

# Antioxidant Activity of the Essential Oils of *Thymbra capitata*, *Origanum vulgare*, *Thymus mastichina* and *Calamintha baetica*

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## Abstract

Some aromatic plants such as *Thymbra capitata*, *Origanum vulgare*, and *Calamintha baetica* are used in some Portuguese traditional meat dishes, particularly in rabbit meat, tomato salads, fish food, escargot, and olives. In the present work, the antioxidant ability of the essential oils extracted from *T. capitata*, *O. vulgare*, *C. baetica*, and *Th. mastichina* cultivated in a field of the Regional Direction of Agriculture of Algarve (Portugal) was studied. The oils, extracted by hydrodistillation, were analysed by gas chromatography and gas-chromatography coupled to mass spectrometry. The antioxidant activity was determined using a modified thiobarbituric acid-reactive substances (TBARS) method, measuring the scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical method, determining the reducing power method, and monitoring the chelating effect on ferrous ions method. The results showed that the oils from *O. vulgare* and *T. capitata* had practically the same capacity of preventing the lipid oxidation of those of BHT and BHA. *T. capitata* oil was the most effective for the scavenging effect on DPPH as any capacity for this effect was practically absent in the oils of *Th. mastichina* and *C. baetica*. These results can be explained by the relative high concentrations of phenol compounds in those plants. The essential oil isolated from *C. baetica* possessed the best ability of chelating ferrous ions, something practically inexistent in the remaining other plant samples and the BHT and BHA. The reducing power of the essential oils was much lower than those observed for the synthetic antioxidants, nevertheless among the oils, those from *O. vulgare* and *T. capitata* were the most effective. The chelating effect of the *C. baetica* oil can be used as a synergistic antioxidant, that is, this oil can be used together with a phenolic antioxidant in the food product, but at lower levels.

## INTRODUCTION

In cellular systems, lipid peroxidation is of great importance, especially in bio-membranes where most of the oxygen-activating enzymes are present. These enzymes are responsible for the oxidative stress, leading to the formation of reactive oxygen species (ROS). Even with the protection mechanisms of cells, lipid peroxidation occur that, in some circumstances, can overcome the cellular defence system. The membrane

structures are destroyed and the function of the cell organelles is loosening with the lipid oxidation. Receptors present in the membrane are also released or inactivated. Induction of lipid peroxidation is linked to several diseases: rheumatoid arthritis, atherosclerosis, ischemia, carcinogenesis, and aging (Jadhav et al., 1995).

The oxidation of lipids in foodstuffs is also problematic, because it renders the product unacceptable for human consumption owing to the development of off-flavour (Madsen and Bertelsen, 1995). To avoid the lipid peroxidation, it is usual to add synthetic substances with antioxidant activity of low cost and high stability: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), gallats and *tert*-butylhydroquinines (TBHQ) in defined concentrations (Miguel *et al.*, 2003a). In the search of plants as a source of natural antioxidants, some medicinal plants and fruits have been extensively studied due to their antioxidant activities. Herbs and aromatic plants, which are largely spread and used in the Mediterranean countries, are of commercial interest for their essential oils (Parejo *et al.*, 2002).

A large number of reports concerning the antioxidant ability of spices and aromatic plants have been published. Several methods can be used and many times the comparison of the results of different experiments is complicated.

Some aromatic plants such as *Thymbra capitata*, *Origanum vulgare* and *Calamintha baetica* are used in some Portuguese traditional meat dishes, particularly in rabbit meat, tomato salads, fish food, “escargot” and olives. The antimicrobial and antioxidant activities of the essential oils isolated from some of these plants were already assayed by us, using different model systems (Miguel *et al.*, 2003a; Miguel *et al.*, 2003b; Miguel *et al.*, 2005; Faleiro *et al.*, 2005).

In the present work, the antioxidant ability of the essential oils of *T. capitata*, *O. vulgare*, *C. baetica* and *Th. mastichina* were followed through four different methods: antioxidant activity by modified thiobarbituric acid-reactive substances (TBARS) method, scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical method, reducing power method, and chelating effect on ferrous ions method.

