

The Study of antioxidant property of metanolic extracts of *Melissa officinalis* L. and *Salvia officinalis* L. on stability of soybean oil

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Received: 28 August 2014; Accepted: 09 September 2014

Abstract

One of the major changes that occur during food processing and storage is oxidation of oils and fats. PG, TBHQ, BHT and BHA are synthetic antioxidant that main synthetic antioxidants that use in industry. Today it became clear that the undesirable effects of synthetic antioxidants. Therefore, the search for a replacement synthetic antioxidants has led to reviews of various antioxidant herbal sources is effortless. In this study, phenolic compound, rosmarinic acid and their antioxidant activity by DPPH method was measured. Finally, their antioxidant effect studied on stability of soybean oil with Ransimat device. The results showed that extracts was rich of phenolic compound and rosmarinic acid and showed that the extracts could be caused stability of soybean oil and Can be replaced for synthetic antioxidant.

Keywords: Oxidative Stability, *Melissa officinalis* L., *Salvia officinalis* L., rosmarinic acid.

1. Introduction

One of the major changes that occur during food processing and storage is oxidation of oils and fats. This is in addition to the loss of food quality, produces free radicals that are undesirable chemical reactions leads. The most suitable way to prevent oxidation of lipids, or protection against damage caused by free radicals, the use of antioxidants. Antioxidants are compounds that lipid oxidation to delay or even stop it. Antioxidants into two categories: natural and synthetic antioxidants are divided. PG (propyl gallate), TBHQ (tertiary butyl hydro quinon), BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluen) are main synthetic antioxidants that use in industry [1]. Today it became clear that the undesirable effects of synthetic antioxidants such as carcinogenesis and toxicity are [2]. Therefore, the search for a replacement synthetic

antioxidants has led to reviews of various antioxidant herbal sources is effortless. The use of natural antioxidants in the diet reduces the risk of lipid oxidative and enhance the quality and value of the food is a good alternative to synthetic antioxidant is (1 and 3). Also, add natural antioxidants are beneficial effects on human health because it is an important cellular constituents such as DNA, proteins and lipids; sheet from the dangers of oxidative damage of oxygen free radicals maintain [4]. Herbs rich in phenolic compounds with high antioxidant potential, in a variety of foods such as fish, meat, and oils to the process [5,6,7].

Melissa officinalis L. to be used as a spice in food, as well as a medicinal herb to treat headaches, gastrointestinal diseases and rheumatism goes to work [8]. This herb is rich in antioxidant compound such as rosmarinic acid, caffeic acid and terpenes, and has high antioxidant activity. The extract of this plant

can causing of stability of sunflower oil and in concentration of 200 ppm similarity to 600 and 1000ppm of BHT prevention lipid oxidation [5].

Salvia officinalis L. is a medicinal plant and is rich in phenolic compounds and antioxidant activity is high. The dried leaves of *Salvia* as a spice in food are used. This plant is used to give a flovera and aroma in food. Extracts of this plant in the recovery of memory and Alzheimer's treatment is effective. In addition, the extract showed estrogenic and sedative effects, which are also relevant to the treatment of Alzheimer diseases [8].

The study of Zandi et al.(2006) on effect of antioxidant activity of 16 species of *Salvia* on stability of soybean oil demonstrated that this plant can inhibit oil oxidation [9].

The aim of this research, is study on antioxidant activity and effect of methanolic extracts of *Mellissa officinalis L.* and *Salvia officinalis L.* to delay of lipid oxidation of soybean oil. Therefore, phenolic compound, rosmarinic acid and their antioxidant activity by DPPH method was measured. Finally, their antioxidant effect studied on stability of soybean oil with Ransimat method.

2. Materials and methods

2.1. Plant sample collecting. Lemon balm collecting from Babol town forests (52°, 44' , 22" of geographical length and 36°, 34', 15" of geographical width) and Sage collecting from Firozkooh (52°, 46' of length and 35°, 28' geographical width) in May of 2012 and dried.

2.2.Extracted. 0.1 g of dried sample was weighted and extracted with 20 ml 80% aqueous methanol on a banmary 70°C for 90 min. extracts was filtered and used for next measurement [10].

