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Evaluation of phenolic, flavonoid content and antioxidant capacity of the leaves, flowers, seeds and essential oil *of Lavandula officinalis* in comparison with synthetic antioxidants

Shahin Mardani-Nejad*

Department of Science, Mobarakeh Branch, Islamic Azad University, Isfahan, Iran
***Email:** *: shmardaninejad@gmail.com*

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ABSTRACT

Background & Aim: *Lavandula officinalis* is one of great importance due to its aromatic compounds and medicinal uses but not many studies have been done on the antioxidant power of different plant organs.

Experimental: Antioxidant ability of Lavender leaves, flowers, seeds and essential oil based on inhibition of free radicals and nitric oxide, inhibition of linoleic acid peroxidation by ferric thiocyanate and inhibition of malondialdehyde by thiobarbituric acid in compare with synthetic antioxidants Butyl Hydroxy Toluene (BHT) and Butyl Hydroxy Anisole (BHA) were measured.

Results: The phenolic and flavonoid content of the leaf was 96.49±6.35 (µg gallic acid per mg dry weight of the extract) and 39.97±3.36 (µg of catechins per mg dry weight of the extract) respectively, more than other samples. In the study of antioxidant power, plant leaf extract with 48.66±5.5 µg was able to inhibit 50% of DPPH radicals, which had a weaker ability than synthetic antioxidants. The leaf extract of the plant had a higher ability than the synthetic antioxidant BHA to inhibit nitric oxide radicals and its ability was as high as BHT. The ability to inhibit the linoleic acid peroxidation of leaf and flower extracts at the beginning of the functional test showed similar BHT and BHA, at the end, the ability of leaf extract was stronger than BHA and weaker than BHT. The inhibitory potential of malondialdehyde leaf extract (82.66±1.5%) was better than BHA and weaker than BHT. Pearson correlation coefficients between phenolic content and antioxidant capacity of samples were high. Examination of leaf essential oil using GC-MS technique showed the presence of phenolic compounds in the plant.

Recommended applications/industries: Due to the dangers of synthetic antioxidants in the food industry, the results of this study could introduce another application of this plant in terms of strong antioxidant properties.

1. Introduction

Oxidative stresses occur as a result of an imbalance between the free production of free radicals and antioxidant defense mechanisms. Oxidation of living cell membrane lipids is one of the most important goals of free radicals in living organisms. Under these conditions, not only the membrane structure and function are affected, but also some oxidation products such as malondialdehyde can react chemically with biomolecules and have destructive effects on cells and hereditary material. Therefore, the presence of free radicals, especially peroxides, play a key role in the pathogenesis of a number of diseases such as cancer, diabetes, cardiovascular disease and other diseases (Engwa, 2018). In most countries, synthetic antioxidants such as butyl hydroxy anisole (BHT) and butyl hydroxy toluene (BHA) are widely used as food additives to prevent oxidative degradation of these products (Schillaci *et al.*, 2014). Synthetic antioxidants are cost-effective and available and are considered for their stability and high performance. In recent years, the use of synthetic antioxidants as chemical additives has been limited due to their toxicity and potential carcinogenicity (Lourenço *et al.*, 2019).

