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COMPARISON OF ANTI-INFLAMMATORY AND ANTIOXIDANT CAPACITY OF ALCHOLIC EXTRACTION OF FRAXINUS FXCELSIOR AND MELILOTUS OFFICINALIS PLANT

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Abstract

Antioxidants are protecting the body against oxidative stress ca used by free radicals. Long-term use of anti-inflammatory drugs causes adverse effects such as stomach ulcers, intestinal ulcers and anemia in finally. Plants are a valuable compound that can be used in the treatment of many diseases. The global purpose of this is investigating the antioxidant and anti-inflammatory activity of Melilotus officinalis and Fraxinuse × celsior which are commonly used in traditional medicine. In this study, the anti-inflammatory activity investigated through inhibition bovine albumin protein denaturation and the antioxidant activity was investigated by applying cupric ion reducing assay (cuprac assay). The results showed both plants have the antioxidant and anti-inflammatory effect. The highest amount of phenolic compound per gram of dry extract of Fraxinus excelsior was 0.04 mg and also this plant has the highest antioxidant effect (0.714± 0.045). The highest amount of flavonoid compounds in Melilotus officinalis was 9.23 mg and this plant has the highest anti- inflammatory effect (0.21 ± 0.033). There was directly relation between the antioxidant and anti-inflammatory properties, and phenolic and flavonoid content of plant extracts.

Key words: phenolic compounds, Fraxinus Excelsior, flavonoids, antioxidant, anti-inflammatory, Melilotus Officinalis.

Introduction

The history of treating diseases with medicinal herbs dates back to the history of human life on the planet. According to the judgment of experience, science, and according to the requirements of the time, many medicinal herbs are being treated with medicinal herbs. The general tendency of the community to use herbal medicines and treatments, and in general, natural products, especially during the good years, has been rising and the most important of which are the evidence of the adverse effects of chemical drugs on one, on the other hand, the creation of environmental contaminants that threaten the planet (Cowan, 1999). In addition, according to the World Health Organization, about 80% of the world's population lives in developing and poor countries, due to the high cost of synthetic drugs, the inaccessibility and side effects of these drugs, their major therapeutic needs. They supply medicinal herbs. In recent years, these factors have led to extensive research on the specific species of these plants that have a beneficial effect on many human diseases. Currently, 25% of the drugs in the world drug market are herbal sources (Magaji *et al.*, 2008, Huang *et al.*, 2009). One of the most recognized causes of many diseases (arthrosclerosis, aging, cancer, Alzheimer's and Parkinson's disease) is oxidative stress caused by free radicals. The most important degradation effects of free Radicals are the onset of lipid peroxidation, which leads to the destruction of cell membranes (Asghari *et al.*, 2011), It also damages other biological molecules, such as proteins and nucleic acids, and leads to mutations in the genes. The most important defenses against free radicals are antioxidants.

The imbalance between the production of free radicals and antioxidants will lead to free radicals attacking biological molecules. The physiological role of antioxidants in the collection of free radicals. These compounds combine free radicals, especially superoxide and hydroxyl anions (Koksal *et al.*, 2008, Zihreh *et al.*, 2015). Plants contain valuable compounds that, in addition to increasing

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the quality and nutritional value of food, are also used in other ways, such as beverages, dyes, cosmetics, pharmaceuticals and therapies. Some plants contain a significant amount of natural antioxidants. Following their use, it was observed that the antioxidant capacity of the plasma significantly increased (Kahkonen *et al.*, 1999). Antioxidants are in both synthetic and natural forms. Scientists and nutritionists have always sought to find natural compounds with anti-oxidant properties to reduce the effects of free radicals on the body. Anti-oxidants do not have side effects of synthetic antioxidants. On the other hand, some plants also have anti-inflammatory agents that can be used to treat various types of inflammation, skin infections, rheumatoid arthritis, fever and infections (Anilkumar *et al.*, 2010, Homayoni *et al.*, 2015). Inflammatory diseases typically include osteoarthritis, lupus erythematosus, asthma, and rheumatoid anomalies, arthritis, and rheumatoid arthritis. Long-term use of drugs used to suppress inflammatory reactions can lead to complications, including gastric ulcer and subsequently anemia (Palasuwan *et al.*, 2006). Due to the side effects of these drugs, the tendency to identify plant compounds with anti-inflammatory properties has increased. The use of Melilotus officinalis plants and Fraxinus excelsior is common in traditional Iranian medicine for various reasons. The purpose of this study was to evaluate the comparative and screening of antioxidant potency and anti-inflammatory potency of the alcoholic extract of these two herbs.

