

An investigation of anti-oxidant properties of salvia, conducting beta-carotene bleaching assay

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Abstract. Due to the large amount of oils, *Salvia* (officinalis species) has always been one of the most popular medicinal plants used in traditional medicine. In this theses, the aerial parts of the plant *Salvia officinalis* were collected from greenhouses of Shiraz city and dried in shade for 15 days. Its essential oil was extracted using Clevenger method and analyzed using Gas Chromatography –Mass Spectrometer. Twenty compound was analyzed in the essential oil of salvia as the main compounds, including; cis-Thujone (31.35%), Eucalyptol (13.11%), Globulol (2.33%), trans-Thujone (10.5 %), camphor (21.05%), Isoborneol (1.69%), α -pinene (4.54%), β -pinene (1.68%), α -Humulene (2.71%). High concentration of some oxygen containing compounds like thujones, Eucalyptol, and camphor in the essential oil of salvia may be the reason of its antioxidant, anti-fungal and anti-inflammatory properties. Therefore, this plant must be examined for its biological and treatment uses. In this study we examined the anti-oxidant activity of salvia by beta-carotene -Linoleic acid system. This essential oil demonstrated a great anti-oxidant activity.

Keywords: Identification, anti-oxidant activity, *Salvia*, Clevenger method

INTRODUCTION

In the traditional medicine of Iran, salvia is used to treat diabetes. This herb is also used to aid digestion. It is diuretic, anti-seizure, anti-fever, disinfectant and is also very useful for the heart. In the traditional Chinese medication, this herb is used to treat diabetes mellitus and Angina pectoris and in the traditional Turkish medication it is used to treat rheumatoid arthritis. Given the wide range of *Salvia*'s consumption and its numerous applications, researchers have studied the different uses of this herb such as its antioxidant effects, anti-diabetic, anti-cholinesterase effects, and etc. Thus, it is needed to study the volatile compounds of salvia's species, especially those which are native of Iran, so that it could be used more effectively.

Salvia, the officinalis¹

Salvia is an herbaceous perennial plant which has tap root and numerous branches. Its height is between 50-80 centimeters. New branches' colors are dark green and they are covered with some thick gray fluff. As the plant grows, its stem becomes woody and its color turns into brown. The leaves are long and pointy. The lower leaves have long petioles, while the upper leaves of the stem have short petioles. The upper side and the lower side of the leaves are covered with some delicate fluff. The flowers are blueish purple, pink or white and are gathered on the top of the stems in especial circles. There are 5-8 flowers in every circle. Its fruit is like a hazelnut and its color is dark or light brown.

¹ *S. Officinalis*

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The diameter of the crown is about 55 centimeter. The weight of 1000 seeds is 55.61 grams and the germination period is 12 days. This plant belongs to the class of Lamiales and familia of Lamiaceae.

Its ecological needs are met in a modified, porous, fertile and relatively humid place. It grows best in rich clay, drained soils which are exposed to the sun. It is a Mediterranean plant which need heat and dry weather during growth. It also is a heat resistant plant. Salvia freezes in winter and in a temperature below 15 ° C and dies within 5-6 days. Hot air and a kind of soil which contains calcium are suitable for the plant and play an effective role in active factors. Sandy soils, lacking nutrients, and cold and humid areas limit the growth of this plant. Soil's PH range for salvia is 4.9-8.2, and the most suitable PH is 6.4. This plant lives 5-7 years and is economically efficient for 4 years. Its seed germinates in a temperature of 12-15 °C (Aineshi, 1370).

Recently, using methods of Clevenger and Drying, researchers have extracted the essential oil of salvia officinalis and identified its compounds by gas chromatography. They have realized that the percentage of extracted compounds are different in every method (baj, 2013²).

Classification of biological properties

Terpenoides have different biological properties and are classified according to these properties(Bakkali et al., 2008)³. Some of these properties are stated in the following:

A) Cytotoxic Activity

Terpenoides engaged in significant cytotoxic activities, for instance two terpenoides extracted from salvia involved in some significant cytotoxic activities⁴(Don et al., 2006). Also, in another study of salvia three terpenoides were extracted that showed a reasonable cytotoxic activities.

B - Antibacterial activity

Dr. Roustaian and et al. extracted compounds from different parts of salvia that engaged in antibacterial activities⁵(Esmaeili et al., 2008).

