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Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol

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Abstract

Antioxidant activities of three pure compounds: carnosic acid, rosmarinic acid and sesamol, as well as two plant extracts: rosemary extract and blackseed essential oil, were examined by applying DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radical-scavenging assays and the ferric thiocyanate test. All three test methods proved that rosemary extract had a higher antioxidant activity than blackseed essential oil. The order of antioxidant activity of pure compounds showed variations in different tests. This was attributed to structural factors of individual compounds. Phenolic contents of blackseed essential oil and rosemary extract were also determined. Rosemary extract was found to have a higher phenolic content than blackseed essential oil. This fact was utilised in explaining the higher antioxidant activity of rosemary extract.

Keywords: Antioxidant activity; Rosemary extract; Blackseed essential oil; Carnosic acid; Rosmarinic acid; Sesamol

1. Introduction

There has been an increasing interest in the use of natural antioxidants, such as tocopherols, flavonoids and rosemary (*Rosmarinus officinalis* L.) extracts for the preservation of food materials in recent years (Bruni et al., 2004; Frutos & Hernandez-Herrero, 2005; Hras, Hadolin, Knez, & Bauman, 2000; Williams, Spencer, & Rice-Evans, 2004), because these natural antioxidants avoid the toxicity problems which may arise from the use of synthetic antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG) (Amarowicz, Naczk, & Shahidi, 2000; Aruoma, Halliwell, Aeschbach, & Loligers, 1992). Plants, including herbs and spices, have many phytochemicals which are potential sources of natural antioxidants, e.g. phenolic diterpenes, flavonoids, tannins and phenolic acids (Dawidowicz, Wianowska, & Baraniak, 2006). These compounds have antioxidant, anti-inflammatory and anticancer activities (Lee, Hwang, & Lim, 2004). Phenolic compounds are also thought to be capable of regenerating endogenous α-tocopherol in the phospholipid bilayer of lipoprotein particles back to its active antioxidant form (Rice-Evans, Miller, & Paganga, 1996).

The greatest level of attention among herbs and spices as sources of antioxidants has been focussed on rosemary. In earlier studies, sage (*Salvia officinalis* L.) and rosemary were shown to have similar patterns of phenolic compounds and their antioxidant activity was attributed mainly to their carnosic acid, carnosol and rosmarinic acid components (Frankel, Huang, Aeschbach, & Prior, 1996; Okamura, Fujimoto, Kuwabara, & Yagi, 1994; Thorsen & Hildebrandt, 2003).

Blackseed (*Nigella sativa* L.) is another plant used as a source of antioxidant. It has been used traditionally, especially in the middle East and India, for the treatment of asthma, cough, bronchitis, headache, rheumatism, fever, influenza and eczema (Burits & Bucar, 2000) and for its

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antitumor, antihistaminic, antidiabetic, anti inflammatory and antimicrobial activities for years. Many of these activities have been attributed to quinone constituents in the seed (Ghosheh, Houdi, & Crooks, 1999). Earlier experimental studies demonstrated the inhibition of nonenzymatic lipid peroxidation in liposomes by both the fixed oil and thymoquinone which is the main compound in blackseed essential oil (Houghton, Zarka, de las Heras, & Hoult, 1995).

Sesamol is one of the components of lignans, which are a class of unusual compounds found in sesame oil (Yoshida & Takagi, 1999) and are being studied among the main antioxidative components in sesame seeds. It has been known for many years that sesame oil is highly resistant to oxidative deterioration as compared to other edible oils (Mohamed & Awatif, 1998), possibly due to the presence of antioxidative components of lignans, including sesamol.

It is important to compare the antioxidant activities of plant extracts, which may contain more than one antioxidant component, with those of individual pure antioxidants, in order to determine possible synergistic interaction among the antioxidants. However, due to the complexity of antioxidant activity in foods, the evaluation of the activities of pure compounds and plant extracts of complex plant matrices should be based on the data obtained under the same experimental conditions.