Materials and method

Preparation and extraction of plants

Melilotus officinalis plants and Fraxinus excelsior were collected from local stores and then were identified by the Ferdowsi University of Medicinal Plants Research Institute and the Herbarium Code for Plant Melilotus officinalis (13133) and for Fraxinus excelsior (13232). Then the available contaminants were separated, the plants were dried and completely milled and powdered. Then, to 50 g of powdered plants, 96% pure ethanol solvent was added to a ratio of 1 to 5 and placed on a shaker for 24 hours at room temperature. After this time, the extracts were concentrated by vacuum distillation apparatus. The dried extracts were kept at 4^0C for the duration of the experiment (Ramman *et al.*, 2006, Adedapo *et al.*, 2001, Taylor *et al.* 1996).

Determine antioxidant activity

To measure the antioxidant capacity of the extracts, we used cuprac assay (Apak *et al.*, 2008). For this purpose, a solution of 1×10^{-2} molar copper chloride, a solution of 1 molar ammonium acetate with pH = 7 and a solution of 7.5×10^{-3} molar neocuproine (2 and 9 dimethyl-1 and 10 phenanthroline) was prepared. In each test tube, they mixed the equal ratios of the solutions prepared with the plant extract and the test tubes were kept at room temperature for 30 minutes. Absorption of solutions at 450 nm wavelength was measured by spectrophotometer. An extract-free test tube was used as control [16]. The experiments were performed in three replicates and the mean was reported.

Evaluation of anti-inflammatory activity

This test was performed based on the method of inhibiting protein transformation (Hayouni *et al.*, 2007). According to this method, a 1% solution of cow albumin was prepared and equal amounts of plant extract and cow albumin were added to the test tube and the pH of the solution was controlled by hydrochloric acid 1N. The specimens were incubated for 20 minutes at 37^0C, and then the specimens were placed in an incubator for thermal shock for 20 minutes at 51^0C. The absorbance of the solutions was measured at 660 nm in comparison with the control solution. The control solution is the cow albumin (Sakat *et al.*, 2010).

Determine the amount of phenolic compounds

To measure the phenolic compounds, the Folin - Ciocalteu reagent was used (Hayouni *et al.*, 2007). According to this method, 100 µl of the extract was added at a concentration of 1 mg / ml to 500 µL of the folin reagent, and after 1 minute, 1.5 ml of 20%, sodium bicarbonate 20%, was added to each tube and then The samples were placed at room temperature for 120 minutes. The absorbance of the samples was read at 760 nm by a spectrophotometer. The standard curve was prepared by solutions of 50 to 500 mg/L of Gallic acid in ethanol (fig. 1). The total phenol content was equally expressed in Gallic acid equivalent (mg Gallic acid/g of extract weight), which is a reference compound for determining the phenolic content (Hayouni *et al.*, 2007).

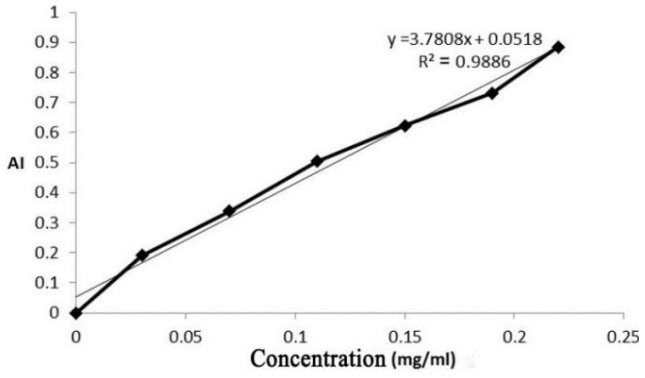

Fig 1: Gallic acid standard curve graph by Folin -Ciocalteu method

Determine the amount of anthocyanin compounds

To measure the amount of anthocyanins, the 0.02 g of dry plant tissue with 4 ml of HCl solution containing % 1 ethanol was pulverized in a mortar Chinese. The solution was stored in a refrigerator for 24 hours. The solution was then centrifuged for 10 minutes at 13000 rpm. The supernatant liquid phase was removed and absorption of solutions was read on wavelengths of 530 and 657 nm. The control solution was Hydrochloric acid (1% ethanol) as control (Mita *et al.*, 1997). The anthocyanin level for each extract was calculated using the equation 1. (Mita *et al.*, 1997).

Eq. (1):$A= A_{530}-(0.25\times A_{657})$

A: Absorption of the solution (the index numbers represent the wavelengths in which absorption is measured).

