



Tunisian essential oils as potential food antimicrobials and antioxidants and screening of their element profile

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Abstract

The potential employment of 18 commercial Tunisian essential oils (EOs) as natural food preservatives was investigated. Their antimicrobial activity was evaluated against two food-borne bacteria (*Escherichia coli* O157:H7 and *Klebsiella pneumoniae*) by the agar-well diffusion method and, subsequently, by determining the minimum inhibitory concentration (MIC); while their antioxidant activity was assayed by the DPPH test. Also, the main volatile constituents and inorganic elements were elucidated and discussed in relation to the biological activities. Excluding the wormwood EO, all EOs inhibited the growth of at least one reference strain. In particular, spearmint, sage and rosemary EOs had the lowest MICs against *E. coli* O157:H7 (0.09, 0.09, 0.07 mg mL⁻¹, respectively); whereas sage EO was effective against *K. pneumoniae* at 0.41 mg mL⁻¹. All EOs displayed also a promising antioxidant activity. Specifically, spearmint, bigroot geranium, and sage EOs showed the lowest IC₅₀ values (0.024, 0.046 and 0.052 mg mL⁻¹), and a behavior similar to that of butylated hydroxytoluene. EOs exhibited variegated and peculiar element profiles. Among minerals, Mg and K were in the range 1.72–14.12 mg Kg⁻¹ and 0.57–20.90 mg Kg⁻¹; while Cu and Fe were generally the most abundant trace essential metals (0.07–1.02 mg Kg⁻¹ and 0.20–2.98 mg Kg⁻¹, respectively). Very low and safe levels of heavy metals were also revealed. A statistical correlation analysis pointed out a significant positive correlation between some elements (K, Cu, and Fe) and the biological activities displayed. In conclusion, the selected Tunisian EOs had a marked potential applicability as antibacterial and antioxidant additive in food industry.

Keywords Tunisian essential oils · Natural food preservatives · Antibacterial agents · Antioxidant agents · Volatile compounds · Inorganic elements

Introduction

In the last decades, an escalated demand for minimally processed food characterized by a higher safety and quality and extended shelf life as well, have led to a negative consumer perception for synthetic preservatives, thus generating interest toward naturally occurring alternatives [1]. Ideally, a

natural preservative should (i) be effective at low concentration, (ii) be economical, (iii) induce no sensory changes in the product, (iv) inhibit a wide range of pathogenic and spoilage microorganisms, and (v) be non-toxic [2].

Natural additives may be obtained from animals [3, 4], bacteria [5, 6], algae [7, 8], fungi [9], and not least higher plants. In this respect, a remarkable diversity of species [10], as well as derived by-products [11–13] and essential oils, make the plant kingdom the most abundant source of natural food preservatives.

Essential oils (EOs), in particular, are extracted from the plant material (e.g., leaves, barks, stems, roots, seeds, flowers or fruits) of ~ 17,000 species, belonging to the most common angiosperm families, such as Lamiaceae, Myrtaaceae, Asteraceae, Zingiberaceae and Rutaceae, [14] and of a variety of gymnosperm species from families such as Pinaceae, Cupressaceae and Taxaceae [15]. They are known to be

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complex mixtures of volatile secondary metabolites, which are marked by a high structural diversity (e.g., hydrocarbons, alcohols, aldehydes, esters, ketones, terpenes, and phenolics) conferring them a lipophilic nature, a distinctive odor and taste, and not least precious antimicrobial and antioxidant properties [2, 16].

EOs have always been regarded throughout the history with peculiar interest and it is generally accepted that they have been produced since the dawn of humanity for a variety of uses. Aside from the undeniable and solid application of EOs in the manufacture of perfumes and cosmetics, many uses have been lost over times, while others have strengthened, such as the employment of EOs for protecting the agri-food commodities from microbial, and oxidative deterioration [16, 17].

From this perspective, scientific literature has reported a variety of EOs characterized by a more or less pronounced biological activity against food-borne pathogens and radical scavenging effects as well, both *in vitro* and in food systems [18–20]. As natural preservatives, EOs would benefit from a wide consumer acceptance, as they are (i) a bio-resource that may reduce the use of synthetic additives, (ii) characterized by a relatively high volatility, ephemeral, and biodegradable traits, (iii) generally recognized as safe (“GRAS” category) according to the US Code of Federal Regulations [21], and (iv) in line with the principles of green economy [16, 17]. Nevertheless, some limitations still exist. The addition of EOs could induce sensory changes in food, due to the peculiar and pronounced odor and flavor. However, such a flaw may be overcome by masking the EO presence with other approved aromas, as suggested by previous research [1, 22]. Additionally, the chemical variability of EOs, related to factors such as geographical origin, pedoclimatic context, age, variety and harvesting time of the plant, and, not least, extraction technology, may in turn influence their overall quality and biological activity [23]. Hence, the chemical profile of a certain EO should be characterized and, as much as possible, standardized to avoid a potential deviation from the expected outcome [16].

