J. Mater. Environ. Sci. 7 (8) (2016) 2908-2912
ISSN : 2028-2508
CODEN: JMESCN

Antioxidant properties of *Artemisia herba-alba* Asso., *Mentha pulegium* L. and *Origanum compactum* Benth. essential oils

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Received 10 May 2016, Revised 01 Jul 2016, Accepted 02 Jul 2016
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Abstract

There is an increasing demand for natural products to replace synthetic antioxidant preservatives. The present work investigates the antioxidant activities of three commercial essential oils (*Artemisia herba-alba*, *Mentha pulegium* and *Origanum compactum*) from Morocco by three different methods, namely DPPH free radical scavenging, β-carotene/linoleic acid and TBARS assays. In all methods investigated, *O. compactum* essential oil exhibited higher antioxidant activity than that of other tested essential oils. This activity could be attributed to the phenolic compounds present at high concentrations in this oil. In conclusion, *O. compactum* essential oil may be worth further investigation and could be a good natural alternative to chemical additives.

Keywords: *Artemisia herba-alba*, *Mentha pulegium*, *Origanum compactum*, antioxidant activity, essential oils.

Introduction

Free radicals are molecules with unpaired electron in their outer orbit. Free radicals are formed naturally in the body and are important intermediates in natural processes involving control of vascular tone, cytotoxicity and neurotransmission [1]. However, at high concentrations, free radicals can cause damage to all cell structures including DNA, proteins, and cell membranes, which cause many diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, cardiovascular diseases, chronic inflammation, aging and other degenerative diseases in humans [2,3]. Oxidation, mediated by free radical reactions is also responsible for the spoilage and quality deterioration of unpreserved food rich in unsaturated fatty acids, and many synthetic antioxidant components have showed toxic and/or mutagenic effect [4,5]. Therefore, there is a growing interest in the natural substances with antioxidant properties, which are supplied as food components or as specific preventative pharmaceuticals [6].

Essential oils have received special attention due to their multifunctional properties other than their classical roles as food additives and/or fragrances. Besides the antibacterial, antifungal, and anti-inflammatory activities [7-12], many essential oils also have been confirmed to possess the antioxidant activity [13,14].

Because of its geographical location, Morocco has favorable conditions for the development of a rich and varied flora with a significant potential of medicinal and aromatic plants. The exploitation of these natural biological resources became an important and promising sector for the country. *Artemisia herba-alba* (white wormwood), *Mentha pulegium* (pennyroyal) and *Origanum compactum* (oregano) are among the plants more commonly used in the production of essential oils and aromatic extracts [15].

In this context, the aim of this study was to investigate the antioxidant activities of *A. herba-alba*, *M. pulegium* and *O. compactum* essential oils by three complementary methods, namely: DPPH free radical scavenging, β-carotene-linoleic acid and thiobarbituric acid reactive species (TBARS) assays.

2. Materials and methods

2.1. Essential oils

Essential oils used in this study were provided by Santis Company. They were extracted by steam distillation from flowering aerial parts (flowers, leaves and stems) of *A. herba-alba*, *M. pulegium* and *O. compactum*. Chemical composition of these essential oils was determined in our previous work. The major components were: α-thujone (59.07%) for *A. herba- alba* essential oil, pulegone (78.07%) for *M. pulegium* essential oil, carvacrol (43.97%), p-cymene (17.78%) and thymol (11.56%) for *O. compactum* essential oil [16,17]

2.2. DPPH free radical scavenging activity

The DPPH radical scavenging assay was determined according to the method reported by Brand-Williams et al. [18] with some modification. 100 µL each essential oil at different concentration or Methanol (negative control) were mixed with 1.3 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution (0.004%). Absorbance measurements were read at 517 nm, after 30 min of incubation in the dark at room temperature. Ascorbic acid was used as positive control, and Methanol as negative control. All analyses were carried out in triplicate and results were expressed as mean ± SD. The percentage of inhibition of the DPPH radical was calculated according to the formula:

$$\%\,\boldsymbol{Inhibition} = \left(\frac{\boldsymbol{Ab - Aa}}{\boldsymbol{Ab}}\right) \times \mathbf{100}$$

Where Ab is the absorption of the blank sample and Aa is the absorption of the tested oils. The sample concentration providing 50% inhibition (IC50) was calculated by plotting inhibition percentages against concentrations of the sample.

2.3. β-carotene / linoleic acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [19]. 25 mg of linoleic acid and 200 mg of Tween 40 were added and mixed to 1 mL of the chloroform solution with 0.5 mg of β-carotene. After chloroform was removed by using a rotary evaporator at 40 °C, 100 mL of distilled water was added with vigorous agitation. 2.5 mL of the emulsion obtained was transferred to tubes containing 350 µL of various concentrations of tested essential oils. Butylhydroxytoluene (BHT) was used as positive control and Methanol as negative control. The absorbance values were measured at 490 nm after 1 h, 2 h, 3 h, 4 h, 6 h, 24 h and 48 h of incubation at ambient temperature. Antioxidant activity (AA) was calculated using the formula:

$$\boldsymbol{AA}\% = (\boldsymbol{Ae/Ac}) \times \mathbf{100}$$

Where Ae is the absorbance in the presence of essential oils and Ac is the absorbance in the presence of BHT used as positive control. All tests were carried out in triplicate.