Determine the amount of flavonoids

The amount of flavonoids was measured using an aluminum chloride photometry method (Chang *et al.*, 2002). According to this method, 0.5 milliliters of each extract with 1.5 milliliters of ethanol and 0.1 milliliters of aluminum chloride 10% and 0.1 milliliters of 1 molar potassium acetate and 2.8 milliliters of the distilled water was mixed and stored at room temperature for 30 minutes, Absorption at 415 nm was read by a spectrophotometer. Different concentrations of quercetin 12-100 μg/ml in the ethanol were used for the standard curve. The flavonoid content was expressed in the form Eq quercetin / gram of dried extract dry weight (fig. 2).

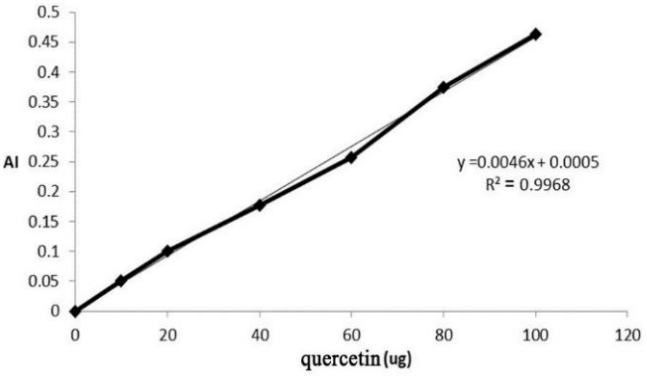

Fig 2: Quercetin standard curve graph by chlorometric method of aluminum chloride

Method of statistical calculation

Statistical tests were performed using SPSS (version 22) and Microsoft Office Excel (2007) software. All the data in the present study were expressed as mean ± standard deviation (Mean ± Sem). $P<0.05$ was considered as a significant difference.

Results

Determination of phenolic, flavonoid and anthocyanin compounds

The results of the experiments on phenolic and flavonoid compounds confirmed the presence of high concentrations of these compounds in the ethanolic extract of Melilotus officinalis and Fraxinus excelsior plants (table 1).

Table 1: The amount of phenolic and flavonoids in the ethanolic extract of the Melilotus officinalis and the Fraxinus excelsior.

Extract	Phenolic compounds (mg/gr)	Flavonoid compounds (mg/gr)
Ethanol Melilotus officninalis	0.029	9.239
Ethanol Fraxinus excelsior	0.047	0.97

The highest amount of phenolic compounds in the Fraxinus excelsior plant were 0.047 mg / g and the highest levels of flavonoids in the fungus Melilotus officninalis were 9.239 mg/g.

The amount of anthocyanin compounds were determined according to equation 1 for ethanol extract of Melilotus officninalis 7.8 mg/grand for the ethanol extract of Fraxinus excelsior 21.05 mg/gr.

Antioxidant activity: Antioxidant capacity Melilotus officninalis and Fraxinus excelsior plants are presented in table 2. In thistest, Measured Regenerative ability of cuprous ions (Cu^{1+}) from Cupric ions (Cu^{2+}) in the presence of copper Reagent.

This is a practical, fast, selective, and suitable method for many antioxidants, regardless of their chemical composition.

In this way, the antioxidants of the thiol group, such as glutathione and non-protein thiols, can be measured (Koksal *et al.*, 2008). This method is applicable to all

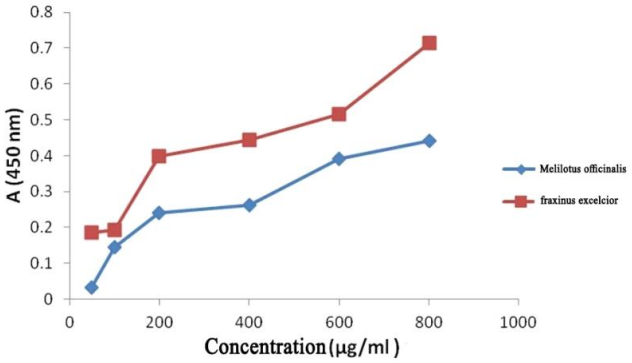

Fig 3: Concentration-based adsorption curve for the effect of the Melilotus officinalis plant and the Fraxinus excelsior.

lipophilic and hydrophilic antioxidants and can be used for all biological samples (Apak *et al.*, 2002).

Table 2 : The antioxidant activity of ethanolic extract of Melilotus officninalis and Fraxinus excelsior.