Besides the unquestionably relevant study of the volatile-active fraction, the screening of inorganic elements may also contribute to reliably prove the effectiveness and safety of EOs in food. The presence of such minor components in EOs may depend on variables such as the genotype of the plant, nature of the soil, climatic conditions, agronomic techniques, and not least storage and extraction procedures [24]. Certain metals (e.g., Fe, Cu, and Zn) are notoriously provided with antimicrobial and/or antioxidant activity [25–27] and may positively affect the biological activity of EOs; while other elements (e.g., Cr, Ni, As, Cd, and Pb) are known to cause toxic effects at relatively low levels through the entire food

chain [28, 29] and may threaten the safety of EOs in the same way as other organic contaminants [24, 30–33].

Aim of this study was to evaluate a variety of EOs available on the Tunisian market as potential food preservatives, by elucidating the *in vitro* antimicrobial and antioxidant activity in relation to volatiles already known for their marked bioactivity. Also, a screening of minerals and essential trace metals, along with the assessment of potentially toxic elements, was performed by inductively coupled plasma mass-spectrometry (ICP-MS), to assess the potential correlation of such elements with the biological activity, and to evaluate the safety of investigated EOs as well.

Material and methods

Plant materials and extraction of EOs

Various materials from 18 Mediterranean plant species was sampled from February 2019 to October 2019 in the area surrounding Mahdia city (Mahdia governorate, North-Eastern Tunisia) (Table 1). The relative voucher specimens were botanically identified and deposited at the Herbarium of the Institute of Applied Sciences and Technology of Mahdia (University of Monastir, Tunisia). Three homogeneous aliquots of plant material from each species were collected at the characteristic balsamic time over the entire study period, so that each EO was represented by triplicate samples.

The EOs were produced by Aroma CAP company (Mahdia, Tunisia), and they are also currently available on the Tunisian market. Briefly, around 50 g of fresh plant material were packed in a reactor (capacity 1.5 L), connected via Pyrex tubes to an electrical steam generator and a conventional condenser. The condenser was in turn connected to a receiving Clevenger-type apparatus to enabling the continuous collection of the condensate mixture EO-distilled water. In this system, pressurized steam was circulated through the plant material at a flow rate of 25 g min⁻¹. Hence, the vapors of the pure EO along with steam were condensed and received in the Clevenger-type apparatus, where the EO film laying on the surface of the distilled water was skimmed off the top. The extraction procedure was conducted until no more EO was obtained. Then, the EO was collected in amber vials, dried under anhydrous Na₂SO₄ and stored in a cool place (4 ± 1 °C), away from any light source, until further use. For every type of EO, the yield and the volatile profile were provided in Table 1. In particular, the percent yield was calculated by dividing the EO weight with the initial plant weight (w/w), whereas the percent composition of the main volatiles was determined by an internal GC-MS method (see Online Resource 1 for further details).

Table 1 List of the Tunisian EOs investigated in this study

| No. | Plant species | Plant material | Common name | % Yield ($n=3$) | % Composition ($n=3$) |
|-----|--|----------------|--------------------|-------------------|--|
| 1 | <i>Cupressus sempervirens</i> L | Leaves | Cypress | 1.6 | α -Pinene (35.89), δ -3-carene (15.65), α -terpinolene (5.12), myrcene (4.11), β -pinene (2.38) |
| 2 | <i>Eucalyptus globulus</i> Labill | Leaves | Tasmanian Blue Gum | 2.0 | 1,8-Cineol (37.76), camphene (23.62), α -pinene (14.16), globulol (5.18), β -pinene (3.42) |
| 3 | <i>Mentha spicata</i> L | Leaves | Spearmint | 1.9 | Carvone (77.14), limonene (10.55), 1,8-cineol (3.03) |
| 4 | <i>Mentha pulegium</i> L | Leaves | Pennyroyal mint | 2.9 | Pulegone (58.66), neo-menthol (23.11), iso-menthone (15.72), menthone (5.65) |
| 5 | <i>Mentha \times piperita</i> L | Leaves | Peppermint | 1.0 | Menthol (38.12), mentone (28.19), carvone (10.89), 1,8-cineole (8.41) |
| 6 | <i>Laurus nobilis</i> L | Leaves | Laurel | 0.8 | 1,8-Cineol (58.10), α -terpinyl acetate (13.26), sabinene (10.4), α -pinene (6.32), β -pinene (5.8), α -terpineol (4.11), |
| 7 | <i>Geranium macrorrhizum</i> L | Leaves | Bigroot geranium | 1.3 | Germacrone (22.17), trans- β -elemenone (15.81), γ -elemene (3.17), thymol (2.08), γ -curcumene (1.97) |
| 8 | <i>Pinus sylvestris</i> L | Needles | Scots pine | 0.7 | α -Pinene (36.51), δ -3-carene (18.72), camphene (10.57), limonene (7.37), β -pinene (4.28), sabinene (2.01), α -terpineol (1.32) |
| 9 | <i>Salvia officinalis</i> L | Herbs | Sage | 0.8 | Camphor (25.05), α -thujone (20.27), 1,8-cineol (14.31), β -thujone (5.96), α -humulene (3.78), borneol (2.97) |
| 10 | <i>Thymus vulgaris</i> L | Herbs | Thyme | 2.8 | Thymol (37.10), p-cymene (28.37), γ -terpinene (20.17), carvacrol (4.34) |
| 11 | <i>Artemisia herba-alba</i> Asso | Herbs | Desert wormwood | 0.7 | α -Thujone (16.73), chrysanthenone (9.56), β -thujone (7.01), trans-pinocarveol (5.18), camphor (4.32), filifolone (2.17) |
| 12 | <i>Rosmarinus officinalis</i> L | Herbs | Rosemary | 1.3 | 1,8-Cineol (44.48), α -pinene (15.85), camphor (10.70), borneol (8.95), linalool (5.16), β -pinene (2.37) |
| 13 | <i>Citrus paradisi</i> L | Fruits | Grapefruit | 1.5 | Limonene (93.19), β -pinene (3.18), myrcene (1.9) |
| 14 | <i>Citrus limon</i> L | Fruits | Lemon | 1.4 | Limonene (76.25), β -pinene (9.52), myrcene (1.3) |
| 15 | <i>Juniperus communis</i> L | Berries | Juniper | 0.6 | α -Pinene (55.69), limonene (6.58), β -pinene (3.88), myrcene (3.18), δ -3-carene (2.46) |
| 16 | <i>Syzygium aromaticum</i> L | flower buds | Clove | 1.8 | Eugenol (78.89), eugenyl acetate (16.96), β -caryophyllene (2.21) |
| 17 | <i>Lavandula officinalis</i> Chax | Flower | Lavender | 1.1 | Linalool (52.61), linalyl acetate (18.05), camphor (10.56), borneol (7.24), α -terpineol (5.13), 1,8-cineol (1.25) |
| 18 | <i>Zingiber officinale</i> Roscoe | roots | Ginger | 2.9 | α -Zingiberene (20.51), α -curcumene (14.52), geranial (11.76), α -farnesene (7.45), camphene (6.66), geranyl acetate (3.74), geraniol (1.02) |