2.4. TBARS assay

Thiobarbituric acid reactive species (TBARS) assay was determined according to the method reported by Okhawa *et al*. [20] using egg yolk homogenates as lipid rich media. The egg yolk (10%, v/v) solution was prepared in KCl (1.15%, w/v). It was homogenized for 30 s and ultrasonicated for 5 min. 500 µL of the homogenate were added to 100 µL of essential oil dissolved in methanol and the volume was made up to 1 mL with distilled water. 0.5 mL of this mixture was added to 0.5 mL of trichloroacetic acid (20%, v/v) and 1 mL of TBA (0.67%). Samples were vortexed and left in a 100°C water bath for 15 min. After cooling, 4 ml of n-butanol was added to each tube then extensively vortexed and centrifuged at 3000 rpm for 15 min. Absorbance of the organic upper layer was measured at 530 nm. Antioxidant Index percentage (AI %) was calculated using the following formula:

$$\boldsymbol{AI}\,\% = (\boldsymbol{A0 - A1/A0}) \times \mathbf{100}$$

Where A0 is the absorbance of the sample completely oxidized and A1 is the absorbance of test sample.

2.5. *Statistical analysis*

All the experiments were conducted in triplicate and data are presented as mean ± standard deviation (SD). Statistical analysis was performed by Analysis of Variance (ANOVA) using SPSS 20.0 software. Significant

differences between means were determined by Tukey post hoc tests. For the percentage values, to ensure homogeneity of variance, the data has been arsine transformed before statistical analysis. Differences at $P < 0.05$ were considered statistically significant.

3. Results and discussion

Essential oils are complex of mixture with different functional groups, polarity and chemical behaviors, and this could lead to scattered results, depending on the test employed. Therefore, the use of multiple methods is necessary in the assessment of antioxidant activity.

The tested essential oils were subjected to screening for their possible antioxidant activity by three test systems, namely DPPH free radical scavenging, β-carotene/linoleic acid and TBARS assays.

In DPPH test, the antioxidants react with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant [21]. The concentrations that led to 50% inhibition (IC50 values) are given in Table 1. The scavenging ability of all the samples showed a concentration-dependent activity profile. Lower IC50 indicating the higher antiradical activity. The strongest free radical scavenging activity was exhibited by *O. compactum* essential oil with an IC50 value of 0.021 ± 0.004 mg/mL. In fact, this essential oil was significantly more active than ascorbic acid used as positive control (IC50 = 0.14 ± 0.001 mg/mL). *M. pulegium* essential oil presented interesting antiradical activity (IC50 = 16.03 ± 1.74 mg/mL) while *A. herba-alba* showed the lowest DPPH scavenging ability (IC50=77 ±3.69 mg/mL).

Table 1: Antioxidant activity of essential oils by DPPH test

Antioxidant test	*A. herba-alba*	*M. pulegium*	*O. compactum*	Ascorbic acid
DPPH assay (IC_{50} mg/mL)	77 ± 3.69a	16.03 ± 1.74b	0.021 ± 0.004c	0.14 ± 0.001d

Values represent mean ± standard deviation of three replicates. Different letters (a-d) indicate a significant difference between the antioxidant activities ($p < 0.05$).

In β-carotene/linoleic acid model system, β-carotene undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. As a result, β-carotene is oxidized and broken down in part; subsequently the system loses its chromophore and characteristic orange color, which is monitored spectrophotometrically [22]. Results are given in Table 2.

Table 2: Antioxidant activity of essential oils by β-carotene/linoleic acid test

Antioxidant test	*A. herba-alba*	*M. pulegium*	*O. compactum*	Ascorbic acid
β-carotene/linoleic acid assay (I%)	61.89% ± 0.55a	69 % ± 0.76b	84.55% ± 1.02c	98.13% ± 0.94d

Values represent mean ± standard deviation of three replicates. Different letters (a-d) indicate a significant difference between the antioxidant activities ($p < 0.05$).

These results are consistent with data obtained from the DPPH test, *O. compactum* essential oil was the most active (I% = 84.55± 1.02 %), although it was less potent than the reference antioxidant BHT (I% = 98.13 ± 0.94%). This was followed by *M. pulegium* essential oil which showed a moderate activity (I% = 69 ± 0.76 %), while *A. herba-alba* essential oil showed the weakest activity potential (I% = 61.89 ± 0.55 %)

The TBARS assay measures by spectrophotometry at 530 nm the formation of the pink pigment produced by the reaction of thiobarbituric acid (TBA) with Malondialdehyde (MDA). Malondialdehyde (MDA) is an indicator of lipid peroxidation formed during oxidative degeneration as a product of free oxygen radicals. The intensity of the pink pigment formed from MDA-TBA condensation indicates the extent of lipid peroxidation [23]. The results showed that the tested essential oils exhibited a dose-dependent inhibition of TBARS formation. As can be seen from the Table 3, results are in agreement with those obtained in the previous tests. *O. compactum* essential oil was the most active (I50 = 305.32 ± 3.21 mg/mL) followed by those obtained from *M. pulegium* (I50 = 922.79 ± 1.50 mg/mL) and *A. herba-alba* (I50 = 985.94 ± 1.72 mg/mL) which were less effective.