Among the plant compounds with antioxidants, phenolic compounds are widely distributed in most plants. Phenols are a wide range of plant foods and beverages found in fruits, leaves, roots, seeds, and other parts of the plant (Stoilova *et al.*, 2007; Dai *et al.*, 2010). The antioxidant properties of phenolic compounds are mainly due to their reducing power and chemical structure, which enables them to neutralize free radicals, form complexes with metal ions, and quench singlet and triplet oxygen molecules. Phenolic compounds inhibit lipid oxidation reactions by giving electrons to free radicals and play an important role in food storage and human health (Parcheta *et al.*, 2021). Lavender belongs to the family Lamiaceae and belongs to the genus Lavand. There are more than 30 species of this genus that are distributed in the Mediterranean region, India and southwestern Asia. The species under study is also known as *Lavandula vulgaris* and *Lavandula vera*. Lavere meaning to wash and clean, the reason for this name is that different species of these plants were used in ancient times to clean and perfume baths or in makeup. Shrub-shaped plants, covered with star hairs, have a narrow, long, pointed woody base, the flowers are blue and provide a number of three with relatively long distances at the ends of flowering branches. At the base of each of the flowers of this plant, broad branches are seen (Prusinowska and Smigielski, 2014). The calyx leads to five equal teeth and has 13-15 vein-like lines, usually a tubular calyx ending in two lips, the upper lip into two lobes, and the lower lip into three lobes. It has 4 flags and a pistil has a carpel. Has a tonic effect on the stomach, diaphoretic, bile, windbreaker, energetic, seizure, anti-worm, anti-flatulence, colic, jaundice and liver disorders, nausea, general weakness, coma, epilepsy, treatment of nerve weakness, relief Heart palpitations in patients with neurological disorders, asthma, pertussis, and eliminating vaginal discharge is very helpful. Its essential oil has many medicinal and industrial values, including in perfumery, cosmetics, soap making and natural dye production. This plant is not native to Iran and has probably been imported to Turkey and Palestine to this country (Mardaninezhad *et al.*, 2003).

In the research that was done on plant leaf essential oil, 26 compounds made up 84.5% of plant leaf essential oil. Borneol 23.6%, Cineol 17.6%, Camphor 12.6%, were the most constituent compounds of plant leaf essential oil (Shafaghat *et al.*, 2012). Examination of available sources showed that not many studies have been done on antioxidants in different parts of the plant and the need for antioxidant studies in different parts of the plant seems absolutely necessary.

2. Materials and Methods

2.1. Chemicals

Folin–Ciocalteu, Butyl Hydroxy Toluene (BHT), Butyl Hydroxy Anisole (BHA), Trichloroacetic acid (TCA), Aluminum chloride were obtained from German Merck. Linoleic acid, Sodium nitroprusside, Potassium ferrocyanide, Free radicals (DPPH), Gallic acid were prepared by Sigma chemical company. All other chemicals were of high purity.

2.2. Plant materials and preparation of ethanolic extract

The plant samples were obtained from the Natural Resources Center of Jehad, Isfahan Province in the late summer of 2020 with the completion of vegetative growth and flowering of the plant, while confirming the botany of the studied species, leaves, flowers and seeds of the plant were collected and dried in an oven at 40 °C for 72 hours and stored in a powder mill and in the biology and chemistry laboratory of Mobarakeh Branch Azad University. The extract was extracted from powdered samples in a ratio of 1:1 with 85% ethanol after stirring for 2 hours at 40 °C. After this time, the filtered mixture and the remainder were mixed with the same proportion of solvent mixture. The filtered solutions of both stages were mixed together, concentrated at 40 °C and dried by a desiccator. Extracts were stored at -50 °C until the experiment with the help of facilities of Azad University of Science and Research (Khademi *et al.*, 2015).

2.3. Preparation of plant leaf essential oil

Plant leaf essential oil was performed using Clevenger apparatus. N-pentane was used as a quantitative solvent. Essential oil was extracted for 2 hours each time. The essential oils were collected in dark containers and stored in a cool place.

2.4. Identification of lavender leaf essential oil compounds by Gas chromatography–mass spectrometry (GC-MS)

Plant leaf essential oil was analyzed by Gas chromatography–mass spectrometry (GC-MS) of Isfahan college of Pharmacy. Characteristics and

conditions of gas chromatography-mass spectrometry device (GC-MS) used in the research are listed in Table 1.

Table 1. Specifications and conditions of Gas chromatography–mass spectrometry (GC- MS) device used in the research**.**

GC device model	Varian 3400
Column type	Cpsil 8
Manufacturer	Chrompack
Column length	30 m
Carrier gas	He
The inner diameter of the column	0.25 mm
Layer thickness	0.25 mm
Column tube temperature	50°c
The final temperature of the column	260°c
Temperature program speed	5° c/min
MS device model	Icons 50
Type of analyzer	Quodrupole
Ionization room temperature	150°c
Ionization energy	70 ev
Publication stream	750uA
Type of injection	Splitless
Criminal range	35-400
Speed of spectroscopy	1/Scan/Sec
Injection site temperature	280°c