For every EO, yield ($n=3$, %) and composition of the main volatiles ($n=3$, %) are also indicated

Material and reagents

For antimicrobial assay: Mueller–Hinton agar (MHA) and Mueller–Hinton broth (MHB) and dimethyl sulfoxide (DMSO) were from Merck (Darmstadt, Germany).

For antioxidant assay: 2,2-diphenyl-1-picrylhydrazyl (DPPH), and butylated hydroxy toluene (BHT) were supplied by Sigma-Aldrich (Steinheim, Germany).

For multi-element analysis: nitric acid (HNO_3 , 65% v/v) and hydrogen peroxide (H_2O_2 , 30% v/v) were of Suprapur grade and purchased from J. T. Baker (Mallinckrodt

Baker, Milan, Italy). Ultrapure water ($< 5 \text{ mg L}^{-1}$ Total Organic Carbon (TOC) was obtained through a Barnstead Smart2Pure 12 system (Thermo Scientific, Monza, Italy). Commercial standard solutions of inorganic elements were from Supelco (Bellefonte, PA, USA). A stock standard solution of Hg ($\text{Hg}(\text{NO}_3)_2$ in 10% HNO_3 , 2 mol L^{-1} , 1000 mg L^{-1}) available from Merck-Millipore (Darmstadt, Germany) served for DMA measurements. Before use, the equipment for sample collection, handling and storage, as well as laboratory glassware and polytetrafluoroethylene (PTFE) digestion vessels, were washed with 5% HNO_3 for

at least 12 h, rinsed with ultrapure water, and then dried at room temperature.

Antimicrobial assay

Reference strains of undesirable Gram-negative food-borne bacteria such as *Escherichia coli* O157:H7 (ATCC 43895) and *Klebsiella pneumoniae* (ATCC 700721), were maintained in their appropriate agar slants at 4 °C throughout the study and used as stock cultures.

Relative inocula were prepared by culturing stock strains overnight at 37 °C in MHB. The resulting suspensions were diluted with MHB to match with 2 McFarland turbidity standard ($\sim 6 \times 10^8$ cfu mL⁻¹).

The in vitro antibacterial activity of selected EOs was then evaluated by the agar-well diffusion method and, subsequently, by the determination of the minimum inhibitory concentration (MIC). Briefly, the fresh overnight inoculum of every reference strain ($\sim 10^8$ cfu mL⁻¹) was plated onto MHA in three directions by sterile swabs. Wells (6 mm diameter) were punched in the plates using a sterile stainless steel borer and filled with 40 µl of every EO with 2% DMSO (v/v) to enhance EO solubility, oxytetracycline (40 µl, positive control), or DMSO (40 µl, negative control). The plates were incubated at 37 °C for 24 h, and the radius of the zone of growth inhibition around the wells was measured with a Vernier caliper. Three replicates were carried out for every EO against every test microorganism.

