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Original Article

Synergistic Effect of *Zataria multiflora* Boiss. and *Bunium persicum* (Boiss.) B. Fedtsch. Essential Oils on Linseed Oil Oxidative Stability

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Abstract

We studied the antioxidant activities of the essential oils *of Zataria multiflora* Boiss. (ZEO) and *Bunium persicum* (Boiss.) B. Fedtsch (BEO) in mixed form on linseed oil using ($DPPH^{\bullet}$), ($ABTS^{\bullet+}$), H_2O_2 scavenging, and reducing power assays. After calculation of IC_{50} for ZEO and BEO separately, interactions of the essential oils were investigated at the form of mixture and the results were given in isobologram. The interactions between antioxidant effects of ZEO, BEO, TBHQ, and α-tocopherol; and isobologram results showed synergistic effect for $DPPH^{\bullet}$ except for BEO with TBHQ and α-tocopherol (1:1) and for $ABTS^{\bullet+}$ in ZEO with TBHQ (1:1). However, we could not find any synergistic effect for H_2O_2 scavenging and reducing power assays in any of the interactions. Statistical results showed that the best antioxidant levels of reductive oxidation were 600 ppm for ZEO and BEO, and 20 ppm for TBHQ in mixed form in linseed oil.

Key words: *Bunium persicum* essential oil, Isobologram, Linseed oil, Synergism, *Zataria multiflora* essential oil

Introduction

Fat oxidation plays an important role in food chemical changes [1]. Essential oils are natural source of phenolic compounds [2] and many studies in the field of antioxidants and antiradicals have been conducted on these compounds. Mixture of essential oils can have synergistic effect [3]. The potential synergistic effect in combination of the substances is increased. The multiplying activity and effectiveness of the substances in comparison with their effects when used separately are increased. So that on combination, the total effects are stronger than expected [3]. There are several studies about investigation of the synergistic effects of essential oils. In different fields of study, it is very common to detect and separate the components of a substance from drugs, herb, and even essential oils by focusing on their specific effects.

Some studies showed that consuming 90 ppm of mint essential oil and 50 ppm of caraway essential oil has positive effects on the gastrointestinal system [4]. Christoph *et al*. studied the *in vitro* combinational effect of melaleuca oil (*M. alternifolia*, *M. quinquenervia*) and manuka (*Leptospermum scoparium*) that contain significant amount of β-triketone [5]. Various spectra of Indian essential oils have been evaluated with different approaches and the results have shown that some of these essential oils in proportion of 1:1 show synergistic effects [6]. An important aspect of the synergy is evaluation of their effects with other chemical components. Interaction and combination effect of fennel, basil, and anise with benzoic acid or methyl-paraben (an ester from p-hydroxy benzoic acid) were studied by Fyfe *et al*. [7]. Their research showed that fennel and paraben have reasonable activity in eradication of microorganisms.

This study aims to investigate the antioxidation activity of combination of two essential oils, *Z. multiflora* and *B. persicum*, in the mixture form.

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Material and Methods

Materials

Chloroform, acetic acid, chloridric acid, potassium iodate, starch, sodium thiosulfate, hexane, TBA, BHT, TBHQ, potassium persulfate, ethanol, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium ferricyanide, trichloroacetic acid, ferric chloride, ascorbic acid, and H_2O_2 from Merck (Germany); α-tocopherol and $ABTS^{\circ+}$ from Fluka; cyclohexane from Riedel-deHaen, potassium iodide from Applied Chem., and $DPPH^{\cdot}$ from Sigma. To prepare the linseed oil (*Linum usitatissimum*), the seeds were purchased from local market. Then, the seeds were cleaned, ground, and poured in some cartouches, then some hexane-soaked cotton sheets were put upon them, and the oil extraction was performed in the room temperature. The essential oils of *Z. multi flora* and *B. persicum* were purchased from Plant- Essence Company (Gorgan, Iran).

Determining the Antioxidant Capacity

$DPPH^{\cdot}$ test

Electropositive potential of the essential oils was measured by bleaching method using $DPPH^{\cdot}$ violet ethanol solution. The method was performed according to Shahsavari *et al.* (2008) with some modifications in the method as follows [9]:

From each concentration of *Z. multiflora* (0.1 to 3 mg/ml) and *B. persicum* (0.2 to 4 mg/ml) essential oils, a 2-ml ethanol preparation was made, and then it was added to 1 ml of 0.2 molar solution of $DPPH^{\cdot}$, and finally after 1 hour, the reductive absorption at 517 nm was measured. Ethanol singly was used as the blank sample. Inhibition percentage was calculated by the following formula:

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

Where A_{blank} and A_{sample} are control (which contains all materials except the testing compound) and sample absorptions, respectively.

$ABTS^{\circ+}$ free radical decolorization assay

The antioxidant capacity was measured by radical cation of ABTS according to Cai *et al.* [10]. In order to produce the radical cation of ABTS, a 7-mM $ABTS^{\circ+}$ solution was prepared and then 2.45 mM of potassium persulfate was added and the solution was left for 12-16 h at the room temperature, two reagents react stoichiometrically at a ratio of 1:0.5. In the following, subscript /or… this solution was diluted by ethanol until 0.7 ± 0.05 absorption at 734 nm. Finally, 0.1 ml of the sample was added to 3.9 ml of the $ABTS^{\circ+}$ solution, and the absorption was read after 15 min. All data are the mean of three determinations and IC_{50} was calculated for each of them. In addition, vitamin C (µg/ml) was considered as the reference standard.