Testing the activity by more than one assay is desirable because different methods measure different characteristics of the antioxidant. Several approaches are used to test the antioxidants in foods and biological systems. Some consist of oxidizing a lipid or lipoprotein substrate under standard conditions and assessing the activity by various methods to determine how much oxidation is inhibited. Some of them, which are called free radical trapping methods, measure the ability of antioxidants to intercept free radicals, as reviewed by Frankel and Meyer (2000).

The purpose of the present work is to determine the antioxidant activities of carnosic acid, rosmarinic acid and sesamol as pure antioxidant compounds and of rosemary extract and blackseed essential oil as complex plant matrices by DPPH$^{\cdot}$ radical assay, ABTS$^{\cdot+}$ radical assay, ferric thiocyanate test (FTC) in a linoleic acid emulsion and measurement of total phenolic content.

2. Materials and methods

2.1. Materials

All solvents used in the experiments were HPLC grade and purchased from Merck. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical, carnosic acid (90%), and linoleic acid were obtained from Sigma, while 2,2′-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and sesamol were from Fluka. Trolox and rosmarinic acid (97%) were from Aldrich. All other reagents were of analytical grade.

Rosemary leaves were collected from Akdeniz university campus area in May and dried at 25–30 °C for 3 days without applying any heat treatment to minimize the loss of active components. Dried leaves were separated from the branches and kept in deep freeze at −20 °C until use.

Blackseed was purchased from a local market and stored in deep freeze at −20 °C until use.

2.2. Preparation and analysis of rosemary extract

Dried rosemary leaves were blended in a blender and were then subjected to Soxhlet extraction using methanol as the solvent. Fifty grams of the plant material and 250 ml of methanol were used in the extraction. Methanol containing the extract was then filtered through Whatman paper (GF/A, 110 mm) and the solvent was vacuum-distilled at 40 °C in a rotary evaporator. The remaining extract was finally dried in a vacuum oven at 30 °C for two hours to ensure the removal of any residual solvent. Final extract was a dark green powder. It was analyzed for carnosic acid and rosmarinic acid by HPLC as described below and kept in a deep freeze at −20 °C under N_2 atmosphere until use.

HPLC analysis of rosemary extract for carnosic acid and rosmarinic acid was done according to the procedure described by Okamura et al. (1994). The analysis was performed with an Agilent 1100 series HPLC instrument equipped with an autosampler. The column was a Hypersil ODS C_{18} type with a 5 μm particle size, 4.6 × 250 mm i.d., used with Hypersil ODS 4.0 × 20 mm i.d. 5 μm guard cartridges. The separation was isocratically undertaken with a mobile phase consisting of 0.1% (w/v) aqueous *ortho*-phosphoric acid and acetonitrile (40:60) at a flow rate of 1 ml/min. Column temperature was 27 °C. The detector was a DAD (230.4 nm) and the injection volume was 5 μl.

2.3. Preparation and analysis of blackseed essential oil

Blackseed essential oil was prepared according to the procedure described by Burits and Bucar (2000); 75 g of blackseed were crushed and extraction was applied using about 220 ml of light petroleum ether (b.p. = 40–60 °C) in a Soxhlet apparatus. The extraction was continued for four hours and repeated until sufficient oil was collected. The solvent was then removed under reduced pressure and temperature. The brownish residue in the flask was steam-distilled with a Clevenger apparatus. Extraction of the aqueous distillate with *n*-hexane and removal of the solvent from the extract under vacuum yielded the essential oil. It was analyzed for thymoquinone by HPLC, as described below, and kept in deep freeze at −20 °C under N_2 atmosphere until use.

HPLC analysis of blackseed essential oil for thymoquinone was conducted according to the procedure reported by Ghosheh et al. (1999). The column was an Alpha Bond C_{18} type, 10 μm, 300 × 3.9 mm. The isocratic mobile phase consisted of H_2O:methanol:2-propanol in the ratio of

10:9:1 by volume. Column temperature and the flow rate were 28 °C and 1 ml/min, respectively. The detector was a DAD (254.4 nm) and the injection volume was 5 μl.