The MIC was assessed by the microbroth dilution method, according to what already described by Duarte and colleagues [34] with slight modifications. Serial two-fold dilutions of every EO (from 0.05 to 5.00 mg mL⁻¹) were prepared in MHB supplemented with 2% DMSO (v/v) and seeded in 96-well plates (200 µL per well). Then, every reference strain was inoculated into every EO dilution at a final bacterial density of 5×10^5 cfu mL⁻¹ (which was achieved by adding 10 µL per well of the $\sim 10^8$ cfu mL⁻¹ bacterial suspensions mentioned above). Wells with MHB with 2% DMSO but not EO and inoculated with bacterial suspensions were considered as positive control; while wells with EO diluted in MHB with 2% DMSO without bacteria were used as negative control. The MIC was defined as the lowest EO concentration able to inhibit the bacterial growth after 24 h incubation at 37 °C, and it was determined by measuring the absorbance at 625 nm with a microplate reader (BIO-RAD 680, Hercules, CA, USA). The MIC values were defined as the lowest concentration of compounds whose absorbance was comparable with the negative control wells. Three replicate tests were carried out for every EO against every test microorganism.

DPPH assay

The in vitro antioxidant activity of EOs was determined by the DPPH assay according to what previously described by Albergamo and colleagues [11, 12] with slight modifications. Different dilutions of every EO in methanol were prepared (0.05, 0.1, 0.3, and 0.5 mg mL⁻¹ or 0.5, 1, 3, and 5 mg mL⁻¹, in dependence of the EO type) and, then, 60 µL of every dilution was mixed with 2.940 mL of a methanolic DPPH solution (0.05 mmol L⁻¹). The mixture was kept in the dark at room temperature (23 ± 2 °C) for 30 min and read at 515 nm against a blank sample (methanol solution, negative control) using an UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan). Inhibition percentage of the DPPH• radical was calculated according to the following equation:

$$\text{Inhibition \%} = \frac{\text{Ac}(0) - \text{Ac}(t)}{\text{Ac}(0)} \times 100, \quad (1)$$

where Ac(0) is the absorbance of control DPPH solution at $t=0$ min and Ac(t) is the absorbance after addition of sample at $t=30$ min. The food additive BHT was used as positive control. Triplicate measurements were conducted for every EO sample. The IC₅₀ value, which represents the EO concentration required to cause 50% inhibition of DPPH radical, was calculated by linear regression analysis from the obtained % inhibition values and was expressed in mg mL⁻¹ of EO.

Sample preparation and multi-element analysis

Concerning sample preparation, every EO was digested using the closed-vessel microwave digestion system Ethos 1 (Milestone, Bergamo, Italy). Briefly, an EO aliquot (0.3 g) was accurately weighted into a PTFE vessel, and then digested with 8 mL of HNO₃ and 2 mL of H₂O₂. A temperature program, consisting of 0–180 °C in 15 min (step 1), and 180 °C held for 15 min (step 2), and a constant microwave power of 1000 W, was adopted. After cooling down to room temperature, the digested sample was diluted up to 25 mL with ultrapure water. Blank solutions (HNO₃ and H₂O₂, 8:2 v/v) were processed in the same way. The screening of minerals (i.e., Na, Mg, and K), trace essential elements (i.e., Mn, Fe, Cu, and Zn), and potentially toxic elements (i.e., Ni, Cr, Al, As, Cd, and Pb) was performed by means of iCAP Q (Thermo Scientific, Waltham, MA, USA), a quadrupole ICP-MS, equipped with an ASX-520 autosampler (Cetac Technologies Inc., Omaha, NE, USA). As already reported in previous works [35, 36], the ICP-MS instrument was tuned before analysis, and the employed method was optimized to reduce spectral (polyatomic and isobaric) and non-spectral (matrix effect) interferences that could significantly

influence the multi-element determination. The following operating parameters were adopted for the element analysis: incident radio frequency (rf) power 1500 W; plasma gas flow rate [argon (Ar)] 15 L min⁻¹; auxiliary gas flow rate (Ar) 0.9 L min⁻¹; and carrier gas flow rate (Ar) 1.10 L min⁻¹. The instrument was operated with helium (He) as collision cell gas (4 mL min⁻¹), and was equipped with a spray chamber (2 °C). The injection volume and the sample introduction flow rate were set at 200 µL and 1 mL min⁻¹, respectively. Spectra acquisition occurred in full scan mode (dwell time 0.5 or 0.1 s point⁻¹, based on the analyte). All sample were screened in triplicate along with analytical blanks and data acquisition occurred through Qtegra™ Intelligent Scientific Data Solution (Thermo Scientific™). For quantification purposes, a six-point calibration curve was built up for each analyte. To this purpose, multi-element standard solutions were prepared, serially diluted, and injected in six replicates along with analytical blanks.