Hydrogen peroxide scavenging activity

The scavenging activity of hydrogen peroxide was determined for the two essential oils according to Büyükbalci and Nehir (2008) with some modifications [11]: One ml of 0.1- 0.6 and 0.4 - 1.2 mg/ml of ZMEO and BPEO essential oils were mixed with 2.4 ml of 0.1 M phosphate buffer (pH=7.4), respectively. Then, 0.6 ml of 43 mM hydrogen peroxide in the same buffer was added and the absorption was read after 40 min at 230 nm.

Reducing power

Fe-reducing power of the essential oils (EOs) was determined according to the method of Hsu *et al.* (2006) with some modifications [12]. One ml of various concentrations of the sample was mixed with 500 µl of potassium ferricyanide (1% w/w in water) and 500 µl of 0.2 M phosphate buffer, and left for 20 min in water bath at 50 °C. After cooling the solution, 500 µl of trichloroacetic acid (10% w/w) was added and the solution was centrifuged for 10 min at 3000 rpm. After that, 500 µl of transparent supernatant was picked up and then 100 µl of ferric chloride was added. Finally, the solution absorption was read after 30 min at 700 nm. The increase in absorption of reaction mixture indicates an increase in reducing power of the essential oils.

After calculation of IC_{50} for each essential oil separately, interactions of the essential oils were investigated at the form of mixture and the results were given in isobologram.

Analysis of isobolographic calculations can be summarized in the following five steps:

- Determinate of IC_{50} of each component separately and using logarithmic curves of dose-response.
- Selection of fixed proportions of the desired dose for combination and calculation of IC_{50add} that theoretically shows 50% inhibition of the desired mixture.

- Calculation of IC_{50}, which is obtained experimentally from the desired proportions in the test.
- Comprise / comparison IC_{50} and IC_{50add} statistically with T-test method according to Tallarida's study (2000) [8].
- Drawing isobologram to observe interactions among different combinations in the test.

Effect of essential oils of ZEO and BEO on oxidative stability of linseed oil

Different amount of ZEO and BEO (0.6, 1, 1.4, and 1.8 mg/ml), TBHQ at two levels (0.01 and 0.02 mg/ml), and BHT at two levels (0.1 and 0.2 mg/ml) were added to linseed oils. After that, peroxide value and thiobarbituric acid index were measured in days 0, 3, 6, 9, 12, and 15 at 60 °C according to the methods of AOCS and Madson *et al.* (1998) [13,14]. All data are the mean of three measurements of the test.

Statistical analysis

Microsoft Excel spreadsheets were used to draw the forms and diagrams, and also to present the results in isobologram. Total concentrations were analyzed using Student's t test. The data were analyzed by SPSS software ver. 16.

Result and Discussion

DPPH˚ test

In the DPPH˚ test, in order to evaluate of the interaction between test samples at different proportions of the two essences (v/v) including 3:2, 2:3, 1:1, 1:3, 3:1 and also the interaction between the essential oils and TBHQ and α-tocopherol in 1:1 proportion, the substances were mixed together. Tables 1-3 and Figs. 1-4 provide the results of these interactions.

As indicate at Table 1 and Fig. 1, mixture of the two essential oils in proportions examined has synergistic effect.

Result of mixture of each essential oil with the synthetic antioxidant (TBHQ) is demonstrated in the Table 2.

Isobologram related to the interaction between ZEO and TBHQ is provided in Fig. 2. The isobologram giving the interaction between BEO and TBHQ is not provided.

The results of interaction between essential oils and synthetic antioxidant in 1:1 proportion indicate synergistic effect only between ZEO and TBHQ.

Table 3 and Fig. 3 show synergistic interaction between α-tocopherol and ZEO but BEO shows an antagonistic effect with α-tocopherol. Isobologram of each mixture can be seen Fig. 3 and Fig. 4.

Table 1 Interaction between two essential oils in determined proportion in DPPH test

Sample	Proportion	IC_{50add} (ppm)	IC_{50mix} (ppm)	(IC_{50}mix/IC_{50add}) α
(ZEO)A:(BEO)Z	1:1	1151.5 ± 29.9	423.6 ± 41.9	0.36
	1A:3Z	1335.7 ± 39.9	716.1 ± 32.9	0.53
	3A:1Z	967.2 ± 25.2	231.0 ±16.4	0.23
	2A:3Z	1225.4 ± 33.5	411.1 ± 26.4	0.33
	3A:2Z	1077.8 ± 27.1	183.9 ± 12.2	0.17

a is equal to index of interaction and α<0.7 shows Synergistic and α>1.3 shows antagonism between two samples of the test. Significant differences at the level of 95% exist among all samples between IC_{50add} and IC_{50}.

Table 2 Interaction between two essential oils and TBHQ in determined proportion in DPPH test

Sample	Proportion	IC_{50add} (ppm)	IC_{50mix} (ppm)	α
A :TBHQ	1:1	392.3 ± 14.4	179.6 ± 20.2	0.45
Z :TBHQ	1:1	760.8 ± 26.2	29241.5± 4304.3	38.47

There are significant differences at level of 95% between IC_{50} and IC_{50add} for all samples.

