lavandulifolia* has a good antioxidant property, according to the results derived from studying the revival percentage, and

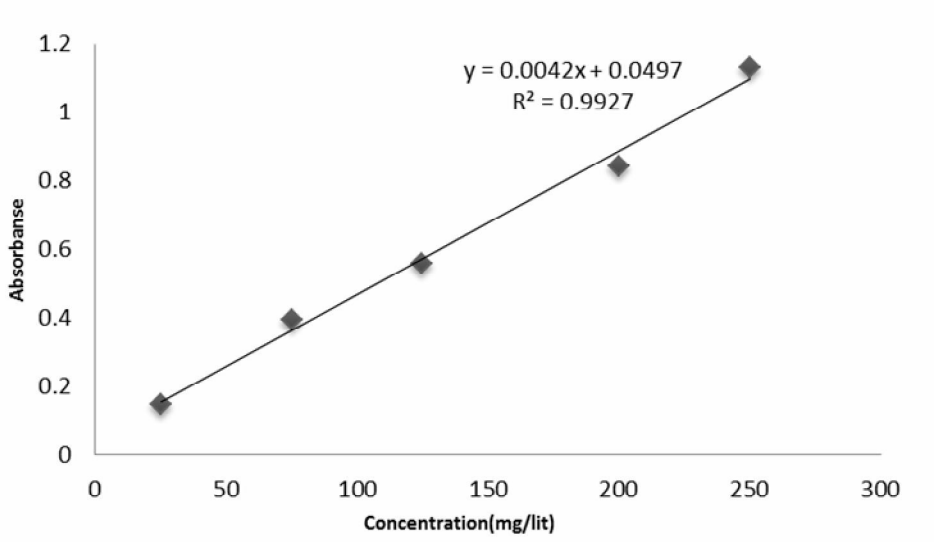

Chart 3. Of antioxidant activity of ethyl acetate extract of *Stachys lavandulifolia* using total phenolic compound.

can be considered as a good alternative to synthetic antioxidants.

Phenolic compounds are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. They are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. Although similar to alcohols, phenols have unique properties and are not classified as alcohols (since the hydroxyl group is not bonded to a saturated carbon atom). They have higher acidities due to the aromatic ring's tight coupling with the oxygen and a relatively loose bond between the oxygen and hydrogen. Accordingly, they have been classified as a secondary metabolite and considered to play an important antioxidant role against many diseases. These compounds have a high antioxidant capacity making them able to trap more free radicals.

Phenolic compounds, including flavonoids, vitamins and pigments are nutritionally important for their antioxidant activities having anti-mutation and anti-tumor properties. The largest and best studied natural phenols are the flavonoids.

The content of phenolic compounds was increased by increased absorbance using Folin-Ciocalteu method (total phenolic compounds).

In a study conducted by Semnani M and et al (2006) , the *Stachys lavandulifolia* extract was found to have high capacity of antioxidant using DPPH method. The same results were obtained by Ghafouri and *et al*. (2012) using both (DPPH) and (TPC) method. These results were also consistent with those of studies conducted by Jamshidi and *et al*. (they conducted a research on Mazandaran native plants, including *Stachys lavandulifolia* to study the phenolic compounds and antioxidant activities).

Comparing these results with those obtained from *Stachys lavandulifolia*, it was found that this plant has a high antioxidant capacity because of phenolic and polyphenol compounds. The reduction or increase in the amount of antioxidant is due to geographic diversity and difference in the amount of rainfall received in different parts of the country.

The results derived from this study and those of previous ones from different parts of the country showed that the *Stachys lavandulifolia* has a good antioxidant property which is undoubtedly due to its compounds and compositions. There is high amount of phenol in *Stachys lavandulifolia*. Generally foods contain complex mixtures of polyphenols. (Some polyphenols are specific to particular food (flavanones in citrus fruit, isoflavones in soya, phloridzin in apples); whereas others, such as quercetin, are found in all plant products such as fruit, vegetables, cereals, leguminous plants, tea). Phenolic and polyphenolic compounds have high antioxidant activity. Taking into account all these parameters, we can use the extract of this plant as a natural antioxidant which is intended to be used in

pharmaceutical, food and agricultural industries.

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