2.5. Determination of total phenolic compounds (TPC) content

The contents of all phenolic compounds were measured by Folin–Ciocalteu method (Singleton *et al.*, 1999). Thus, 1 ml of ethanol was added to 10 mg of dry extract powder of each of the studied organs or leaf essential oil and mixed well with vortex. Then 200 µl of dilute folin reagent (1:10) was added to 40 µl of this extract and mixed well with a mixter. Then 3120 µl of twice ionized water was added to it and then 600 µl of 7.5% sodium carbonate was added after 5 minutes. After stirring for 15 seconds, the samples were kept in a dark place at 23 ° C for 90 minutes and the absorbance of the samples was measured at 760 nm using a spectrophotometer (Uv/Vis T90 PG).

The standard curve was plotted based on 50 to 1000 µg/ml gallic acid and the amount of plant phenolic compounds equivalent to µg of gallic acid in 1 mg of dry extract was measured:

$$T=(CV)/M$$

In this equation, C is the concentration of gallic acid in micrograms per milliliter according to the standard diagram. V is the volume of the extract in milliliters and M is the weight of the extract in milligrams**.**

2.6. Investigation of flavonoid content

Zhishen *et al*. (1999) method was used to measure flavonoids in samples. In this method, 2 ml of double ionized water was added to 500 µl of ethanolic extracts (10 mg/ml) of the studied organ. Then 150 µl of 5% sodium nitrite was added. 6 minutes later 150 µl of 10% aluminum chloride was added. After 6 minutes, 1 ml of sodium molar hydroxide was added. Finally, the contents of the test tube were reduced to 5 ml. After 15 minutes, the absorbance of the samples was read at 510 nm. A standard curve was drawn and the amount of plant flavonoid compounds equivalent to micrograms of catechins in one mg of dry extract was calculated according to the relation:

T= (C.V)/ M

In this regard, C is the concentration of catechins according to the standard curve in micrograms per milliliter, V is the volume of the extract in milliliters and M is the weight of the extract in milligrams**.**

2.7. Investigation of DPPH radical inhibition activity

The study of free radical scavenging activity (DPPH) was performed according to Brand Williams (1995) method. In this method, concentrations of 6.25-400 µg/ml with a volume of 2 ml of the extract of each of the examined organs and synthetic antioxidants were prepared and poured into a test tube, 2 ml of ethanolic DPPH with a concentration of 100 µM was added to each tube and stirred well for 15 seconds using a vortex, absorbed at a wavelength of 517 nm. The samples were kept in the dark and the last absorbance of each sample was measured in 60 minutes. Positive control of the tube contained free radicals without extract. The opposite equation was used to calculate the percentage of free radical scavenging.

%Inhibition = ((Blank adsorption at 517-Extract adsorption at 517) / Blank adsorption at 517)) × 100

The concept of IC_{50} was used to compare the free radical scavenging activity of plant organ extracts with synthetic antioxidants (BHT and BHA) (Diniz *et al.*, 2021).

2.8. Investigation of nitric oxide radical inhibition activity

Nitric oxide radical scavenging activity was investigated by Garrat (1964) method. Salt phosphate was incubated with 0.5 ml of the studied organ extract

or synthetic antioxidants at a concentration of 25-400 mg/ml for 150 minutes at room temperature. Then 0.5 ml of the reaction mixture was mixed with 1 ml of sulfonic acid (0.33% in 20% glacial acetic acid. After 5 minutes, 1 ml of naphthyl ethylene diamine dihydrochloride (0.1%) It was added and mixed and then placed at room temperature for 30 minutes, a pink dye was dispersed in the solution, the absorbance of the solution was measured at 540 nm against the blank, and the inhibition percentage was as follows: The IC_{50} value of the concentration of the sample was able to inhibit 50% of the nitric oxide radical.