Table 3 Interaction between two essential oils and α-Tocopherol in determined proportion in DPPH test

Sample	Proportion	IC_{50add} (ppm)	IC_{50mix} (ppm)	α
A:tocopherol	1:1	394.9 ± 14.4	190.5 ± 13.1	0.48
Z:tocopherol	1:1	763.4 ±26.2	5861.3 ± 429	7.67

There are significant differences at level of 95% between IC_{50} and IC_{50add} for all samples

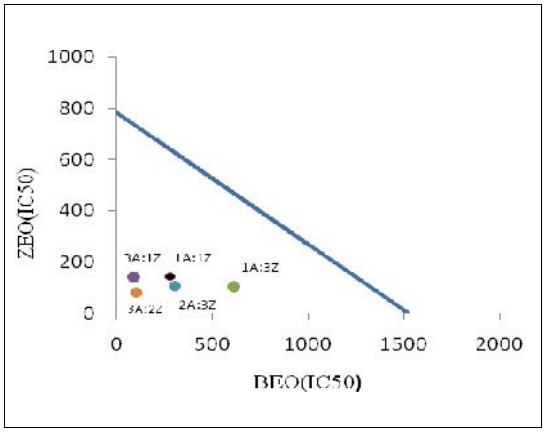

Fig. 1 Isobologram for interaction between two essential oils in determined proportion in DPPH test

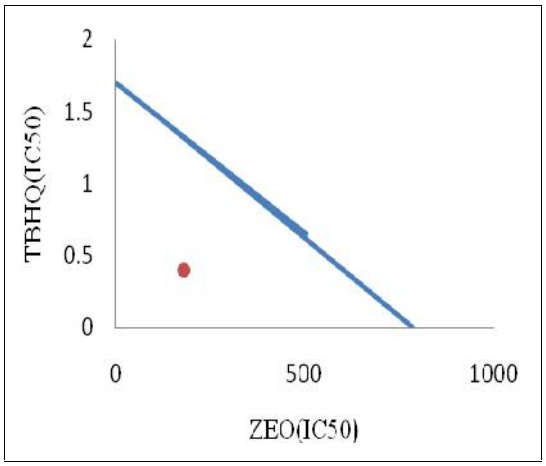

Fig. 2 Isobologram for interaction between ZEO and TBHQ in determined proportion in DPPH test

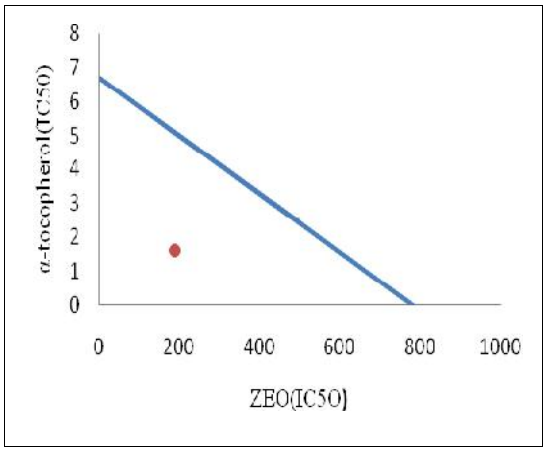

Fig. 3 Isobologram for interaction between ZEO and α-Tocopherol in determined proportion in DPPH test

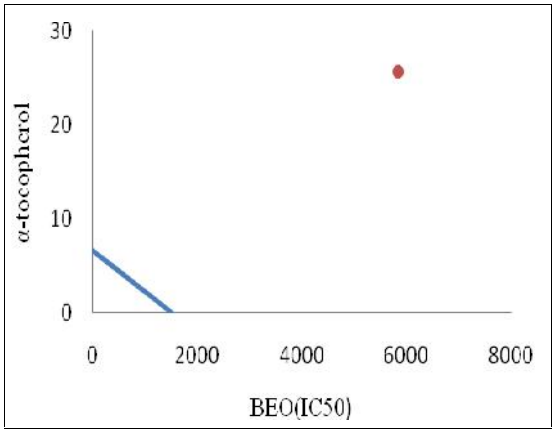

Fig. 4 Isobologram for interaction between BEO and α-Tocopherol in determined proportion in DPPH test

Regarding the unknown mechanism of interaction between these combinations, it seems that in some cases with synergistic effects, the effect is caused by activities of an oxidryl group of an antioxidant with another antioxidant and consequently the increase in formation of radical hydrogen and its reaction with DPPH˚ radical [15].

ABTS$^{\bullet+}$ assay

In this step, similar to the DPPH˚ test, mixtures of ZEO and BEO at different proportions (V/V) and mixture of essential oils with TBHQ and α-tocopherol in a specific proportion were prepared to get a better understanding of the interaction between these two EOs with other antioxidants in antioxidant activity measurements.

Table 4-6 and Figs. 5-9 show the result of interactions among the samples. Fig. 5 illustrates isobologram of two EOs interactions in this test.