Table 3: Antioxidant activity of the essential oils by TBARS assay

Antioxidant test	*A. herba-alba*	*M. pulegium*	*O. compactum*
TBARS (I_{50} mg/mL)	985.94 ± 1.72a	922.79 ± 1.50b	305.32 ± 3.21c

Values represent mean ± standard deviation of three replicates. Different letters (a-c) indicate a significant difference between the antioxidant activities ($p < 0.05$).

Previous studies investigated the antioxidant activity of essential oil and organic extracts of *O. compactum*. In fact, Bouhdid et al. have studied the antioxidant activity of *O. compactum* essential oil by three methods namely: reducing power, DPPH free radical scavenging assay and β-carotene / linoleic acid assay. The results showed that *O. compactum* has a good antioxidant activity in all tests used [24]. Also, Elbabili et al. have studied the antioxidant activity of *O. compactum* essential oil, Ethyl acetate, Petroleum ether, Ethanol extract and decoction using ABTS (acid 2,2'-azino-bis(3-éthylbenz-thiazoline-6-sulfonique) radical scavenging assay and DPPH free radical scavenging. *O. compactum* essential oil showed a high antioxidant activity with an IC50 value of 2 ± 0.1mg/L [25] .

The strong antioxidant activity of *O. compactum* essential oil could be attributed mainly to the high content of phenolic components. Several studies showed the linear positive correlation between the phenolic content and the antioxidant capacity of plant extracts. Phenols are organic compounds that contain a hydroxyl group bound directly to the aromatic ring, and the H-atom of the hydroxyl group can trap peroxyl radicals, preventing other compounds to be oxidized [26]. Phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen or electron donating agents, and metal ion chelating properties [27]. Carvacrol (43.97%) and thymol (11.56%) the main components of *O. compactum* essential oil are shown to act as strong antioxidants [28, 29]. Those phenols are in fact responsible for the antioxidant activity of many essential oils which contain them [30-32]. The other major component p-cymene has slight antioxidant activity [33]. In general, the antioxidant activity of essential oils is the product of additive, synergistic and/or antagonistic effects since they are complex mixtures of several classes of compounds.

At the present work, the antioxidant activity of essential oil of *M. pulegium* from Morocco was higher when compared to that of *M. pulegium* essential oil from Iran, which was able to scavenge 14736 ± 156 µg/mg of the free radical DPPH and caused only 26.01% of lipid peroxidation inhibition [34]. In other study, essential oil of *M. pulegium* from Morocco was effectively able to inhibit the linoleic acid oxidation and caused 66.88 ± 1.1% of inhibition [35]. Previous studies reported a wide variation in the antioxidant activity of *M. pulegium* essential oil. In fact, the essential oil of *M. pulegium* from Tunisia exhibited higher antioxidant activity [36]. On the other hand, *M. pulegium* essential oil and cold water extracts presented poor antioxidant activity, while ethanolic extract and hot water extract were classified as moderate and very strong antioxidants, respectively [37]. Pulegone (78.07%) the major component of our *M. pulegium* oil has been demonstrated to have moderate antioxidant activities [33].

Low antioxidant capacity of *A. herba-alba* essential oil, demonstrated in our study, is in agreement with previous studies [38]. Thujone present in high concentrations in this oil is known for its low antioxidant potential [33]. Similar results were found with essential oils of other *Artemisia* species such as *A. absinthium*, *A. biennis*, *A. cana*, *A. dracunculus*, *A. frigida*, *A. longifolia* and *A. ludoviciana* [39].

Conclusion

In the current study, we have screened antioxidant activities of essential oils of *A. herba-alba*, *M. pulegium* and *O. compactum* from Morocco by three tests, namely DPPH free radical scavenging, β-carotene/linoleic acid and TBARS assays.

The results demonstrate the strong antioxidant activity of *O. compactum* essential oil compared to the moderate and weak antioxidant activities of *M. pulegium* and *A. herba alba* essential oils, respectively. Thus, this study suggests that *O. compactum* essential oil has a huge potential as natural alternative to synthetic preservatives. Further studies are required to evaluate the safety and critical toxicity of this oil for human consumption.

Acknowledgements-The authors are grateful to Prof. Ababou and Prof. Boukachabine (Faculty of Sciences and Technologies of Settat) for their collaboration. Thanks are also due to *Santis* Company for providing essential oils.

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