%Inhibition = ((Blank adsorption at 540- Extract adsorption at 540)/Blanc adsorption at 540)) × 100

2.9. Inhibition of linoleic acid peroxidation activity

The effect of ethanolic extracts on anti-lipid peroxidation was determined by ferric thiocyanate (FTC) method (Kim, 2018). 20 mg/ml of plant samples or standard Butyl Hydroxy Toluene and Butyl Hydroxy Anisole were dissolved in 4 ml. 95% ethanol or linoleic acid (2.51%) was mixed in 4.1 ml of 99.5% ethanol, 8 ml of 0.5 M phosphate buffer with 7 acidity and distilled water (8 ml), 3.9 ml of the mixture was removed and stored in airtight containers at 40 °C in the dark, and 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate were added to 0.1 ml of this solution. Exactly 3 minutes after adding 0.1 mM iron chloride in 3.5% hydrochloric acid to the reaction mixture, the adsorption was measured at 500 nm of the red solution every 24 hours until the control adsorption (phosphate buffer with linoleic acid) was maximal. Reached itself (fifth day). During the oxidation of linoleic acid, the formed peroxides oxidized divalent iron to trivalent. The reacted ions formed a red complex with thiocyanate and had the highest absorption at 500 nm. Percentage of inhibition of linoleic acid oxidation was calculated as follows:

% inhibition = [(increase in sample absorption / increase in control absorption) × 100] -100

2.11. Inhibitory activity of malondialdehyde

The effect of ethanolic extracts on the inhibition of malondialdehyde (MDA) was determined by thiobarbituric acid (TBA) method (Kikuzaki *et al.*, 1993). In this method, during the attraction of the control to the maximum based on iron thiocyanate method (after 120 hours), 1 ml of 20% trichloroacetic acid and 2 ml of 0.67% thiobarbituric acid to 2 ml of extract (standard) Was added and boiled for 10 minutes. The samples were cooled and centrifuged at 3000 rpm and their absorbance was measured by spectrophotometer at 532 nm. Malondialdehyde inhibition activity was calculated based on the following equation (A_0 uptake in the absence of ethanolic extract and positive control): %inhibition = $(1\text{-As} / A_0) \times 100$

2.12. Statistical analysis

Data were analyzed using analysis of variance ($P<0.05$) and means were analyzed by Duncan's multiple range tests. Experimental results were reported as mean ±S.E. All measurements were performed in three replications. Statistical analysis was performed by SPSS software.

3. Results and discussion

3.1. Identification of lavender leaf essential oil compounds

Using Mass chromatography (GC-MS) 46 compounds were identified in lavender leaf essential oil, storage time (RT) in the device (Figure 1), 15 important compounds made up 81.8% of plant leaf essential compounds, percentage and name of each of the main compounds are shown in Table 2.

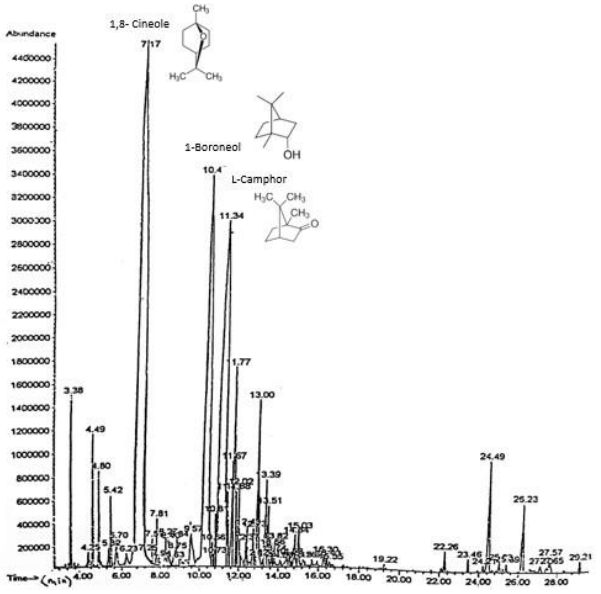

Figure1. Investigation of major chemical constituents of *Lavandula officinalis* leaf essential oil by mass spectrometer (GC-MS)

Cineole with 33.46%, camphor 18.32% and borneol 20.61% were the most important constituents of the plant essential oil. In the research that was done on plant leaf essential oil, 26 compounds made up 84.5% of plant leaf essential oil.

Table 2. Relative percentage of important compounds of lavender lavender leaves essential oil.