2.4. DPPH· radical-scavenging assay

This assay was carried out as described by Blois (1958) with some modifications; 1.5 ml of various dilutions of the test materials (pure antioxidants or plant extracts) were mixed with 1.5 ml of a 0.2 mM methanolic DPPH· solution. After an incubation period of 30 min at 25 °C, the absorbances at 515 nm, the wavelength of maximum absorbance of DPPH·, were recorded as A_{sample}, using a Cary 100 Bio UV/VIS spectrophotometer. A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A_{blank}.

The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\%\ \text{inhibition} = 100(A_{blank} - A_{sample})/A_{blank} \quad (1)$$

Antioxidant activities of test compounds or extracts were expressed as IC_{50}, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH· concentration.

2.5. ABTS·+ radical-scavenging assay

This assay was carried out according to the procedure described by Re et al. (1999). $ABTS^{\cdot+}$ radical cation was produced by reacting 7 mM aqueous ABTS with 2.45 mM (final concentration) potassium persulfate and keeping the mixture in the dark at room temperature for 16 h. Blue–green $ABTS^{\cdot+}$ was formed at the end of this period. The solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm, the wavelength of maximum absorbance in the visible region. Test materials were dissolved in and diluted with ethanol such that, after the introduction of an accurately measured volume of each dilution into the assay, they produced a 10–90% decrease in the absorbance of the blank solution at 734 nm. After adding 35 μl of the test solution to 3.5 ml of $ABTS^{\cdot+}$ solution having $A_{734} = 0.70 \pm 0.02$, absorbance was recorded up to 6 min in 1 min intervals. Results were expressed as trolox equivalent antioxidant capacity (TEAC) at 1, 4 and 6 min. TEAC is defined as the mM concentration of a trolox solution whose antioxidant activity is equivalent to the activity of 1.0 mM test solution. In order to find TEAC values, a separate concentration response curve for standard trolox solutions was prepared.

2.6. Ferric thiocyanate test (FTC)

The antioxidant activity analysis using ferric thiocyanate was performed according to the procedure reported by Lee et al. (2004); 0.006 g of each test material was dissolved in 0.12 ml ethanol and 2.88 ml of a 2.51% linoleic acid solution in ethanol. Then, 9 ml of a 0.04 M phosphate buffer (pH 7.0) were added to this solution. The mixture was kept at 40 °C in a stoppered test tube in the dark for 72 h. A 0.1 ml aliquot was taken from the mixture every 24 h and diluted with 9.7 ml of 75% ethanol, followed by the addition of 0.1 ml of 30% ammonium thiocyanate. Exactly 3 min after adding 0.1 ml of 0.02 M $FeCl_2$ in 3.5% HCl, the absorbance for the red colour was measured at 500 nm. The ratio of this absorbance to the absorbance of a blank without any test material was taken as a measure of ability of the test material to inhibit lipid peroxidation which is, in turn, a measure of antioxidant activity.

2.7. Total phenolic content

The total phenol content of plant extracts was determined using Folin–Ciocalteu reagent (FCR) according to the procedure reported by Singleton, Orthofer, and Lamuela-Raventos (1999) with some modifications. One mili liter of each plant extract solution, prepared in ethanol at a concentration of 0.1 mg/ml was mixed with 7.5 ml of FCR which was diluted 10-fold with distilled water. After standing at room temperature for 5 min, 7.5 ml of 60 mg/ml of aqueous Na_2CO_3 solution were added. The mixture was kept at room temperature for 2 h and then the absorbance was measured at 725 nm. The results were expressed in gallic acid equivalents (GAE), determined utilizing a separately prepared absorbance versus concentration curve for gallic acid.