## **MATERIALS AND METHODS**

### **Plant Material**

Plants grew up in the experimental field of “Direcção Regional de Agricultura do Algarve” (DRAALG), all of them in three different blocks with 25 plants each, in order to get a representative sample. All these plants are endogenous of Algarve. After flowering, plants were cut and dried in a solar drier in the same conditions.

### **Isolation of Essential Oils**

The oils were isolated from dried materials by hydrodistillation, for 4 h, using a Clevenger-type apparatus (Anonymous, 1996).

### **Sample Analysis**

The samples were injected after diluting 20 µl of oil into 1000 µl of n-pentane in a 20 ml vial containing 65 µm PDMS-DVB (polydimethylsiloxane – divinylbenzene) coated fibre. SPME fibre was then placed inside the head space of the vial for 20 min at  $20 \pm 2$  °C. The fibre was subsequently withdrawn into a needle and transferred to the injector of the GC and/or GC-MS, where the absorbed constituents were thermally desorbed from the fibre over 5 min. The percentage composition of the plant oil was computed by the normalisation of GC peak areas compared with standards.

### **GC-MS Analysis**

A Shimadzu 17-A chromatograph equipped with Shimadzu QP-500 mass spectrometer was used. The separation was achieved using a J&W Scientific DB-1701P column of 30 m x 0,25 mm i.d. and 0,25 µm of film thickness. GC oven temperature was programmed from 40 °C (5 min), to 230 °C at a rate of 5 °C/min and then 5 min at 230 °C. The carrier gas was helium with a column-head pressure of  $1.4 \times 10^5$  Pa.

Mass spectra were recorded in the electron impact (EI) mode at 70 eV, scanning the m/z 30 to 300. Interface temperature was 250 °C. Data acquisition and data processing were carried using Class5K programme.

Peaks in total ion current (TIC) or Multi Ion Chromatogram (MIC) profiles for both analyses were characterized or tentatively identified from their mass spectral data using National Institute of Standards and Technology (NIST12 or NIST62) and Wiley 229 mass spectrometry libraries. Identification was confirmed using standard compounds when available.

### **GC Analysis**

Gas chromatographic analysis was done on a Hewlett Packard 5890 Série II equipped with a FID detector and using helium as the carrier gas. The constituents were separated on 30 m x 0,25 mm i.d., 0,25 µm film thickness DB-1701P column from J & W Scientific. The injector temperature was set at 250 °C and all injections were made in split mode (split 30:1). The column was initially maintained at 50 °C for 5 min with subsequent increases to 210 °C at a rate of 5 °C/min and finally held for 5 min. FID Detector temperature was set at 270 °C. Data acquisition and data processing using Chromulan programme.

### **Antioxidant Activity**

For the DPPH assay, 50 µL samples of various concentrations of essential oils and tested substances in methanol were added to 2 ml of 0.004 % methanol solution of DPPH. After a 5 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in was calculated as percent inhibition =  $(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100$ .

For the TBARS assay, two sets of experiments using a modified TBARS assay were used to measure the antioxidant activity of essential oils and tested substances without and with the lipid peroxidation inducer 2,2'-azobis-(2-amidinopropane) dihydrochloride (ABAP). In both cases, egg yolk homogenates, prepared as described by Dorman et al. (1995), were used as a lipid-rich media. The antioxidant activity was measured spectrophotometrically at 532 nm as described by Baratta et al. (1998). All values were expressed as antioxidant index, where the control was completely peroxidized and each oil and tested substance demonstrated a comparative percentage of antioxidant protection. The antioxidant protection was calculated as antioxidant protection =  $(1 - t/c) \times 100$ , where c is the absorbance of the fully oxidized control and t is the absorbance of the test sample.

For determining the chelating activity, various concentrations of essential oils and tested substances in methanol were mixed with 3.7 ml of methanol and 0.1 ml of 2 mM  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ . The chemical reaction was allowed to proceed for 30 sec and then 0.1 ml of 5 mM ferrozine was added. After the solution was mixed and left stand for 10 min at room temperature, the absorbance of the mixture was read at 562 nm. A lower absorbance indicated a higher chelating power. Ethylenediaminetetraacetic (EDTA) was used as positive control, and an untreated sample served as the negative control (Mau et al., 2003).