No.	Retention Time	Compound	%
1	3.37	Xylene	1.00
2	4.49	Alpha Pinene	0.97
3	4.80	Camphene	0.82
4	5.41	Beta Pinene	1.00
5	7.14	1,8 Cineole	33.46
6	8.84	Linalool	0.97
7	9.58	Cyclohexane	1.08
8	10.46	Camphor	18.32
9	11.32	1-Boroneol	20.61
10	11.43	Terpinene-4-ol	0.86
11	11.77	P-Cymen-8-ol	4.81
12	12.01	Myrtenol	1.27
13	12.99	Terpinene Acetate	2.42
14	13.38	Benzaldehyde	1.09
15	24.48	Caryophyllene oxide	1.59
			81.8

Borneol 23.6%, Cineol 17.6%, Camphor 12.6%, were the most constituent compounds of plant leaf essential oil (Shafaghat *et al.*, 2012). The difference in effective compounds can be related to the different weather conditions of the cultivation areas.

3.2. Total phenol and flavonoid content

The results showed a significant difference between ethanolic extract of leaf and flower and plant essential oil in terms of phenolic content. The highest content of phenolic compounds in plant leaves was measured with 96.49±6.35µg of gallic acid per mg of dry weight of the extract based on the standard curve. In flowers 79.69±2.94µg of gallic acid per mg dry weight of the extract and the lowest values were measured in essential oil of leaf (65.5±2.55) and seed (54.2±4.55), respectively (Figure 2).

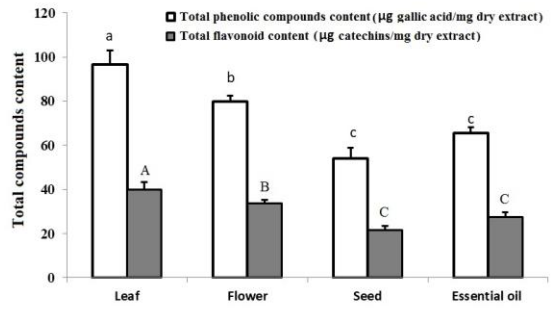

Figure 2. Total phenolic and flavonoid content Lavender (*Lavandula officinalis*) leaves, flowers, seeds etanolic extract and essential oil of leaves. Different letters are significantly different from each other at ($P<0.05$).

The results showed a significant difference between ethanolic extract of leaf and flower with plant essential oil in terms of flavonoid content. The highest content of total flavonoid content in plant leaves with 39.97±3.36 equivalent of µg of catechin in mg of dry weight of the extract and the lowest amount in essential oil (27.53±2.02) and seed etanolic extract (21.65±1.68) were measured (Figure 2).

Phenolic compounds have shown extensive antioxidant activity in plant-derived foods (Shahidi *et al.*, 2015). Studies have shown that increasing levels of phenolic compounds in the diet can lead to diseases significantly reduce (Lourenço *et al.*, 2019). Phenolic content according to Folin–Ciocalteu method and flavonoid content according to aluminum III calorimetric method showed that although the leaves, flowers, seeds and leaf essential oil of the plant have high phenol and flavonoid content, the phenolic and flavonoid content of plant leaves was higher than other organs. Flavonoids have been shown to have significant antioxidant activity extensively in plant-based food products (Rodriguez-Garciaet *et al.*, 2019). Studies have shown that increasing flavonoid levels in the diet can cause a significant reduction in disease (Ahn-Jarvis and Parihar, 2019). In a study, the content of phenolic compounds was reported to be 85.38 µg gallic acid in the dry weight of aerial parts of plant (Toloeallah *et al.*, 1997), This study showed high levels of phenolic compounds in the plant. The studies performed in this study also pointed to high phenolic compounds in plant organs, especially in leaves (96.49 equivalent µg gallic acid in the dry weight) and anti-radical and inhibitory function of lipid peroxidation in plant organ extracts. In a study on the content of phenolic compounds in leaves, stems and flowers of *Lavandoula x intermedia* species, the content of phenolic compounds in leaves was reported more than other mentioned organs (Blazekovic *et al.*, 2010) which was consistent with the results of this study.