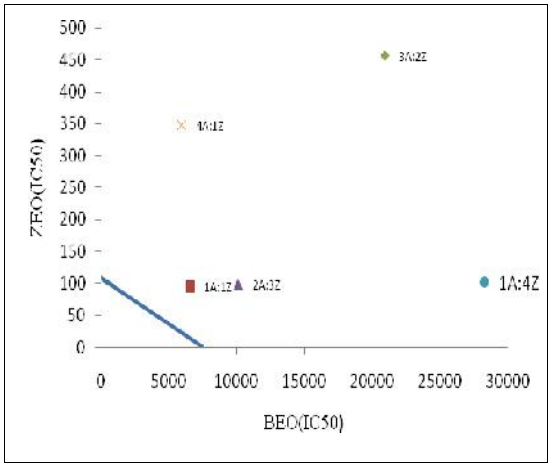

Fig. 5 Isobologram for interaction between BEO and ZEO in determined proportion in ABTS test

Table 4 Interaction between two essential oils in determined proportion in ABTS assay

Sample	Proportion	IC_{50add} (ppm)	IC_{50mix} (ppm)	(IC50mix/IC50add) α
ZEO:BEO	1:1	3804.1 ± 68.9	6652.7 ± 229.5	1.74
	1A:4Z	6021.0 ± 110.3	28379.1 ± 6657.7	4.71
	4A:1Z	1587.3 ± 27.6	6309.5 ± 1755.9	3.79
	2A:3Z	3065.2 ± 82.7	10232.9 ± 2471.2	3.33
	3A:2Z	4543.1 ± 55.1	21379.6 ± 1801.5	4.70

There are significant differences at level of 95% between IC_{50} and IC_{50add} for all samples

Table 5 Interaction between two essential oils and TBHQ in determined proportion in ABTS test

Sample	Proportion	IC_{50add} (ppm)	IC_{50mix} (ppm)	α
ZEO :TBHQ	1:1	73.5 ± 0.5	52.0 ± 4.7	0.7
BEO :TBHQ	1:1	3768.3 ± 69*	7188.9 ± 2298.3*	1.9

*There are not significant differences at level of 95% between IC_{50} and IC_{50add} for samples

Table 6 Interaction between two essential oils and α-Tocopherol in determined proportion in ABTS test

Sample	Proportion	IC_{50add} (ppm)	IC_{50mix} (ppm)	α
A:tocopherol	1:1	89.1 ± 3.8	132.8 ± 16.8	1.49
Z:tocopherol	1:1	3783.9 ± 0.69*	4432.6 ± 723.8*	1.17

*There are not significant differences at level of 95% between IC_{50} and IC_{50add} for samples

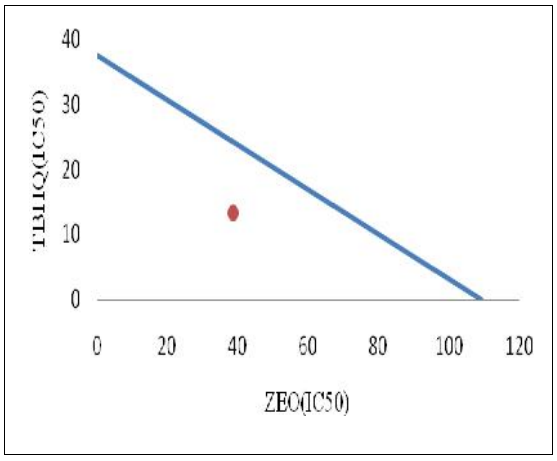

Fig. 6 Isobologram for interaction between ZEO and TBHQ in determined proportion in ABTS test

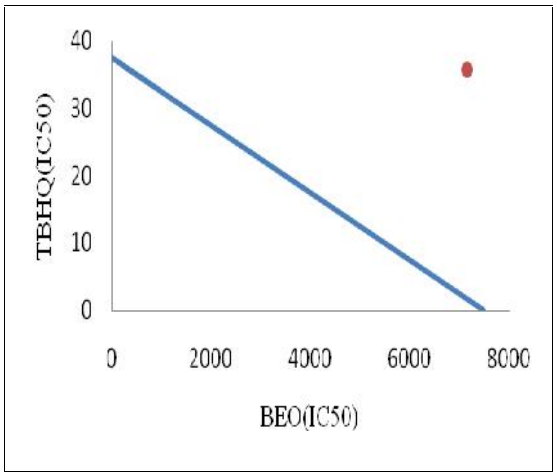

Fig. 7 Isobologram for interaction between BEO and TBHQ in determined proportion in ABTS test

With regard to Tables 4-6 and Figs. 5-9, it is concluded that there is an antagonistic interaction between ZEO and BEO. In addition, it is observed that ZEO has a synergistic effect only with TBHQ, and antagonistic interaction with α-tocopherol.