ICP-MS has itself established as a powerful tool for the monitoring of inorganic elements in a great variety of matrices owing to an excellent sensitivity and accuracy, as well as a simultaneous multi-element measurement capability assured by a wider linear dynamic range [37–42]. However, the direct determination of Hg by ICP-MS notoriously suffers from the so-called “memory effect”, attributed to a combination of sample introduction, spray chamber and nebulizer effects, and leading to a non-linear calibration, long wash-out times, decreasing sensitivity with time, and signals dependent on the matrix as well [43]. As a result, the Hg determinations were performed according to the EPA method 7473 (SW-846) [44], based on the principle of the thermal decomposition amalgamation-atomic absorption spectrophotometry (TDA-AAS), and contemplating the use of the DMA-80 automatic mercury analyzer (Milestone S.r.l., Bergamo, Italy). In this respect, EO sample (100 mg) was introduced into a sample boat, dried at 200 °C for 3 min, and chemically and thermally decomposed at 650 °C for 2 min. The Hg content was determined measuring the absorbance at 253.7 nm and exploiting an external calibration procedure. Triplicate measurements were carried out for every sample.

Both the ICP-MS and DMA procedures were analytically validated and demonstrated to be more than satisfactory to reliably detect inorganic elements in the selected EOs (see Online Resource 1 for further details).

Statistical analysis

Descriptive data analysis, including mean and standard deviation (sd), was carried out. A statistical comparison of every EO in terms of microbiological and antioxidant activity, as well as element profile, was performed by one-way ANOVA, followed by Tukey’s honestly significant difference

(HSD) post hoc test. All analyses were conducted by means of the SPSS Statistics Software Version 2.0 (IBM, New York, USA), and their statistical significance was accepted at $p \leq 0.05$. A Pearson correlation analysis was carried out to investigate the potential relationship between inorganic elements and biological activities characterizing a given EO.

Results and discussion

It is generally accepted that the bioactivity of an EO, in terms of antimicrobial and antioxidant properties, is mainly regulated by its volatile constituents and, not least, by their mutual interaction. In fact, these components may act by permeating the bacterial wall and cytoplasmic membrane and inducing a variety of cellular responses aimed to determine the cell death. Attributing the biological activity of an EO to an individual volatile compound; however, it is quite speculative, as (i) the most abundant compounds and/or the volatiles present at trace levels may be responsible for the final outcome, and (ii) the potential synergistic and antagonistic impact deriving from their combination should be also taken into account [45].

Antimicrobial activity

Among the huge variety of food-borne microorganisms susceptible to EOs, *E. coli* O157:H7 and *K. pneumoniae* were selected for the present study. Specifically, *E. coli* O157:H7 is an enteric pathogen typically involved in food-borne illness outbreaks throughout the world, as it is found in a variety of foodstuffs such as meat, milk, yogurt, vegetables, fruits, and derived products [46]. *K. pneumoniae* is well known as cause of community-acquired bacterial pneumonia. Contaminated sprouts, vegetables, seafood, and other animal meat products are typically considered as the main food sources of *Klebsiella* infection [47, 48].

Almost all tested EOs inhibited the growth of at least one of the selected bacteria (Fig. 1). In particular, 15 EOs (i.e., cypress, tasmanian blue gum, spearmint, pennyroyal mint, peppermint, laurel, bigroot geranium, Scots pine, sage, thyme, desert wormwood, rosemary, lemon, clove, lavender and ginger) were bioactive against both microorganisms; while 2 EOs (i.e., grapefruit and juniper) showed antibacterial activity only against *E. coli* O157:H7. On the other hand, the desert wormwood EO failed to inhibit any of the tested strains.

As expected, no growth inhibition was observed for the negative control (2% DMSO); whereas the positive control (oxytetracycline) showed a high activity against *E. coli* and *K. pneumoniae* (respectively, 23.68 mm and 21.45 mm). Only the ginger EO caused even greater, and significantly different, growth inhibition against both bacterial strains (*E.*