To determine reducing power, various concentrations of essential oils and tested substances in methanol were mixed with 2.5 ml of 200 mM sodium phosphate (pH 6.6)

and 2.5 ml of 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 min followed by the addition of 2.5 ml of 10 % TCA and centrifugation at 22 g for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionised water and 0.5 ml of 0.1 % ferric chloride. The absorbance was measured at 700 nm. Higher absorbances of the reaction mixture indicated greater reducing powers (Duh et al, 2001).

## RESULTS AND DISCUSSION

### Chemical Composition of the Essential Oils

The main group of constituents present in the essential oil were monoterpenoids (Table 1) with *Th. mastichina*, and *T. capitata* containing mainly 1,8-cineole (41.0 %) and carvacrol (78.0 %), respectively. The two primary constituents of *O. vulgare* essential oil  $\gamma$ -terpinene (34.4 %) and thymol (31.8 %) and the primary constituents of *C. baetica* were 1,8-cineole and isopulegone at 30.4 % and 36.4 %, respectively.

### Antioxidant Activity of the Essential oils

Lipid oxidation was evaluated using more than one method because this oxidation occurs through several steps producing various types of products. The TBARS measure of lipid degradation by spectrophotometry at 532 nm monitors the formation of the pink pigment produced by the reaction of thiobarbituric acid (TBA) with malonaldehyde (MDA), a secondary lipid peroxidation product. Antioxidant indexes of the oil samples as well as those of the synthetic antioxidants generally used in food industry (BHA and BHT) in the absence of the lipid inducer. *T. capitata* and *O. vulgare* showed similar lipid antioxidant activity, comparable with the activity of BHT and BHA (Table 1).

The antioxidant index of *O. vulgare* oil was even better than BHA since all tested concentrations exceeded 90 %, results not detected for BHA primarily at the lowest concentrations (100 and 250 mg/l) assayed. *Th. mastichina* and *C. baetica* oils were less effective as antioxidants than other samples, probably due to their chemical composition in which the phenolic compounds do not predominate (thymol and carvacrol for *O. vulgare* and *T. capitata*, respectively). The presence of the inducer ABAP, in some way, restricted the capacity for preventing lipid oxidation (Fig. 2), except for the highest concentrations of *C. baetica* oil (Fig. 2).

Reduction of the DPPH radical, which accepts an electron of hydrogen radical to become a stable diamagnetic molecule, was determined by the decrease in its absorbance at 517 nm by antioxidants. Fig. 3 shows the scavenging effect of oil samples and the synthetic BHA and BHT. *T. capitata* and *O. vulgare* were the best samples in scavenging of the DPPH radical, however, not at a level comparable with the synthetic BHA and BHT. The differences of one percent between BHT, BHA, and *T. capitata* and *O. vulgare* oils were more significant for the lowest tested concentrations (Fig. 3). For the TBARS method and the DPPH method the oil samples of *Th. mastichina* and *C. baetica* showed the lowest free radical scavenging activity, meaning these oils are ineffective as hydrogen donors and as primary antioxidants by reacting with the lipid radical.

The chelating capacity of  $\text{Fe}^{2+}$  was absent in samples of BHA, BHT, *T. capitata* and *O. vulgare* (Fig 4). Our results were in agreement with previous studies (Chung et al., 2002) showed that BHT had no detectable  $\text{Fe}^{2+}$ -chelating activity. Only the essential oil of *C. baetica* showed a significant, detectable, concentration dependent  $\text{Fe}^{2+}$ -chelating effect, reaching a maximal value at 750 mg/L (42 %). Such results can only be explained by the chemical composition of the oils since the main components

are substantially different (Table 1). The *Th. mastichina* oil had some concentration dependent chelating activity, but was inferior to that observed for *C. baetica*.