3. Results and discussion

3.1. Analysis of plant extracts

HPLC analysis of rosemary extract showed that it contained about 6% of carnosic acid and 8% of rosmarinic acid. Blackseed essential oil was found to contain about 12% of thymoquinone by HPLC analysis. Total phenolic contents of rosemary extract and blackseed essential oil were 162 and 28.2 mg GAE/g, respectively.

3.2. DPPH radical-scavenging

DPPH· is a stable radical showing a maximum absorbance at 515 nm. It can readily undergo reduction by an antioxidant (AH) which can be demonstrated by the following reaction (Frankel & Meyer, 2000),

$$\text{DPPH}\cdot + \text{AH} \rightarrow \text{DPPH} - \text{H} + \text{A}\cdot \quad (2)$$

Because of the ease and convenience of this reaction, it now has widespread use in free radical-scavenging assessment (Siddhuraju, 2007; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Bryne, 2006; Wu, Shiau, Chen, & Chiou, 2003). The disappearance of the DPPH radical absorption at 515 nm by the action of antioxidants is taken as a measure of antioxidant activity.

In the present study, the ability of test materials (pure antioxidants and plant extracts) to scavenge DPPH· was assessed on the bases of their IC_{50} values, defined above as the concentration of test material to decrease the absorbance at 515 nm (or concentration) of DPPH· solution to half of its initial value. These IC_{50} values were obtained utilizing a calibration curve prepared by plotting percent inhibition values calculated by Eq. (1) as a function of concentration of the test material. IC_{50} values of carnosic acid, rosmarinic acid, sesamol, rosemary extract and blackseed essential oil are given in Table 1. It can be seen that carnosic acid shows the highest antioxidant activity among the pure antioxidants according to the DPPH·-scavenging test. On the other hand, the antioxidant activity of rosemary extract is almost 10 times more effective (in DPPH· in scavenging) than that of blackseed oil. This can be attributed to the higher phenolic content of the former (162 mg GAE/g) than that of the latter (28.2 mg GAE/g). The close correlation between antioxidant activity and phenolic content of extracts obtained from various natural sources has been demonstrated by many workers (Liu et al., 2007; Verzelloni, Tagliazucchi, & Conte, 2007). It was reported that the solvent used in extraction may also be important in the antioxidant activity of the extract, depending on the phenolic content. For example Liu et al. (2007) found that phenolic and flavonoid contents of an endophytic *Xylaria* sp. were higher in methanol extracts than in hexane extracts.

With regard to antioxidant activity, based on the DPPH· test of pure compounds, it seems surprising that carnosic acid, with two phenolic hydroxyl groups, has a higher antioxidant power than rosmarinic acid, with four phenolic hydroxyl groups (see Fig. 1 for the structure of pure compounds). Similar unexpected results, based on the number of phenolic hydroxyl group in the structure of antioxidant molecule, were reported in the literature. For example, in the work of Brand-Williams, Cuvelier, and Berset (1995) on kinetic behaviour and antiradical power (based on $1/IC_{50}$ values) of 20 compounds toward their reaction with DPPH·, interesting results were found. Ascorbic acid, containing no phenolic hydroxyl group and known as a poor antioxidant, reacted rapidly with DPPH·, reaching a steady state in less than 1 min, whereas rosmarinic acid and δ-tocopherol, containing 4 and 1 phenolic –OH groups, respectively, showed only intermediate kinetic behaviours, reaching a steady state after 5 and 30 min, respectively. Again in the same work, it was found that caffeic acid, carrying two phenolic –OH groups, had a higher antiradical power than rosmarinic acid, carrying 4 phenolic –OH groups. It is clear that the number of phenolic –OH groups present in the structure of an antioxidant molecule is not always the only factor determining its antioxidant activity. Positions of phenolic –OH groups, presence of other functional groups in the whole molecule, such as double bonds and their conjugation to –OH groups and ketonic groups, also play important roles in antioxidant activities and have been demonstrated by Rice-Evans et al. (1996) in their extensive study on structure-antioxidant activity relationships of flavonoids and phenolic acids. In general, the above factors can be termed as the tension at the phenolic –OH groups. It is seen in Fig. 1 that the tension at the phenolic –OH group is increasing in the following order: Rosmarinic acid < sesamol < carnosic acid. In carnosic acid, two adjacent phenolic –OH groups are strained with an adjacent carboxylic group. In sesamol, one phenolic –OH group is strained with two oxygen atoms, although not adjacent. So, the order of the degree of strain on phenolic –OH groups of antioxidants is reflected in their antioxidant activity according to the present DPPH· test. Recently, the polarity and hydrophobicity of antioxidants, besides the above mentioned factors, were found to play important roles in their activity, especially in biomembrane systems (Wu, Huang, Lin, Ju, & Ching, 2007). Of course, in the present study, it is difficult to arrive at any general conclusions about structure-antioxidant activity relationships with the limited number of antioxidant molecules under consideration.