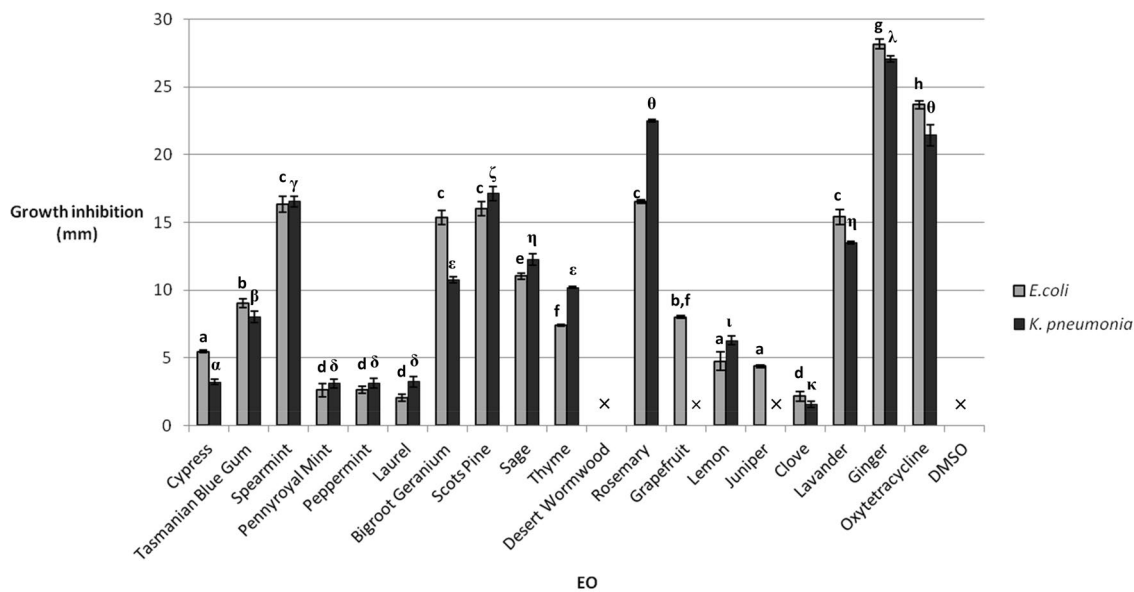


Fig. 1 Growth inhibition (mm) of 18 Tunisian EOs against *E. coli* O157:H7 and *K. pneumoniae*. For each EO type, results are expressed as mean \pm standard deviation of triplicate samples, where

coli O157:H7: 28.17 mm, and *K. pneumoniae*: 27.7 mm, $p < 0.05$); whereas rosemary EO had a behavior similar to that of oxytetracycline, when considering *K. pneumoniae* (22.51 mm, $p > 0.5$). In particular, geranial, geranyl acetate and geraniol from ginger EO (Table 1) have already proved to be active against Gram-negative bacteria, including *E. coli* O157:H7 [49, 50]; while the monoterpeneoid 1,8-cineol characterizing the rosemary EO was notoriously effective against *K. pneumoniae* [51].

Spearmint, bigroot geranium, Scots pine, rosemary, and lavender EOs were marked by high and similar inhibitory activities against *E. coli* O157:H7 (respectively, 16.33, 15.37, 16.01 and 15.40 mm, $p > 0.05$). In this respect, a variety of volatiles, such as carvone (in spearmint EO), thymol (in bigroot geranium EO), 1,8-cineol (in spearmint, rosemary and lavender EOs), linalool (in rosemary and lavender EOs), α -terpineol (in lavender and Scots pine EOs), and limonene (in spearmint and Scots pine EOs) may justify their bioactivity (Table 1; Fig. 1). On the other hand, the growth of *K. pneumoniae* was significantly inhibited by Scots pine EO (15.40 mm, $p < 0.05$), and, to follow, by sage and lavender EOs (13.50 mm and 12.24 mm, $p > 0.05$). There is no literature evidence about the effectiveness of volatiles characterizing these EOs on *K. pneumoniae*. However, a previous study focused on the desert wormwood EO revealed that the combination of four main components (i.e., 1,8-cineol, camphor, β -thujone, and α -thujone) was very active against gram-negative bacteria [52]. Also, the combination 1,8-cineol and camphor may act in a synergistic manner and improve the antimicrobial activity [53].

each sample was analyzed three times. The symbol 'x' means that the EO did not inhibit the growth of the bacterial strain(s)

Accordingly, the interesting antimicrobial activity of the Tunisian sage and lavender EOs against the Gram-negative *K. pneumoniae* may be explained by these peculiar volatile combinations (Fig. 1; Table 1). However, the absence of antibacterial activity in the desert wormwood EO from this study, would be due to the variation in quantity of compounds such as, camphor, β -thujone, and α -thujone, and to the absence of 1,8-cineol as well [52].

Table 2 summarizes the antibacterial effects, evaluated in terms of MICs, of the investigated EOs against *E. coli* and *K. pneumoniae*. The MICs ranged from 0.07 to 4.37 mg mL⁻¹ when the EOs were tested against *E. coli* O157:H7, and from 0.22 to 4.87 mg mL⁻¹ when they were assayed against *K. pneumoniae*, confirming that slightly higher EO concentrations were needed to inhibit *K. pneumoniae*. The highest MICs were obtained for the grapefruit EO against *E. coli* O157:H7 (4.37 mg mL⁻¹, $p < 0.05$), and for cypress, bigroot geranium, and thyme EOs against *K. pneumoniae* (respectively, 4.30, 4.77, and 4.87 mg mL⁻¹, $p > 0.05$). Conversely, the lowest MICs were showed by spearmint, sage, and rosemary EOs against *E. coli* O157:H7 (0.09, 0.09, 0.07 mg mL⁻¹, $p > 0.05$), and sage EO against *K. pneumoniae* (0.41 mg mL⁻¹, $p < 0.05$). Tasmanian blue gum, spearmint, Scots pine, and lemon EOs tested against *K. pneumoniae* revealed intermediate and non-significantly different MICs (from 1.14 to 1.79 mg mL⁻¹, $p > 0.05$), thus, demonstrating of inhibiting the growth of such bacteria at similar concentration. The same goes for clove, cypress, and thyme EOs, which showed intermediate and similar MICs against *E. coli*