Tube No.	Extract solution µg/ml	Antioxidant activity of Melilotusofficinalis plant nm(SEM ± Avg)	Antioxidant activity of Fraxinus excelsior nm (SEM ± Avg)
1	50	0.032±0.016	0.185±0.03
2	100	0.146±0.013	0.192±0.03
3	200	0.241±0.021	0.398±0.024
4	400	0.263±0.027	0.445±0.017
5	600	0.392±0.039	0.516±0.022
6	800	0.442±0.025	0.714±0.045

The level of antioxidant activity of the extract of plants was measured at concentrations of 50 to 800 µg. In this study, based on the results of table 2, it was found that both Melilotus officinalis and Fraxinus excelsior had antioxidant activity and also the comparison showed that the highest antioxidant effect was on the Fraxinus excelsior plant at 800 µg is 0.714 ± 0.045 nm, and antioxidant effect of Melilotus officinalis plant is lower than the Fraxinus excelsior plant (fig. 3). There was also a direct relationship between increasing the concentration and increasing the antioxidant effect (Ga *et al.*, 2011). Particularly, there was a significant difference between Melilotus officinalis extract and Fraxinus excelsior ($p< 0.05$).

Determination of anti-inflammatory activity

Anti-inflammatory activity of the extract of plants was measured at concentrations of 50 to 800 µg. In this test, the ability of plants is measured in preventing deformation of protein.

Based on the results of table 3, both plants showed anti-inflammatory and anti-oxidant effects and increased the concentration of adsorption (figure 4). At a

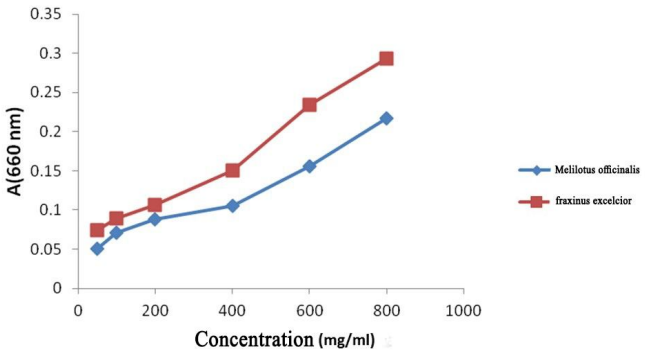

Fig. 4: Absorption curve based on concentration for anti-inflammatory effect of the Melilotus officinalis and Fraxinus excelsior.

Table 3: Anti-inflammatory activity of the ethanolic extract of the Melilotus officinalis and Fraxinus excelsior.

Tube No.	Extract solution µg/ml	Anti-inflammatory activity of Melilotusofficinalis plant nm(SEM ± Avg)	Anti-in flammatory activity of Fraxinus excelsior nm (SEM ± Avg)
1	50	0.051±0.010	0.074±0.006
2	100	0.071±0.008	0.089±0.010
3	200	0.088±0.001	0.106±0.011
4	400	0.105±0.012	0.150±0.020
5	600	0.153±0.023	0.234±0.010
6	800	0.217±0.033	0.293±0.025

concentration of 800 µg, the absorbance of the Melilotus officinalis was 0.217± 0.033 nm and for the Fraxinus excelsior plant was 0.293 ± 0.225 nm. In this method, the lower the absorption rate, the lower the turbidity and increase the plant's ability to prevent deformation of protein and increase the anti-inflammatory capacity, so the Melilotus officinalis plant at a concentration of 800 micrograms has a more anti-inflammatory effect than the Fraxinus excelsior plant (Pesca-manca *et al.*, 2002).

Conclusion

In this study, the antioxidant and anti-inflammatory potency of the alcoholic extract of the two Melilotus officinalis plants and Fraxinus excelsior, which are commonly used in traditional medicine, have been evaluated. Based on the results of table 2, it was found that both Melilotus officinalis and Fraxinus excelsior have antioxidant activity and also, according to table 2, the highest antioxidant effect of Fraxinus excelsior was found at 800 µg concentration and Is equal to 0/714 ± 0/045 and the antioxidant effect of Fraxinus excelsior plant is more than the Melilotus officinalis plant, in particular, there was a significant difference between extract of Melilotus officinalis and Fraxinus excelsior ($p <0.05$), and there was a direct correlation between increase Concentration and increased antioxidant activity (Ga *et al.*, 2011). In a study by Braga *et al.*, On a Melilotus officinalis plant with radical activity inhibition, a chemical strain on human neutrophil fragmentation and a lipoparoxidase method was determined that this plant has an antioxidant effect and is effective in eliminating oxidative stress (Braga *et al.*, 2013) Also, in the phytochemical study of this plant, using the two-dimensional thin-layer chromatography (Karzakowa *et al.*, 2010). The presence of phenolic compounds was confirmed in this plant (Krzakowa *et al.*, 2010). In the study by Middleton *et al.*, The effect of the antioxidant on the Fraxinus excelsior plant was compared with the black anemone and black alder plants, and the results indicated that the effect of the antioxidant on the Fraxinus excelsior was more than the other two