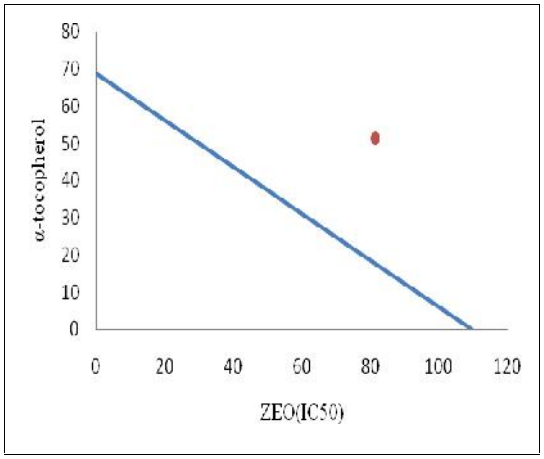

Fig. 8 Isobologram for interaction between ZEO and α-Tocopherol in determined proportion in ABTS test

In terms of the interactions of BEO with TBHQ and α-tocopherol, it is observed that there is not a significant difference between their IC_{50mix} and IC_{50add}. This indicates that BEO do not have any interaction with TBHQ and α-tocopherol at the proportion of 1:1. To explain the mechanism of interaction between effective constituents in this test, it seems that TBHQ and ZEO at the proportion of 1:1 show higher activity at the oxidryl groups within them, which result in the synergistic effect [15].

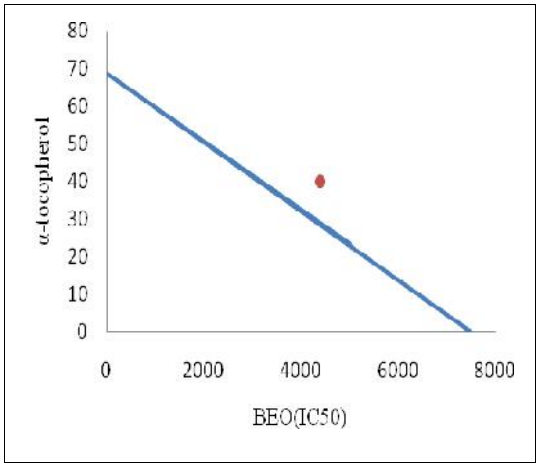

Fig. 9 Iisobologram for interaction between BEO and α-Tocopherol in determined proportion in ABTS test

Hydrogen peroxide scavenging activity

In this test, the interactions between the two essences and also between the essences and TBHQ were evaluated only at the proportion of 1:1 (V/V). The results are provided in the following.

According to the results (Table 7-8 and Figs. 10-12) obtained for the interactions between the two essential oils and TBHQ at hydrogen peroxide scavenging activity assay, it can be concluded that ZEO and BEO have antagonistic effect at the proportion of 1:1. This is also the case for ZEO and TBHQ. However, BEO and TBHQ at the proportion of 1:1 did not show any interaction. To explain the mechanism of interaction between the constituents in this test, it seems that mixture of TBHQ and ZEO, and also TBHQ and BEO at the proportion of 1:1 cause lower activity of oxidryl groups, which result in the antagonistic mechanism observed [15].

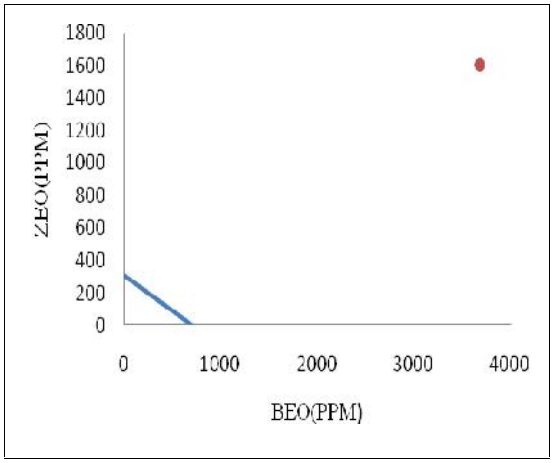

Fig. 10 Isobologram for interaction between BEO and ZEO in determined proportion in Hydrogen Peroxide scavenging activity assay

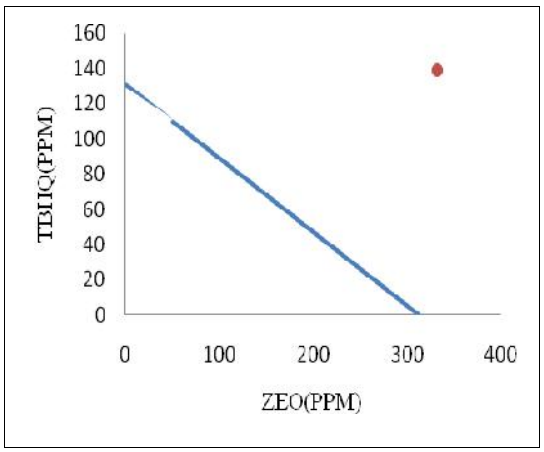

Fig. 11 Isobologram for interaction between ZEO and TBHQ in determined proportion in Hydrogen Peroxide scavenging activity assay

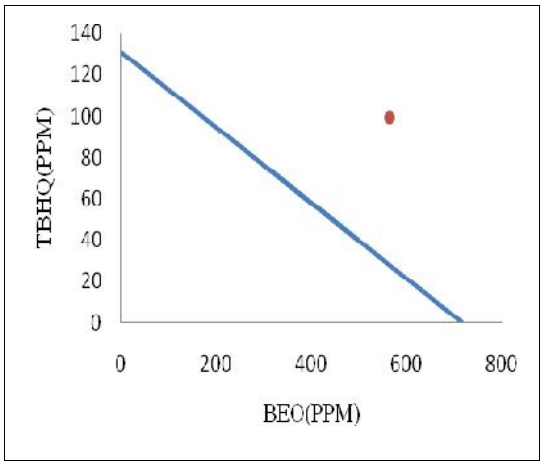

Fig. 12 Isobologram for interaction between BEO and TBHQ in determined proportion in Hydrogen Peroxide scavenging activity assay