Table 2 Minimum inhibitory concentration (MIC) of Tunisian EOs against *E. coli* O157:H7 and *K. pneumoniae*

| EO sample | MIC (mg mL ⁻¹) | |
|--------------------|----------------------------|----------------------------|
| | <i>E. coli</i> O157:H7 | <i>K. pneumoniae</i> |
| Cypress | 2.45 ± 0.13 ^a | 4.30 ± 0.11 ^a |
| Tasmanian Blue Gum | 0.91 ± 0.03 ^b | 1.14 ± 0.19 ^b |
| Spearmint | 0.09 ± 0.00 ^c | 1.79 ± 0.02 ^b |
| Pennyroyal Mint | 1.84 ± 0.33 ^d | 2.21 ± 0.27 ^{b,c} |
| Peppermint | 3.12 ± 0.08 ^e | 2.91 ± 0.20 ^c |
| Laurel | 0.72 ± 0.04 ^b | 2.34 ± 0.13 ^c |
| Bigroot Geranium | 0.76 ± 0.24 ^b | 4.77 ± 0.22 ^a |
| Scots Pine | 0.30 ± 0.01 ^f | 1.74 ± 0.18 ^b |
| Sage | 0.09 ± 0.01 ^c | 0.41 ± 0.10 ^d |
| Thyme | 2.42 ± 0.08 ^a | 4.87 ± 0.58 ^a |
| Desert wormwood | nd | nd |
| Rosemary | 0.07 ± 0.02 ^c | 2.77 ± 0.09 ^c |
| Grapefruit | 4.37 ± 0.46 ^g | nd |
| Lemon | 1.24 ± 0.27 ^b | 1.71 ± 0.18 ^b |
| Juniper | 0.25 ± 0.10 ^f | nd |
| Clove | 2.07 ± 0.11 ^{a,d} | 0.22 ± 0.04 ^d |
| Lavender | 0.24 ± 0.06 ^h | 3.75 ± 0.19 ^e |
| Ginger | 3.18 ± 0.03 ^e | 0.37 ± 0.03 ^d |

For each EO type, results are expressed as mean ± standard deviation of triplicate samples, where each sample was analyzed three times

nd MIC not detected

^{a–f}Different superscript letters in the same column indicate significantly different values ($p \leq 0.05$ by a post hoc Tukey's HSD test); same superscript letters in the same column indicate non-significantly different values ($p > 0.05$ by a post hoc Tukey's HSD test)

O157:H7 (respectively, 2.07, 2.45 and 2.42 mg mL⁻¹, $p > 0.05$, Table 2).

In the last decades, scientific literature has presented few and conflicting outcomes on the bacteriostatic effects of the same EOs against *E. coli* O157:H7 and *K. pneumoniae*. This could be due to variables related to the (i) plant material (geographic origin, pedoclimatic conditions, agronomic practices, harvest time, variety, etc.) and (ii) EO production (i.e., extraction technology), which cannot be undervalued during the standardization of the bioactivity of a certain EO.

Concerning *E. coli* O157:H7, for example, Ghabraie and colleagues [54] found that juniper, laurel, rosemary, thyme, sage, peppermint and Scots pine EOs, with different geographical origins (i.e., Quebec, Hungary, Morocco, Bolivia and Spain), had a bacteriostatic effect only over 10 mg mL⁻¹; however, clove EO from Madagascar showed a MIC similar to that calculated for the same type of EO in this study (1.87 and 2.07 mg mL⁻¹, respectively) [54]. Conversely, commercial thyme and clove EOs from Germany and Canada, and Algerian peppermint EO produced by steam distillation showed much lower MIC values than those

from this work (respectively, 0.00062–0.00125 mg mL⁻¹, 0.1 mg mL⁻¹, and 0.050 µg mL⁻¹) [46, 55, 56].

Highly variable bioactivities of EOs were also perceived toward *K. pneumoniae*, and in general, they were not comparable with those revealed by the Tunisian EOs. High MICs were observed for commercial clove, lemon, and rosemary EOs from India (> 6.4 mg mL⁻¹) [57], and hydrodistillates of thyme and sage with Greek origins (9.51–11.34 mg mL⁻¹ and 207–240 mg mL⁻¹, respectively) [58]. Conversely, Argentines EOs isolated from different phenotypes of rosemary by hydrodistillation, and Portuguese commercial eucalyptol EO revealed a higher bacteriostatic effect, explained by much lower MICs (respectively, 0.020–0.040 mg mL⁻¹ and 0.016 mg mL⁻¹) [51, 59].

Antioxidant activity

The radical scavenging capacity of the Tunisian EOs was reported in Fig. 2. For every EO, the antioxidant activity increased steadily with the employed concentration (0.05–0.5 mg mL⁻¹ or 0.5–5 mg mL⁻¹), and the linear correlation was confirmed by R^2 values between 0.980 and 0.998. However, to make comparison with the DPPH activity of EOs from previous studies easier, the results were expressed by IC₅₀ values, calculated from the relative dose–response curves (Fig. 2). Notoriously, the lower the IC₅₀, the higher the antioxidant activity [60].