2.3.Determination of the total phenolic assay. 0.2 ml of extract was added to 1.8 ml of distilled deionised water (ddH₂O). 0.2 ml of the Folin-Ciocaltu's phenol reagent was added to mixture and shaken. After 5 min 2 ml 7% Na₂CO₃ solution was added to the mixture. The solution was diluted to 5 ml with ddH₂O and mixed. After incubation for 90 min at room temperature, the absorbance against the prepared reagent blank was determined at 750 nm with an UV-VIS Spectrophotometer [11].

2.4.Determination of the rosmarinic acid. The absorbance of extracts was determined at 333 nm with an UV-VIS Spectrophotometer and determined by standard curve [12].

2.5.DPPH assay. For DPPH assay, various concentration of 0.03 to 0.12 mg of extract was diluted to 1 ml with pure methanol. The solution was mixed with 1 ml 0.004% methanolic solution of DPPH. The solution was diluted to 30ml with pure methanol. After 15 min incubation at room temperature the absorbance was read against the prepared reagent blank (1ml DPPH and 2 ml pure methanol) at 517 nm. Inhibition of free radical by DPPH in present (I%) was calculated in following way [13] :

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

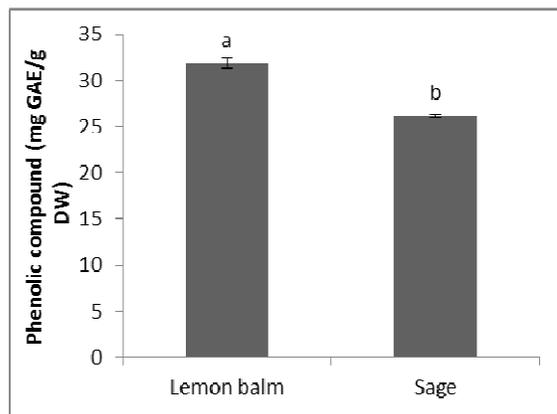
2.6.The study of antioxidant property of methanolic extracts of *Mellissa officinalis L.* and *Salvia officinalis L.* on stability of soybean oil. To study of antioxidant property of extracts on soybean oil stability, two concentrations of extracts was used. 0.1 and 0.2 g (to selection of best effective rate) of dried sample extracted with 20 ml 80% aqueous methanol on a banmary 70°C for 90 min and filtered. 400 ppm 80% aqueous methanol extracts was mixed in refining and without antioxidant soybean oil (20ml of extract mixed in 1 kg of oil). Because, methanol not dissolved in oil, used of Propylene glycol, in exchange for 400 ppm of extract, 800 ppm of Propylene glycol was added to oil. To measure of time of stability of oil used of Ransimat 743 apparatus, that base of its work is measure of amount of products that result of oxidation of oils and fats [13].

2.7. statistical analyse. All of experiment to be accomplished with 3 repetition and data analysed by SPSS19 and graphs was drawn by Excel 2010.

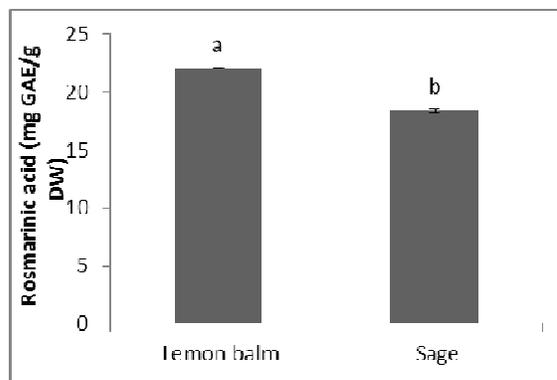
3.Results and discussion

Phenolic compound and rosmarinic acid contents. The results show that these plants are rich in phenolic compound and rosmarinic acid. The content of phenolic compound (a) and rosmarinic acid (b) in Lemon balm was 31 mg GAE/g DW and 22 mgRA/gDW and in Sage, was 26 mg GAE/g DW and 18 mg RA/gDW respectively, as shown in fig 1 with gallic acid and rosmarinic acid as standards.

These results indicate that the higher Phenolic compound and rosmarinic acid contents of the Lemon balm extract compared to the Sage extract.



(a)



(b)

Figure 1. Phenolic compound (a) and rosmarinic acid (b) contents.