The reducing power of essential oils increased with increasing levels (Fig. 5). With BHA and BHT, such an increase in reducing power was observed until 20 mg/L at which point a plateau was achieved. *T. capitata* and *O. vulgare* oils had the best reducing power, but nevertheless were significantly lower than the synthetic BHT and BHA. Practically no reducing power was observed for the oils of *C. baetica* and *Th. mastichina* (Fig. 5). Some reports have noted that the antioxidant effect is concomitant with the development of reducing power (Duh et al., 2001). Therefore, the antioxidant activity of *T. capitata* and *O. vulgare* may be related to their reducing power.

## ACKNOWLEDGEMENTS

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## Literature cited

- Anonymous. 1996. European Pharmacopoeia, 3<sup>rd</sup> ed., Council of Europe, Strasbourg, France. pp. 121-122.
- Baratta, M.T., Dorman, H.J.D., Deans, S.G., Figueiredo, A.C., Barroso J.G., and Ruberto, G. 1998. Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour and Fragrance Journal*, 13:235-244.
- Chung, Y.C., Chang, C.T., Chao, W.W., Lin, C.F. and Chou, S.T. 2002. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. *Journ. Agric. Food Chem.* 50:2454-2458.
- Dorman, H.J.D., Deans, S.G., Noble, R.C., Surai, P. 1995. Evaluation in vitro of plant essential oils as natural antioxidants. *Journ. Essential Oil Res.* 7:645-651.
- Duh, P.-D., Yen G.-C., Yen, W.-J., and Chang, L.-W. 2001. Antioxidant effects of water extracts from barley (*Hordeum vulgare* L.) prepared under different roasting temperatures. *Journ. Agric. Food Chem.* 49:1455-1463.
- Faleiro, L., Miguel, G., Gomes, S., Costa, L., Venâncio, F., Teixeira A., Figueiredo, A.C., Barroso, J.G., and Pedro, L.G. 2005. Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. *Journ. Agri. Food Chem.* 53:8162-8168.
- Jadhav, S.J., Nimbalkar, S.S., Kulkarni, A.D., and Madhavi D.L. 1995. Lipid oxidation in biological and food systems. In D. L. Madhavi, S. S. Deshpande, D. K. Salunkhe, ed. *Food Antioxidants - Technological, Toxicological, and Health Perspectives*.
- Madsen, H.L., and Bertelsen, G. 1995. Spices as antioxidants. *Trends in Food Science & Technology* 6:271-277.
- Mau, J.L., Lai, E.Y.C., Wang, N.P., Chen, C.C., Chang, C.H., and Chyau, C.C. 2003. Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. *Food Chemistry* 82:583-591.
- Miguel, M.G., Figueiredo, A.C., Costa, M.M., Martins, D., Duarte, J., Barroso, J.G., and Pedro, L.G. 2003a. Effect of volatile constituents isolated from *Thymus albicans*, *Th. mastichina*, *Th. carnosus* and *Thymbra capitata* in sunflower oil. *Nahrung/Food*, 47: 397-402.
- Miguel, M.G., Figueiredo, A.C., Costa, M.M., Martins, D., Duarte, J., Barroso, J.G., and Pedro, L.G. 2003b. Effect of the essential oils isolated from *Thymbra capitata* (L.) Cav. on olive and sunflower oils. *Grasas Y Aceites* 54:219-223.
- Miguel, M.G., Falcato-Simões, M., Figueiredo, A.C., Barroso, J.M.G., Pedro, L.G., and Carvalho, L.M.. 2005. Evaluation of the antioxidant activity of *Thymbra*

*capitata*, *Thymus mastichina* and *Thymus camphoratus* essential oils. *Journ. Food Lipids* 12:181-197.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burtillo, J., and Codina, C.. 2002. Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. *Journ. Agric. Food Chem.* 50:6882-6890.

Table 1. Volatile oil constituents of *Th. mastichina*, *T. capitata*, *O. vulgare*, and *C. baetica*.