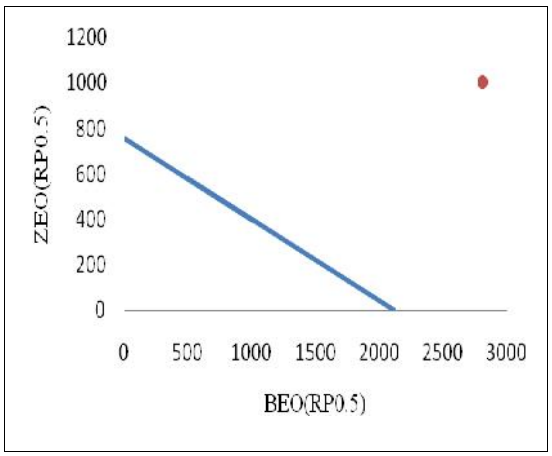

Fig. 13 Isobologram for interaction between BEO and ZEO in determined proportion in reducing power assay

Table 7 Interaction between two essential oils in determined proportion in Hydrogen Peroxide scavenging activity assay

Sample	Proportion	IC_{50add} (ppm)	IC_{50mix} (ppm)	α
ZEO:BEO	1:1	514.3 ± 11.9	5288.5 ± 1666.4	10.28

There are significant differences at level of 95% between IC_{50} and IC_{50add} for all samples

Table 8 Interaction between two essential oils and TBHQ in determined proportion in Hydrogen Peroxide scavenging activity assay

Sample	Proportion	IC_{50add} (ppm)	IC_{50mix} (ppm)	α
A :TBHQ	1:1	221.7 ± 6.1	470.9 ± 43.3	2.12
Z : TBHQ	1:1	423.5 ± 11.1*	645.6 ± 207.9*	1.52

*There are not significant differences at level of 95% between IC_{50} and IC_{50add} for samples

Table 9 Interaction between two essential oils in determined proportion in Hydrogen Peroxide scavenging activity assay

Sample	Proportion	EC_{50add} ($RP_{0.5AU}$) (ppm)	EC_{50mix} ($RP_{0/5AU}$) (ppm)	α
A:Z	1:1	1438.7 ± 14.7	3813.5 ± 324.5	2.65
A :TBHQ	1:1	390.3 ± 13.9	2152.7 ± 851.6	5.51
Z :TBHQ	1:1	1071.6 ± 4.9	13846.3 ± 6655.9	12.9

There are significant differences at level of 95% between IC_{50} and IC_{50add} for all samples

Table 10 Result from peroxide value (meqO_2/kg) in mixture treatments in determined days

Treatment	3	6	9	12	15
CTRL	7.82±0.65 b	10.70±0.24 b	12.92±0.28 d	18.09±0.38 d	23.71±0.27 d
A600:Z600	9.13±0.69 c	11.76±0.20 c	12.86±0.41 d	16.64±0.46 c	20.64±1.02 c
A300:Z300	7.00±0.58 b	10.11±0.28 b	10.79±0.67 c	15.79±0.65 c	18.60±0.55 b
A600:TB20	2.55±0.11 a	3.32±0.22 a	3.55±0.45 a	6.73±0.24 b	8.22±0.61 a
A300:TB10	3.16±0.42 a	3.88±0.54 a	12.08±0.16d	25.07±0.85 e	29.17±0.75 e
Z600:TB20	2.70±0.18 a	3.63±0.68 a	4.62±0.92 b	5.48±0.72 a	7.15±0.68 a
Z300:TB10	2.79±0.71 a	3.42±0.09 a	10.09±0.31 c	18.55±0.29 d	23.98±0.46 d

Table 11 Result from tiobarbituric acid value (mgMDA/kg) in mixture treatments in determined days

Treatment	3	6	9	12	15
ctrl	0.165±0.004 d	0.185±0.009 c	0.232±0.010 e	0.293±0.006 e	0.437±0.006 d
A600:Z600	0.175±0.006 e	0.197±.009 c	0.232±0.005 e	0.268±0.004 d	0.400±0.010 c
A300:Z300	0.161±0.003 d	0.180±0.008 c	0.196±0.003 d	0.255±0.004 c	0.311±0.010 b
A600:TB20	0.069±0.001 b	0.084±0.004 a	0.093±0.008 a	0.151±0.004 b	0.173±0.002 a
A300:TB10	0.083±0.002 c	0.097±0.006 b	0.229±0.003 e	0.459±0.006 f	0.528±0.016 f
Z600:TB20	0.072±0.002 a	0.095±0.002 b	0.113±0.004 b	0.139±0.002 a	0.161±0.004 a
Z600:TB10	0.073±0.001 b	0.093±0.009 ab	0.179±.013 c	0.296±0.002 e	0.466±0.007 e

Tiobarbituric acid value is 0.005±0.004 at zero day and it is considered for all treatment for zero day as a result of same condition of linseed oil.