Also, in a comprehensive study the antibacterial activities were classified into three different species of salvia which are native of Brazil⁶ (Delamare et al., 2007)

C- Antifungal activity

Terpenoides also have aa great antifungal activities, for example in a study on the extracted essential oil of slvia officinalis, antifungal properties were studied⁷ (Abu-Darwish et al., 2013)

D- Antiviral and anti-inflammatory activities

Two Diterpenoid lactones are separated from Indopacific gorgonian and showed anti-inflammatory properties and can be compared with indomethacin in this case. They are also antiviral especialy against influenza virus. This effect of terpenoides resists the activity of cyclooxygenase enzyme.

E- Antioxidant properties

² Tomasz baj et al., 2013

³ Bakkali et al., 2008

⁴ Don et al., 2006

⁵ Esmaeili et al., 2008

⁶ Longaray Delamare et al., 2007

⁷ M. S. Abu-Darwish et al., 2013

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There are a lot of researchers conducted on the antioxidant properties of salvia resulting from the terpenoid compounds (⁸Valifard et al., 2014). One of the ways through which researchers analyze this phenomena will be discussed in the next section.

Bleaching beta-carotene by Lipid peroxy radical to measure antioxidant properties

In this colored combination, beta-carotene is bleached (first reaction) due to oxidation of Lipid peroxy radicals (subsequent to Lipid peroxidation), while the presence of hydrogen's antioxidants prevent it.

Characteristics of *salvia officinalis*

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Preparation of sample plant

Salvia samples were collected from greenhouses of Shiraz city and dried in shade for 15 days. 50 Grams of dried herb was cut into small pieces and transferred to 500 liter balloon containing distilled water. At first, contents were boiled at the temperature of 100 °C for 2.5 hours and then at the temperature of 290 °C, essential oil was extracted from the contents using Clevenger apparatus.

Beta-carotene bleaching assay

Making soluble beta-carotene

To provide soluble beta-carotene, we have to add 0.5 mg beta-carotene to 1 ml chloroform and then add them to 25 microliter linoleic acid and 200 mg 40 Twin as emulsifier.

⁸ Valifard et al., 2014

e

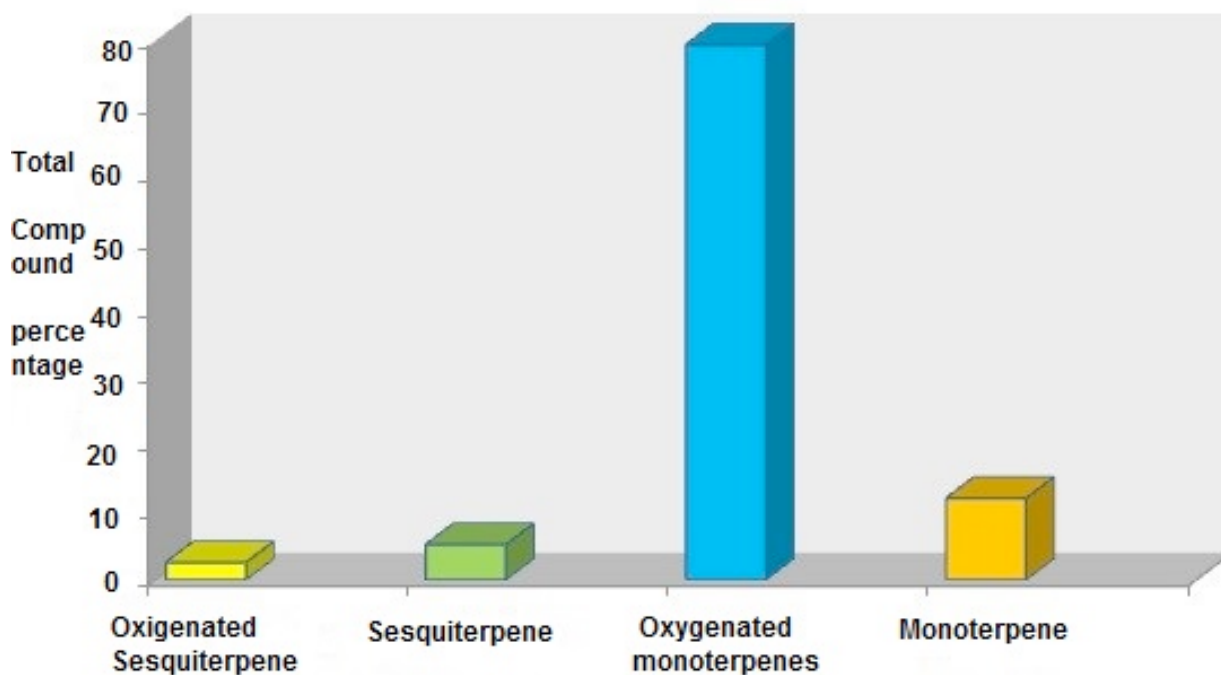


Figure 1. Total compound percentage of compounds in the sample according to each compounds species.