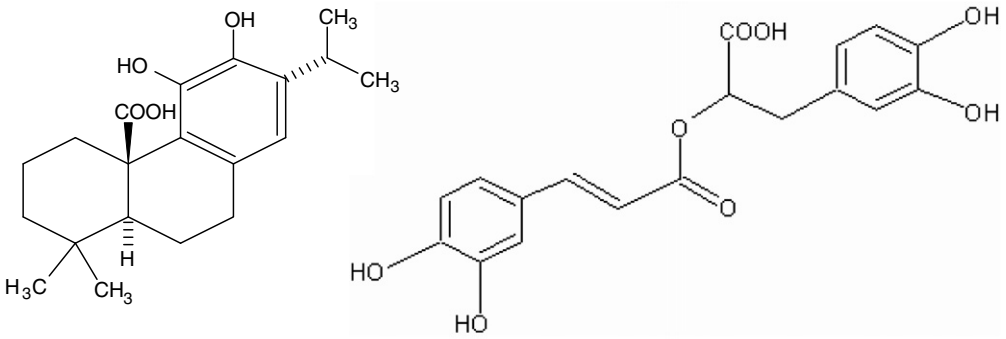
3.3. $ABTS^{\cdot+}$ radical-scavenging

This method measures the antioxidant activity of both water-soluble and lipid-soluble antioxidants, as well as

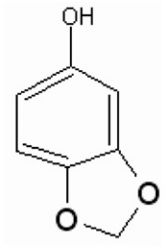
Table 1
Radical-scavenging activities of carnosic acid, rosmarinic acid, sesamol, rosemary extract and blackseed essential oil

Sample	Radical-scavenging activity[a]			
	DPPH·	$ABTS^{\cdot+}$ (TEAC, mM Trolox)		
	IC_{50} (μM)	1 min	4 min	6 min
Rosemary extract	54.0 ± 1.4[b]	15.5 ± 1.1	15.6 ± 1.1	15.7 ± 1.0
Blackseed essential oil	515 ± 20.1[b]	2.0 ± 0.7	2.4 ± 0.3	2.5 ± 0.6
Carnosic acid	33.1 ± 1.7	5.6 ± 1.0	5.7 ± 1.7	5.9 ± 1.2
Rosmarinic acid	72.3 ± 3.3	3.6 ± 0.4	3.7 ± 0.2	3.7 ± 0.7
Sesamol	48.0 ± 3.3	2.4 ± 0.8	2.4 ± 0.7	2.4 ± 0.4

[a] The results are given as mean of three measurements with 95% confidence intervals.
[b] Data presented in μg/ml units. For carnosic acid, rosmarinic acid and sesamol, TEAC is defined as the mM concentration of a trolox solution whose antioxidant activity is equivalent to a 1.0 mM solution. For rosemary and blackseed, TEAC is defined as the concentration of trolox whose antioxidant activity is equal to 1.0 mg/ml of sample solution.