Antioxidant activity. These results indicate that the higher antioxidant activity of the Lemon balm extract compared to the Sage 0.043 mg/ml extract of Lemon balm and 0.061 mg/ml extract of Sage was necessary to obtain 50% of DPPH degradation Fig 2.

The study of antioxidant property of metanolic extracts of *Melissa officinalis L.* and *Salvia officinalis L.* on stability of soybean oil. Results indicate that plant extracts can cause stability of soybean oil. Table 1 shows stability time of soybean oil together various antioxidants in 110°C.

According to data, defined that plant extracts can cause stability of soybean oil and can be successor of artificial antioxidants. Also, results indicate that in higher concentration of extract to have more stability.

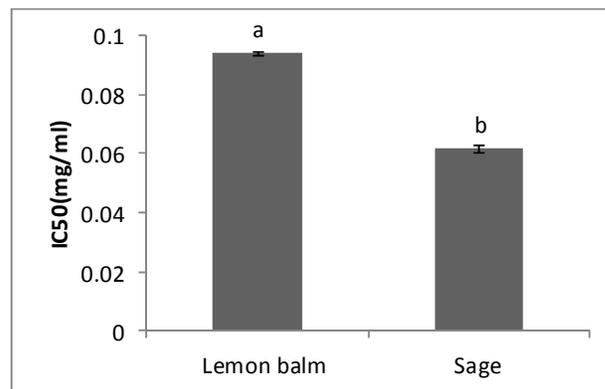


Figure 2. Comparison of antioxidant activity of extracts

Table 1. Time stability of soybean oil with various antioxidants.

Sample	Time (hour)
Soybean oil (example)	5.68
Soybean oil with antioxidant Propylene glycol (PG)	11.68
Soybean oil with Lemon balm metanolic extract (0.2 g dried material)	28.6
Soybean oil with Lemon balm metanolic extract (0.1 g dried material)	18.8
Soybean oil with Sage metanolic extract (0.2 g dried material)	27.2
Soybean oil with Sage metanolic extract (0.2 g dried material)	17.04

Lipid oxidation leading to reduction foodstuffs value of oils. To prevention of oxidation to exist numerous methods, that one of them is use of antioxidants. PG (propyl gallate), TBHQ (tertiary butyl hydro quinone), BHA (butylated hydroxyl anisole), BHT (butylated hydroxyl toluene) are main artificial antioxidants that use in food industry. Nowadays, defined that artificial antioxidants are cause of wickedness charges as cancer and poisoning. Therefore, preparation and identification of natural antioxidant resources is necessary as successor [1, 2]. It is well that plant flavonoids and phenols, are highly effective free radical scavenging and antioxidants. Polyphenols are used for the prevention and cure of various diseases. The phenolic compound have been recognized as antioxidant agent, which act as free radical oxidation terminators [8].

Azizkhani et al (2006), studied effect of complex of natural antioxidants on oxidative stability of Margarin and results demonstrated that complex of *Rosmarinus officinalis L.* extract and Tocopherol to have well effects on stability of Margarin and can to be use as artificial antioxidants successor [14].

Buta et al (2013) to study of antioxidant activity of Lemon balm on stability of sunflower oil demonstrated that Lemon balm extract that is rich of phenolic compound and has high antioxidant activity, to be cause of oxidative stability of sunflower oil [5].

The study of Ghanbari et al (2006), on antioxidant activity of *Rosmarinus officinalis* on stability of Kanola oil demonstrated that *Rosmarinus officinalis* extract is rich of phenolic compound and these compound increase stability of oil [15].

4. Conclusion

In this study, the results demonstrated that the aqueous metanolic extracts of Lemon balm and Sage are rich of phenolic compound and rosmarinic acid content and to have high antioxidant activity and they can to cause stability of soybean oil, but the higher than time of oil stability with Lemon balm extract compared to the Sage extract, that may be correlated to the phenolic compound and rosmarinic acid contents of respective plant extract. The results demonstrated that the higher than ontioxidant activity of the plant extracts compared to artificial antioxidant and can to be successor for that. The results showed that the concentration the more oil will be stabilized. Therefor, the natural antioxidant Can be replaced for synthetic antioxidant.

Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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