3.3. Inhibition of DPPH and nitric oxide free radicals

In controlling 50% of free radicals (IC_{50}), the performance of synthetic antioxidants was better than ethanolic extracts and plant essential oil, however, the performance of extracts, especially leaves, was very close to synthetic antioxidants. The leaf extract of the

plant with 48.66±5.5 µg neutralized 50% of free radicals (Table 3). The leaf extract of the plant showed a stronger performance of the synthetic antioxidant BHA in inhibiting nitric oxide radicals and its ability to neutralize nitric oxide radicals at BHT level was recorded. Ethanolic extract of plant seeds had the least effect on neutralizing nitric oxide radicals (Table 3).

Table 3. 50% inhibition concentration of DPPH and nitric oxide free radicals. Data show means ± S.E., n=3

Sample	DPPH˙ inhibition (IC_{50})		•NO inhibition (IC_{50})	
Leaf	48.66±5.50	b	161.33±13.86	a
Flower	82.33±6.42	c	243.65±6.58	c
Seed	129.33±16.92	d	330.30±9.60	d
Leaf Essential Oil	82.33±3.61	c	261.00±8.08	c
BHT	23.66±3.51	a	156.00±4.58	a
BHA	13.16±1.25	a	193.33±8.62	b

*The letters a, b, etc. indicate a significant difference (at the 95% confidence level) statistically is between samples.

The DPPH radical inhibition model is one of the most widely used methods to evaluate the inhibitory potency of samples (Elmastas *et al.*, 2007). DPPH has a stable nitrogenous center, which results in the DPPH dissolving in ethanol to form a dark purple color that changes to yellow during the hydrogenation or electronization process. Compounds capable of this reaction can be considered antioxidants and act as radical scavengers DPPH radicals react with the antioxidants in the extracts to form a stable DPPHH As a result, its color changes from dark purple to light yellow and the amount of absorption is reduced (Akar *et al.*, 2017).

The results of this experiment showed that ethanolic leaf extract at a concentration of 48.66±5.5, flower extract at 82.33±6.42, essential oil leaves at 101.33±3.51 and seed extract at a concentration of 129.33±16.92 µg per milliliter inhibited half of the radicals. An index called IC_{50} was used in the studies, which indicates the ability of the extract to remove 50% of environmental radicals. This index is inversely related to the anti-radical activity of compounds. The lower the number of this index, the higher the anti-radical activity. As the results showed, this index was lower in the leaf than other organs, indicating a higher ability of the organ to inhibit free radicals. In the existing sources, research in this field was not observed on the vegetative or reproductive organs of the plant, but anti-radical studies of the essential oil of the plant 56 µg per milliliter were mentioned (Ghadri, 2010).

Analysis of essential oil of the plant showed that the phenolic and flavonoid content of the plant is high. High correlation between phenolic content and free radical scavenging has been reported in a number of studies (Khademi *et al.*, 2015; Mardani-Nejad *et al.*, 2016; Lourenço *et al.*, 2019). A high negative correlation of 0.937 between phenolic contents and IC_{50} index in inhibiting DPPH radicals indicated the relationship between the presence of phenolic compounds and this ability in plants. In a study on the ability to inhibit the free radicals of leaves, stems and flowers of *Lavandoula x intermedia* species, they reported that the inhibitory ability of leaf extract was stronger than other organs, which could be due to the high phenolic contents of this organ (Blazekovic *et al.*, 2010).

Sodium nitroprusside is used to inhibit nitric oxide radicals. It produces nitric oxide in aqueous solution and physiological acidity, which is measured by a grease reagent. Nitric oxide inhibitors compete with oxygen to reduce nitric oxide production, so a substance that reduces *nitric* oxide production. Can be considered as an antioxidant and scavenger of this radical (Akbarpour *et al.*, 2013), which is associated with a decrease in pink color and consequently a decrease in absorption compared to the control group. Ethanolic extract of leaves with a concentration of 161.33±13.86, flower extract with 243.66±6.65, seeds extract with a concentration of 330.33±9.6 µg per ml inhibited half of the nitric oxide radicals.