carnosic acid rosmarinic acid



sesamol

Fig. 1. Molecular structures of carnosic acid, rosmarinic acid and sesamol.

extracts from natural sources, through the TEAC value, defined in the materials and methods section. Higher TEAC values demonstrate higher antioxidant activity. In order to obtain TEAC values, a calibration curve for each test material was derived from the percent inhibition (calculated from Eq. (1)) versus concentration plot. Then, using the calibration curves for each test material and for trolox, trolox equivalent antioxidant capacity (TEAC) for each material was obtained and these are given in Table 1. The time response of these values seems to be very small, in 1–6 min, indicating that the reaction between $ABTS^{\cdot+}$ and the inhibitor is almost complete within 1 min. Among the pure antioxidants, the highest activity is seen for carnosic acid (Table 1) according to TEAC values. This is in agreement with $DPPH^{\cdot}$ test results. Rosmarinic acid is intermediate and sesamol is the weakest antioxidant among the three pure compounds according to this test. It should be recalled that the reverse antioxidative order was observed for rosmarinic acid and sesamol according to the $DPPH^{\cdot}$ test. This is expected to originate from specific interactions of rosmarinic acid and sesamol with the radicals $DPPH^{\cdot}$ and $ABTS^{\cdot+}$. Such mismatches of antioxidant activity order between the two methods are also seen in the literature, usually without any satisfactory explanation (e.g. Wu et al., 2007).

TEAC results show that rosemary extract is a much more powerful antioxidant than is blackseed essential oil (Table 1), a result in agreement with $DPPH^{\cdot}$ test results which were explained on the basis of the higher phenolic content of rosemary extract than blackseed oil. Dorman, Peltoketo, Hiltunen, and Tikkanen (2003) reported slightly lower TEAC values (about 10–14) for rosemary extract than our values of about 15. The difference may result from using different solvents for extraction in the two works, acetone in the work of Dorman et al. (2003) and methanol in our work. We have already mentioned the importance of the type of solvent used in extraction, as demonstrated by the recent work of Liu et al. (2007) in which varying phenolic contents were determined in extracts of the same substance obtained using different solvents in the extraction.

3.4. Antioxidant activity in a linoleic acid system

The ferric thiocyanate test determines the antioxidant activity with the measurement of the amount of peroxides formed in a linoleic acid emulsion of antioxidant during incubation (Lee et al., 2004; Singh, Maurya, deLampasona, & Catalan, 2007), as described in the materials and methods section. The absorbances of the systems with various antioxidants and without any test material as control of the systems at 500 nm were plotted as a function of time in Fig. 2. The incubation period was 72 h at 40 °C. It is seen that absorbance increases with time, the highest increase being in the first 24 h for each system. As expected, the highest absorbances are seen for the control system without any test material at all times. In order to be more quantitative, the ratios of absorbance of each test material system to that of control system at 24, 48 and 72 h periods were collected in Table 2. This ratio is used as a measure of antioxidant activity. Obviously, the lower the ratio, the better is the antioxidant activity. An interesting order of antioxidant activity, quite different from that determined by $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ tests seen in Table 1, was observed

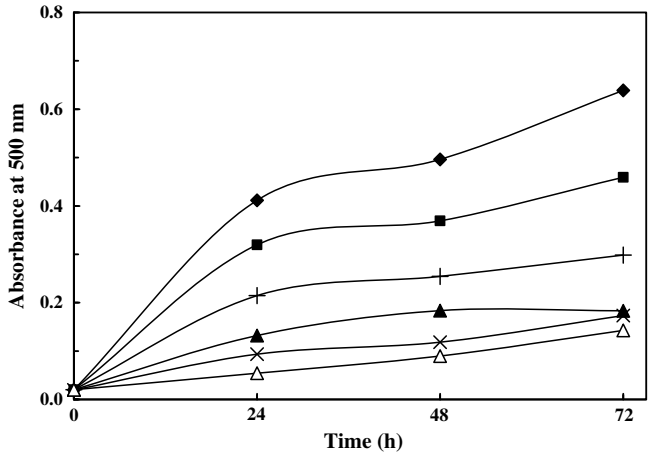


Fig. 2. Absorbance versus time plots for the antioxidant activities of various antioxidants at 500 nm.♦: control; ■: blackseed essential oil; ▲: rosemary extract; ×: carnosic acid; +: rosmarinic acid; Δ: sesamol.