Reducing power assay

Results of interaction between the two EOs and EOs with TBHQ at the proportion of 1:1 (V/V) are provided in Table 9 and Figs. 13-14. Isobologram between the two EOs and ZEO and between EOs and TBHQ are given at the Fig 13 and Fig 14. The isobologram related to BEO are not provided. Because of, an antagonist effect was severe and suitable interaction was not existed.

According to the results, interactions between EOs themselves and between EOs and TBHQ at the proportion of 1:1 are antagonistic interactions. The antioxidant capacity test was based upon electron exchange.

Journal of Medicinal Plants and By-products (2014) 2: 177-185

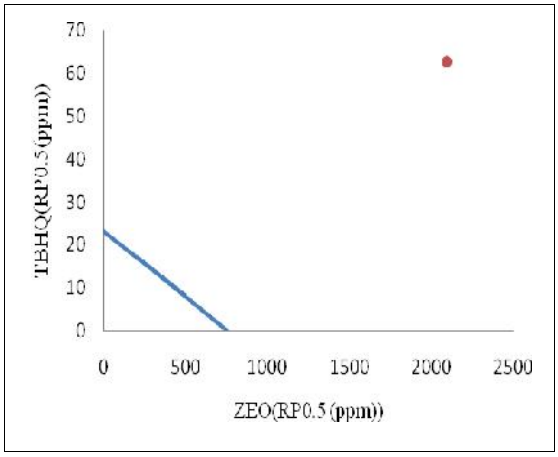

Fig. 14 Isobologram for interaction between TBHQ and ZEO in determined proportion in reducing power assay

With regard to the results of the antioxidant test on the mixtures, it can be concluded that the two EOs at the mixture status had a limited electron exchange with the probe and the two Eos showed synergistic effects only at the DPPH$^{\bullet}$ test. In addition, the result of antioxidant capacity determination showed that BEO has lower capacity to make synergistic interaction at the proportions applied (except for the interaction with BEO at the DPPH$^{\bullet}$ test) and applying TBHQ and α-tocopherol were useless. On the other hand, it must be considered that EOs antioxidant activity is not limited to the phenolic combinations, and other components such as monoterpenes, ketones, aldehydes, and hydrocarbons can cause synergistic or antagonistic effects [16].

Determine peroxide value in mixture treatment

After choosing the best and most effective treatments (Z600, A600, and TBHQ20) in reducing the peroxide value compared with the control, the next step began. It involves mixing the antioxidant effect, which is desirably performed under similar conditions with an early stage to identify the most appropriate concentrations. To this end, treatments of A600:Z600, Z300:A300, TBHQ20:A600, TBHQ10:A300, TBHQ20:Z600 and TBHQ10:Z300 were prepared and placed in the oven at 60 °C.

According to the Table 10 and Figure 15 presented treatment A300: TBHQ10 caused a significant increase in the peroxide value in comparison with the last day of the control. This trend shows an oxidation enhancement in this treatment. Z300: TBHQ300 was not significantly different from the control. The peroxide values of treatments A600:Z600 and A300:Z300 were significantly less than that of the control; however, they could not significantly prevent oil oxidation.

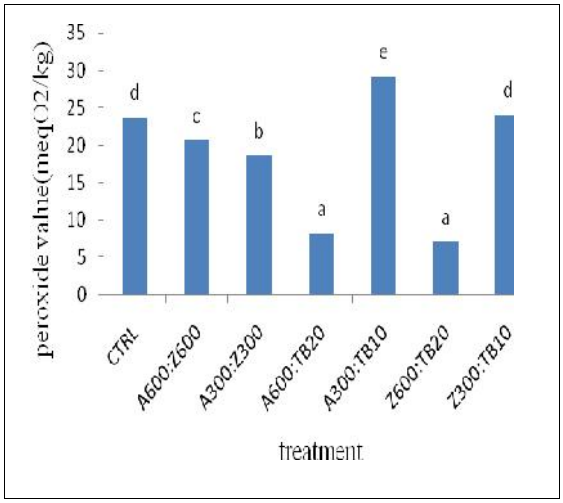

Fig. 15 Comparison of peroxide values treatments on linseed oil in 15th day

Since decreasing the concentrations of the two EOs showed better result in terms of preventing oxidation in linseed oil, it seems that the two Eos could have better effects and proper stability at lower concentrations in the linseed oil. Treatments A600: TBHQ20 and Z600: TBHQ20 could significantly prevent oil oxidation in comparison with the control.

Determine thiobarbituric acid value at mixture treatments

Results of thiobarbituric acid test at mixture status are shown in Table 11. Thiobarbituric acid value is 0.005±0.004 at day zero and it is considered for all treatments at day zero; owing to the same condition of linseed oil.

In previous studies, both ZEO and BEO separately have shown appropriate antioxidant effect on linseed oil stability. In addition, mixture treatments of both ZEO and BEO had better effects on decreasing the linseed oil oxidation, respectively, at 600 ppm with TBHQ and 20 ppm. Therefore, they could increase the oil shelf life.

Rudnik *et al.* (2001) conducted a study to evaluate the linseed oil oxidation stability in nine months. BHA at the level of 0.02% and mixture of α-tocopherol, ascorbylpalmitate, and ascorbic acid at the level of 0.05%, 0.1%, and 0.2% were used, respectively. It was indicated that the mixture used had better antioxidant effect on linseed oil stability [17].