Major component	<i>Th. mastichina</i>	<i>T. capitata</i>	<i>O. vulgare</i>	<i>C. baetica</i>
	(% of the total volatile oil)			
Tricyclene	t			
$\alpha$ -Thujene	0,3	1.5	1.3	0.1
$\alpha$ -Pinene	6.0	0.6	0.1	1.6
Camphene	5.5	0.1	0.3	0.2
$\beta$ -Pinene + <i>trans</i> -sabinene	7.0	0.2	0.5	4.7
$\beta$ - Myrcene	1.2	1.7	1.7	1.0
Monoterpene not identified	0.1	0.2	0.1	
$\alpha$ -Phellandrene			0.3	
$\beta$ -Terpinene	0.3	1.2	3.1	
Limonene	2.6	0.2	0.4	2.2
<i>cis</i> -Sabinene	0.3	0.2	0.2	
<i>trans</i> -Ocimene	0.1	4.8	6.0	
1-8 Cineole	41.0	0.3		30.4
<i>p</i> -Cymene			1.8	0.1
<i>cis</i> -Ocimene	0.9	t	0.5	
$\gamma$ -Terpinene	0.9	5.4	34.4	
3-Octanol				0.2
<i>allocimene</i>			0.1	
Terpinolene	0.2	0.1		
<i>trans</i> -Sabinene hydrate	1.1	0.6	0.1	0.1
$\alpha$ -Terpinolene				1.3
Linalool	3.9	0.7	0.7	
<i>cis</i> -Sabinene hydrate	0.5	0.2		
Citronellal				0.1
<i>trans</i> -Pinocarveol				0.4
Camphor	6.9			
Isopulegol				11.7
Terpinen-4-ol	3.2	0.6	0.8	
Geraniol			0.1	
<i>trans</i> - $\alpha$ -Terpineol	1.2			1.3
Borneol	6.5	0.2	0.3	
Isopulegone				36.4
Thymyl methyl ether			0.8	
<i>cis</i> - $\alpha$ -Terpineol	4.5	0.1	0.2	
Carvacrol methyl ether			1.7	
Isoborneol	0.1			
Myrtenal	t			
Pinocarveyl acetate			0.2	
Dihydrocarvone	0.1			
Pulegone				3.9
Verbonene	0.3			
<i>trans</i> -Caryophyllene	1.1	1.3	2.8	0.4
Thymol		0.2	31.8	
Carvacrol		78.0	0.2	
Germancrene			1.2	
$\beta$ -Bisabolene			2.8	

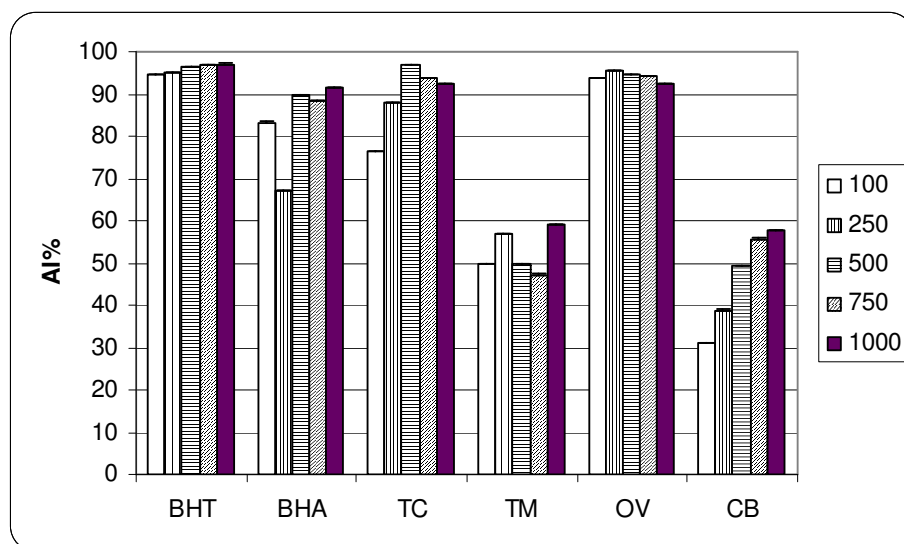


Fig. 1. Antioxidant index (AI%) of the essential oils of *T. capitata* (TC), *Th. mastichina* (TM), *O. vulgare* (OV), *C. baetica* (CB) and the synthetic antioxidants BHT and BHA, in different concentrations (mg/l), using TBARS assay without ABAP.