In this study, an index called IC_{50} was used in the studies, which indicates the ability of the extract to remove 50% of nitrous oxide radicals in the environment. This index is inversely related to the anti-radical activity of *compounds*. The lower the number of this index, the higher the anti-radical activity. As can be seen, the leaf extract had the lowest number among the extracts of other plants and was better in inhibiting nitric oxide radicals. The phenolic content of plant vegetative and reproductive organs can be related to the ability to inhibit nitric oxide radicals. Numerous studies have pointed to this correlation (Mardani-Nejad, 2016; Ahn *et al.*, 2019). A high negative correlation of 0.970 between phenolic contents and IC_{50} index in inhibiting nitric oxide radicals indicated the relationship between the presence of phenolic compounds and this ability in the plant. The leaf extract of the plant showed a stronger performance of the synthetic antioxidant BHA in inhibiting nitric oxide radicals and its ability to

neutralize nitric oxide radicals was similar BHT. In this regard, the inhibitory power of leaf extract was very high and it can be concluded that these extracts with the help of electron donation cause chain reactions. In addition to active oxygen, nitric oxide is also involved in other pathological conditions such as inflammation and cancer. Plants or plant products that can prevent the formation of nitric oxide can play a role in in addition, the trapping activity of this compound can be used to stop the chain reactions caused by the overproduction of nitric oxide in the human health system (Pacher *et al.*, 2007).

3.4. Inhibition of linolenic acid peroxidation

Inhibition of linoleic acid peroxidation in ethanolic extracts, plant leaf essential oil and synthetic antioxidants decreased over time. At 24, 48 and 72 hours after the experiment, no significant difference was observed in the amount of inhibition of linolenic acid peroxidation between leaf and flower extracts with synthetic antioxidants. AT 96 and 120 hours after the experiment leaf extract showed stronger antioxidant power than BHA and weaker than BHT in inhibiting linolenic acid oxidation.The essential oil and seed etanolic extract were significantly weaker than other plant extracts studied (Figure 3).

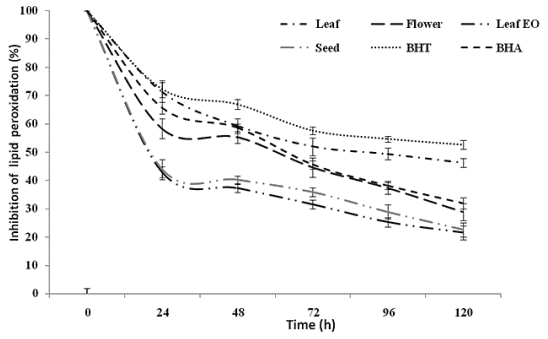

Figure 3. Inhibition of linolenic acid peroxidation by ethanolic extracts Lavender (*Lavandula officinalis*) leaves, flowers, seeds and leaves essential oil in comparison with synthetic antioxidants by ferric thiocyanate method.

One of the most important roles of antioxidants in inhibiting the chain reaction is the oxidation of fatty acids by free radical scavenging (Santos-Sánchez *et al.*, 2019). The high correlation between free radical scavenging and oxidation of linoleic acid by plant leaves indicates the high antioxidant potential of this organ. Membrane lipids are rich in unsaturated fatty acids, making them susceptible to oxidative processes. Arachidonic acid and linoleic acid are good examples for the study of lipid oxidation (Ayala *et al.*, 2014). Inhibition of fatty acid oxidation may be due to the activity of trapping free radicals. The superoxide ion indirectly begins to oxidize fatty acids and lipids because the superoxide anion acts as a radical precursor to oxygen and hydroxyl (Ordonez *et al.*, 2006).

Hydroxyl radicals reduce hydrogen atoms in the cell membrane. These atoms can lead to lipid peroxidation. Ferric thiocyanate method determines the degree of oxidation of fatty acids in the initial stage (Kim, 2018). In this experiment, divalent iron with lipid peroxide radicals is converted to trivalent iron, which reacts chemically with ammonium thiocyanate to produce ferro cyanate, a red complex. Substances that neutralize peroxide radicals ultimately reduce the production of color complexes that can be considered antioxidants. The used extracts showed less absorption than the control group due to the presence of antioxidant compounds and the inhibitory property of the extracts decreased over time due to the accumulation of radicals. The model of inhibition of linoleic acid oxidation by ethanolic extract and essential oil of the leaf plant was in accordance with BHT and BHA.