Lampi *et al.* (1999) studied triacylglycerol oxidative stability of rapeseed oil with both α- and

γ-tocopherols separately and in combination at 40 °C and for 16 days. They evaluated the tocopherol concentrations of 5 - 500 µg/g and reported that in concentrations less than 50 µg/g and higher than 100µg/g, γ-tocopherol was more effective. In addition, synergistic effect was not observed in the combination status in both 5+5 and 10+10 µg/g concentrations. Also, in 500+500 µg/g, α-tocopherol was less stable [18]. Bandarra *et al.* (1999) conducted a study to evaluate the synergistic activity of α-tocopherol (0.04%) and some phospholipids (phosphatidylcholine (PC), phosphatidyl ethanolamine (PE), and cardiolipin (CL) at the concentration of 0.5% as antioxidants in stability of sardine oil. The best antioxidant activity in separate status belonged to PC and in combination status the best synergistic effect belonged to α-tocopherol + PE [19].

Conclusion

According to the results of evaluation of the synergistic effect in antioxidant activity determination tests, the two EOs had synergistic effect only at the DPPH radical test. Moreover, BEO did not have synergistic effect with antioxidants such as TBHQ and α-tocopherol. ZEO did not show synergistic effect with TBHQ and α-tocopherol at hydrogen peroxide scavenging and reducing power tests. Results of the mixture treatments showed that mixture of both Eos at 600 ppm concentration and TBHQ at 20 ppm concentration had the best effect in preventing the linseed oil oxidation. Furthermore, mixture status treatments showed better effects than using them separately.

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References

1. Akon CC. Min DB. Food Lipids: Chemistry, Nutrition and Biotechnology. 3th edn. CRC Press. USA.2008.
2. Dormana HJD, Peltoketo A, Hiltunen R,Tikkanen MJ. Characterization of the antioxidant properties of de-odourisedaqueous extracts from selected laminaceaeherbs, Food Chem.2003;83:255-262.
3. Harris R. Synergism in the essential oil. Inter J Aromatherapy.2002; 12:179-186.
4. May B, Kuntz D, Kieser M, Kohler S. Efficacy of a fixed peppermint/caraway oil combination in non-ulcer dyspepsia. Arzneim-Forsch.1996; 46:1149-1153.
5. Christoph F, Kaulfers PM, Stahl-Biskup E. *In vitro* evaluation of the antibacterial activity of b-triketones admixed to Melaleuca oils. Planta Med.2001;67:768-771.
6. Geda AK. Antibacterial activity of essential oils and their combinations. Fat. Sci. Technol. 1995;97:458-460.
7. Fyfe F, Armstrong F, Stewart J. Inhibition of *Listeria monocytogenes* and *Salmonella enteriditis* by combinations of plants oils and derivatives of benzoic acid: the development of synergistic antimicrobial combinations. Int. J Antimicrobial Agents.1998;9:195-199.
8. Tallarida R. Drug Synergism and Dose-Effect Analysis. Florida, CRC Press. USA. 247p. 2000.
9. Shahsavari N, Barzegar M, Sahari M A,Naghdibadi H.Antioxidant activity and chemical characterization of essential oil of *B. persicum*. Plant Foods Hum Nutr. 2008;63:183-188.
10. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74:2157-2184.
11. Buyukbalci A, Nehir S. Determination of *in vitro* antidiabetic effects, antioxidant activities and phenol contents of some herbal teas. Plants Foods Human Nut.2008;63:27-33.
12. Hsu B, Couper IM, Ng K. Antioxidant activity of hot water extract from the fruit of the doum palm, HyphaeneThebaica.2006;98:317-328.
13. AOCS. In: D. Firestone, (Ed.), *Official Methods and Recommend Practices of the American Oil Chemists' Society* (4thed.). Champaign: AOCS.1989.
14. Madsen HL, Sorensen B, Skibsted LH,Bertelsen G. The antioxidative activity of summer savory *(Satureja hortensis* L.) and rosemary *(Rosmarinus oflcinalis* L.) in dressing stored exposed to light or in darkness. Food Chem.1998;63:173-180.
15. Romano CS, Abadi K, Repetto V, Vojnov AA, Moreno S. Synergistic antioxidant and antibacterial activity of rosemary plus butylated derivatives. Food Chem.2009;115:456-461.
16. Svoboda KP, Hampson JB. Bioactivity of essential oils of selected aromatic plants: antibacterial, antioxidant, antiflammatory and other related pharmacological activities. Available on: www.googlescholar.com.1999.
17. Rudnik E, Szczucinska A, Gwardiak H, Szulc A, Winiarska A. Comparative studies of oxidation stability of linseed oil. Thermochimica Acta.2001; 370:135-140.
18. Lampi AM, Kataja L, Eldin LK, Vieno P. Antioxidant activities of α- and γ-tocopherols in the oxidation of rapeseed oil triacylglycerols. JAOCS.1999;76:749-755.
19. Bandarra NM, Campos RM, Batista I, Nunes ML, Empis JM. Antioxidant synergy of α-tocopherol and phospholipids. JAOCS.1999;76:905-913.