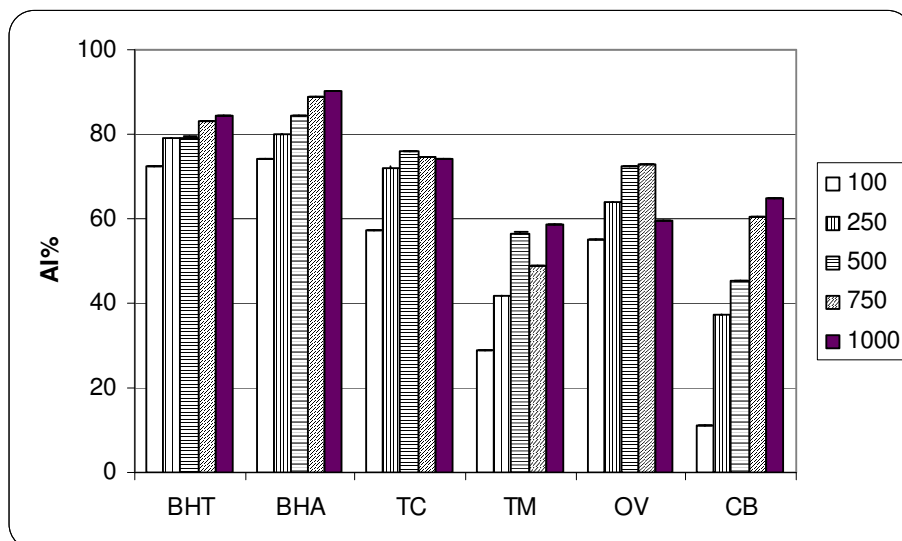


Fig. 2. Antioxidant index (AI%) of the essential oils of *T. capitata* (TC), *Th. mastichina* (TM), *O. vulgare* (OV), *C. baetica* (CB) and the synthetic antioxidants BHT and BHA, in different concentrations (mg/l), using TBARS assay with ABAP.



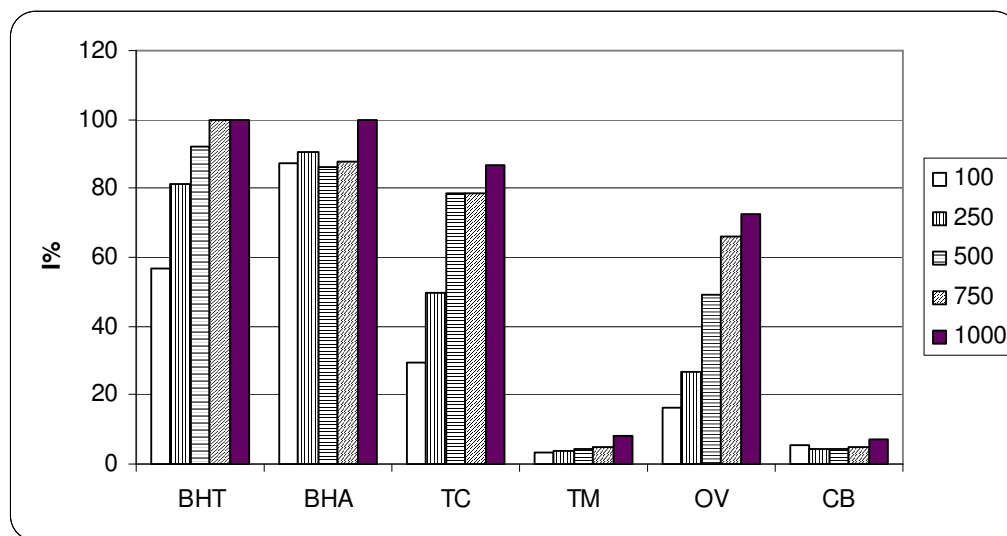


Fig. 3. Scavenging effect of the essential oils of *T. capitata* (TC), *Th. mastichina* (TM), *O. vulgare* (OV), *C. baetica* (CB) and the synthetic antioxidants BHT and BHA, in different concentrations, on DPPH radical.