Table 2
The ratios of absorbances of various antioxidants and extracts to the absorbance of control system at 500 nm determined by FTC test

Sample	$A_{sample}/A_{control}$		
	24 h	48 h	72 h
Rosemary extract	0.27 ± 0.06	0.37 ± 0.12	0.29 ± 0.07
Blackseed essential oil	0.78 ± 0.14	0.75 ± 0.14	0.72 ± 0.11
Carnosic acid	0.23 ± 0.08	0.24 ± 0.08	0.27 ± 0.10
Rosmarinic acid	0.52 ± 0.05	0.52 ± 0.10	0.47 ± 0.06
Sesamol	0.13 ± 0.08	0.18 ± 0.07	0.23 ± 0.13

The results are given as means of three measurements with 95% confidence intervals.

by the FTC test seen in Table 2. The increasing orders of antioxidant activity may be given as rosmarinic acid < carnosic acid < sesamol for the pure antioxidants and blackseed essential oil < rosemary extract for the plant extracts. The order for pure antioxidants may be explained on the basis of their hydrophobicity and thus their solubility in linoleic acid emulsions in accordance with the observation that polar antioxidants are more active in bulk oil systems whereas non-polar antioxidants (hydrophobic) are more active in lipid suspended in aqueous systems as reported by Frankel and Meyer (2000). When the structures of the three compounds in Fig. 1 are compared, it is seen that the most hydrophobic compound is sesamol with a single hydrophilic –OH group, followed by carnosic acid with two –OH groups and a –COOH group and then comes rosmarinic acid with four –OH groups and a –COOH group. It should be noted that solubility or hydrophobicity was not a factor in the previous tests of DPPH$^{\cdot}$ and ABTS$^{\cdot+}$. Rosemary extract had a higher antioxidant activity than blackseed essential oil by a factor of about 2, according to the FTC test. This result shows a parallelism with the results of DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ tests. However, the factor was much higher in the previous tests, about 10 in the DPPH$^{\cdot}$ test and about 6 in the ABTS$^{\cdot+}$ test. The decrease of the factor in the FTC test may again be attributed to the presumably higher hydrophilicity of rosemary extract, with a higher phenolic content, compared to blackseed essential oil.

4. Conclusion

It was found that, between the two extracts studied, rosemary extract had higher antioxidant activity than blackseed essential oil according to all three antioxidant activity tests. This was attributed to the higher phenolic content of the former. The order of antioxidant activity of the three pure antioxidants; rosmarinic acid, carnosic acid and sesamol, showed variations in the three tests. These orders were, rosmarinic acid < sesamol < carnosic acid in the DPPH$^{\cdot}$ test, sesamol < rosmarinic acid < carnosic acid in the ABTS$^{\cdot+}$ test and rosmarinic acid < carnosic acid < sesamol in the FTC test. These variations were attributed to the structural factors of the individual antioxidants.

With the present results it was difficult to reach any conclusion about the additive or synergistic contributions of individual antioxidants to the overall antioxidant activity of plant extracts since not all of their antioxidant components are known. Although, rosemary extract was found to contain about 6% carnosic acid and about 8% rosmarinic acid, it may also contain some other antioxidant components. On the other hand, blackseed essential oil was found to contain a considerable amount of thymoquinone as an antioxidant component which has a hydroquinone structure with low phenolic content. This is confirmed by its lower antioxidant activity than rosemary extract.

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