(Middletona *et al.*, 2010) and in A phytochemical study of this plant was carried out by Baie (Baie *et al.*, 2009). A phenolic compound and nine glycosylated compounds were confirmed in this plant (Baie *et al.*, 2009), all of which can be corroborated by the results of this study. Phenolic compounds in the Fraxinus excelsior plant (0.047), which are listed in table 1, Probably phenolic compounds can be responsible for the antioxidant activity of extracts extracted from two Melilotus officinalis plants and Fraxinus excelsior. It seems that the difference in the antioxidant power of phenolic compounds of different plants is due to the difference in their chemical structure (Waterman *et al.*, 1994). This result is due to The results of other studies are consistent with the direct relationship between high antioxidant activity and phenolic content of rosemary extract (Elmasta *et al.*, 1994). In this study, the anti-inflammatory effect of the Melilotus officinalis plant and the Fraxinus excelsior plant was evaluated using a test for the prevention of proteinalbum in deformaion, and the anti-inflammatory capacity of the plant was measured to prevent the transformation of the second and third building of the protein, which is no matter how much Lower absorption indicates a decrease in turbidity and increased anti-inflammatory capacity of the plant. According to the results of table 3, it was determined that both plants have anti-inflammatory effects and have the most anti-inflammatory effect according to the results of table 3 of the Melilotus officinalis plant at 800 µg and 0.217 ± 0.033 and there is a direct relationship between increased concentration and anti-inflammatory effect. In the studies conducted by Monika and colleagues, the anti-inflammatory effect of the Melilotus officinalis was confirmed (Pesca-manca *et al.*, 2002). In a phytochemical study of the Melilotus officinalis plant by Boni, it was announced that the plant, when it is dry, has a bitter taste and odor of alfalfa due to the presence of coumarin. Its components are melilotine and glycosides of coumarin and essence and flavonoid pigments (Bunney *et al.*, 1992). In another study, the anti-inflammatory effect of Fraxinus excelsior was combined with two spruce and chicory herbs. The results indicated that this The drug has anti-inflammatory effects (Bonaterra *et al.*, 2007). In the phytochemical study of Fraxinus excelsior, various classes of compounds including benzoquinone, coumarin, flavonoids, phenylethanoyde, and indole derivatives and some phenolic compounds have been reported (DNP, 2001). The results can be confirmed by the results of the study. Due to the higher levels of flavonoid compounds in the Melilotus officinalis plant as listed in table 1, it may be possible that the anti-inflammatory effect of both Melilotus officinalis and Fraxinus excelsior can be attributed to the presence of flavonoid compounds Attributed.

According to this study, it was found that both plants have anti-oxidant and anti-inflammatory properties and there are phenolic and flavonoids in both plants, but considering that the amount of phenolic compounds in the Fraxinus excelsior plant has an antioxidant effect and also due to the higher levels of flavonoids in the Melilotus officinalis that have more anti-inflammatory properties, it may be possible that the presence of higher anti-inflammatory properties in the Melilotus officinalis plant may be attributed to the presence of more flavonoid compounds in this plant, as well as the presence of anti-oxidant properties Higher in the herb of the Fraxinus excelsior to a greater extent Connected phenolic compounds in this plant. Nevertheless, further studies are needed to achieve a definitive outcome. In this study, the combined effects of anti-oxidant and anti-inflammatory properties in the Melilotus officinalis and Fraxinus excelsior were observed. Due to the long-term use of these plants in traditional medicine, they can be used in the treatment of diseases whose pathogens Based on inflammation and free radicals. Anti-inflammatory compounds have high ability in the treatment of various diseases, including cancers, Neurological abnormalities and Slow down the aging process (Lealaprakash *et al.*, 2010). In general, the use of screening methods, such as the copper ion resuscitation test and the prevention of the deformation of protein albumin in bovine animals, will provide an opportunity to find plants with new active compounds and to select them for advanced and advanced testing. Considering the higher antioxidant effect in the Fraxinus excelsior plant and the high anti-inflammatory properties of the Melilotus officinalis and the effectiveness of anti-oxidant and anti-inflammatory agents in the process of inflammatory diseases, the study of the combined properties of these two plants as a valuable topic is recommended for further research.

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