3.5. Inhibition of malone dialdehyde

In the study of malondialdehyde inhibition test by plant extracts and essential oil, the results showed that the leaf extract had a higher antioxidant ability to inhibit malondialdehyde than the synthetic antioxidant BHA and similar performance to synthetic antioxidant BHT. Essential oil and seed etanolic extract showed a similar function to inhibit malondialdehyde (Figure 4).

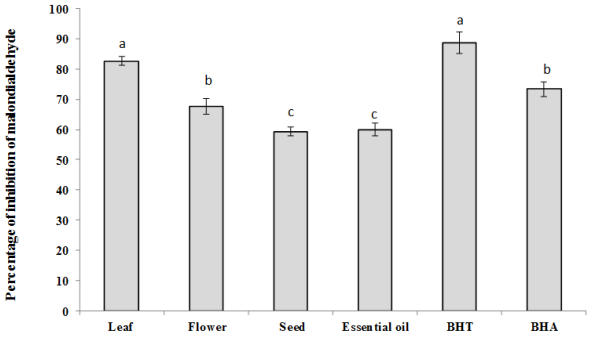

Figure 4. Percentage of inhibition of malondialdehyde ethanolic extracts leaves, flowers, seeds and leaves essential oil of Lavender (*Lavandula officinalis*) in comparison with synthetic antioxidants by

thiobarbituric acid method. Different letters are significantly different from each other at ($P<0.05$).

Thiobarbituric acid method is one of the most widely used methods for determining lipid oxidation in foods. Malondialdehyde is produced during lipid oxidation (Grotto *et al.*, 2009). It can react chemically with biomolecules and exhibit cytotoxic and genotoxic effects. Therefore, the presence of more free radicals, especially peroxides, plays a key role in the pathogenesis of various diseases such as diabetes, cardiovascular disease, cancer, aging and various other diseases (Mehdinezhad and Mohamadi, 2018). This method makes malondialdehyde pink complex with thiobarbituric acid and has a maximum absorption in the range of 535-530 nm. Substances that are able to react chemically with malondialdehyde do not allow it to react chemically with thio barbituric acid and are considered antioxidants. Therefore, the compounds in the extracts prevent the formation of a pink complex due to malondialdehyde. When adsorption control is maximized, malondialdehyde is converted to acid and alcohol, so absorption is not recorded during spectrophotometry (Nagababu *et al.*, 2010). The ability to inhibit the linoleic acid oxidation of leaf and flower extract at the beginning of the functional test showed similar BHT and BHA and at the end of the functional test leaf extract had the same BHT and stronger than BHA. The inhibitory potential of malondialdehyde in leaf extract was better than BHA but weaker than BHT. Leaf malondialdehyde was reported to be more than flowers and stems (Blazekovic *et al.*, 2010), which was consistent with the results of this study. In this ability, leaf extract also showed better performance than flowers and seeds. Based on ferric thiocyanate and thiobarbituric acid methods, a high correlation was observed between inhibition of linoleic acid oxidation and malondialdehyde with phenolic content of organs with coefficients of 0.865 and 0.882, respectively.

4. Conclusion

In this study, a high correlation between total phenolic content and antioxidant capacity of ethanolic extracts of plant organs base on the methods used was evident. The vegetative, reproductive organs and essential oil of *Lavandula officinalis*, especially the leaves of the plant, have a high phenolic and flavonoid content and have a high ability to inhibit free radicals and linoleic acid peroxidation as a fatty acid and a standard for measuring lipid oxidation. Therefore, in addition to the very important applications of the medicinal compounds of this plant, the extraction of strong antioxidant compounds of the plant can be very useful in the food and pharmaceutical industries and can be used as an alternative to synthetic antioxidants. The main use of the plant is flowering and flowering branches and there are not many reports about the use of plant leaves for antioxidant properties and alternatives to food preservatives in the sources. The results of this study can introduce other applications for this important medicinal plant.

5. References

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