Making control soluble

Control soluble is beta-carotene when no antioxidant is added to it.

Making standard soluble

Vitamin E is used as a standard sample.

Beta-carotene bleaching assay on the essential oil

To carry out the test, we must evaporate chloroform solvent under a vacuum condition, after making beta-carotene soluble. Then we add 100 mg distilled water to it. 4 mg of this soluble is added to a tube test containing 4 mg of essential oil. As soon as this emulsion is created, we must read the absorption in zero moment at 470nm using spectrophotometer. Then we incubate these tube tests for 2 hours at a temperature of 50 °C and assess their absorption in regular intervals.

CONCLUSION

To evaluate the anti-radical and antioxidant effect of the essential oil, the bleaching beta-carotene method was used in this study.

This method is one of the most reliable and widely used methods in measuring herbal essential oil's antioxidant activities in vitro. This method can be considered a simulation of cellular Lipid peroxidation in vitro. According to the obtained results, the essential oil of *Officinalis* has a good protective effect against radicals generated by linoleic acid. In fact, the bleaching beta-carotene method, is administered in a emulsion environment; therefore, antioxidant compound's

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activity depends on their polarity. Since less polar antioxidants are accumulated in the lipid environment generally, show more power to polar compounds and are more effective in protecting lipid compounds against free radicals and active kinds ⁹(Denison E and Ie, 2005). However, it should be noted that essential oils are a mixture of various compounds of various chemical groups, so the antioxidant activity of these compounds is in line with the effects resulting from them (with various mechanisms that can sometimes intensify each other's effects, strengthen or weaken). Also the results of a similar study confirm the officinalis' desired potential of antioxidants (Lakhal and et al, 2013).

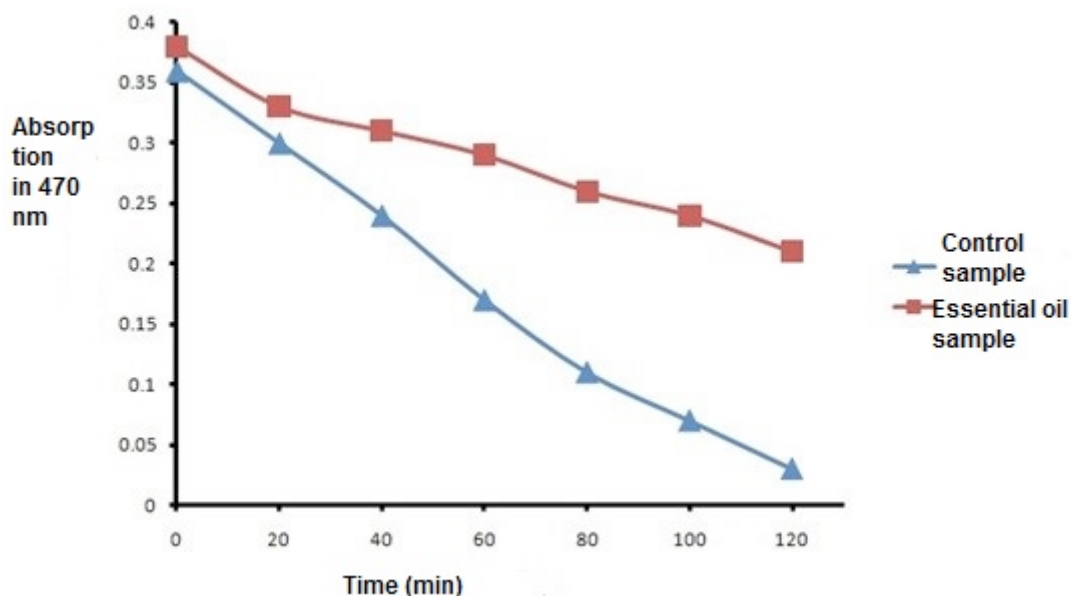


Figure 1. Bleaching beta-carotene chart over time.

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⁹ Denison E and Ie, 2005

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