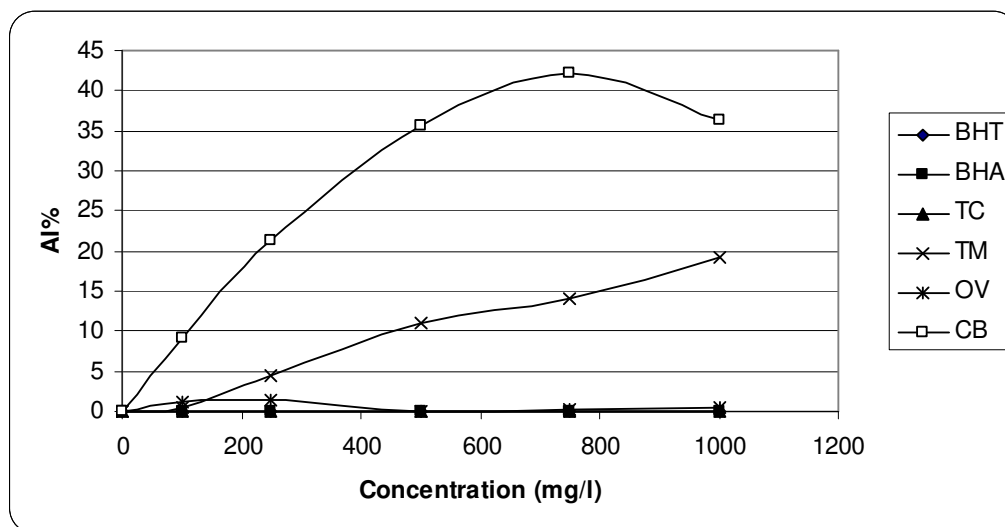


Fig. 4. Chelating activity of *T. capitata* (TC), *Th. mastichina* (TM), *O. vulgare* (OV), *C. baetica* (CB) and the synthetic antioxidants BHT and BHA, in different concentrations, on ferrous ion.

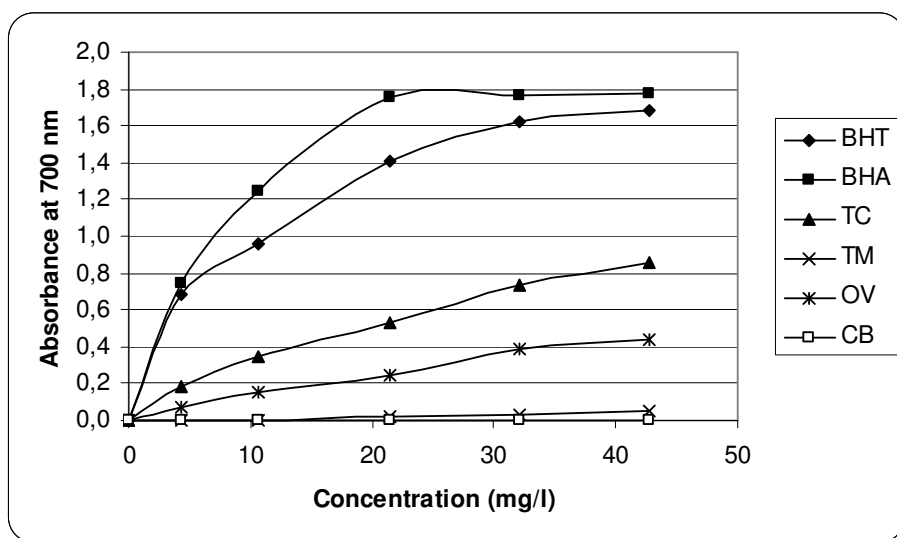


Fig. 5. Reducing power of *T. capitata* (TC), *Th. mastichina* (TM), *O. vulgare* (OV), *C. baetica* (CB) and the synthetic antioxidants BHT and BHA, in different concentrations.