The investigated EOs exhibited varying degrees of scavenging capacities, as the IC₅₀ values were in the range 0.024–4.90 mg mL⁻¹. However, most of them (11 out of 18) revealed a lower, and not comparable, antioxidant activity than the positive control (BHT), as their IC₅₀ values were between 1.53 mg mL⁻¹ (clove EO) and 4.90 mg mL⁻¹ (desert wormwood EO), and they were significantly different from that of the synthetic antioxidant (IC₅₀: 0.032 mg mL⁻¹, $p < 0.05$, Fig. 2).

Other EO types, such as Scots pine, lavender, ginger and thyme, were characterized by a good, but significantly lower scavenging capacity than BHT ($p < 0.05$), being their IC₅₀, respectively equal to 0.30, 0.50, 0.70, and 0.73 mg mL⁻¹ ($p > 0.05$). Such activity may be attributed to volatiles such as α -pinene, sabinene in Scots pine EO [61–63], linalool and camphor in lavender EO [63, 64], thymol and carvacrol in thyme EO [19, 63], and geraniol and camphene in ginger EO [63] (Table 1; Fig. 2).

Only three EOs, namely spearmint, bigroot geranium, and sage, showed the lowest IC₅₀ values (0.024, 0.046 and 0.052 mg mL⁻¹, $p > 0.05$) and, hence, an antioxidant potential not significantly different from that of BHT ($p > 0.05$, Fig. 2). In particular, the synergistic combination of monoterpenes, such as carvone, limonene and 1,8-cineol, may be effective in determining the radical scavenging capacity of spearmint EO [65, 66] (Table 1; Fig. 2).

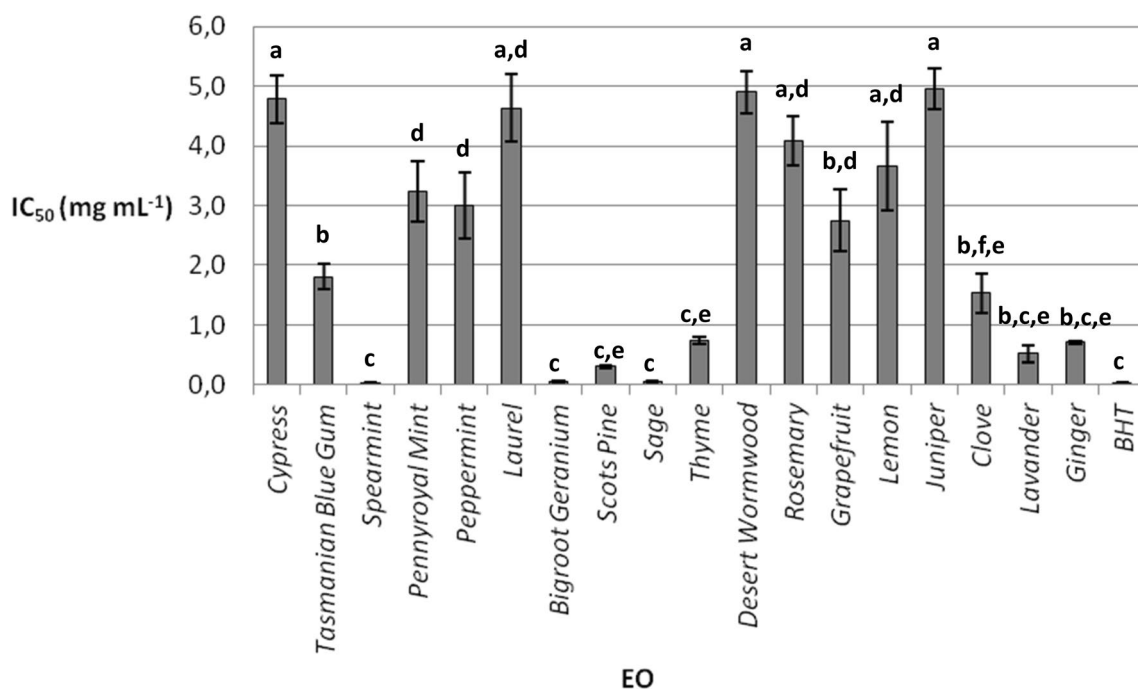


Fig. 2 DPPH activity (IC₅₀, mg mL⁻¹) of 18 EOs from the Mahdia region. For each EO type, results are expressed as mean ± standard deviation of triplicate samples, where each sample was analyzed three times

A comparison of the actual findings with previous studies is somehow arduous, because the DPPH-radical scavenging activity of EOs has been constantly characterized by a marked variability.

Among spices, rosemary EOs with different provenance (i.e., Egypt, Korea, and Tunisia) and obtained by hydrodistillation, had IC₅₀ values between 0.003 mg mL⁻¹ and 0.110 mg mL⁻¹ [65, 67, 68], being the EO with the highest IC₅₀ coming from Tunisia (0.110 mg mL⁻¹) [67]. However, Spanish EOs obtained by steam- and hydrodistillation, surprisingly reported a very scarce scavenging capacity when compared to BHT (IC₅₀ = 17 mg mL⁻¹ vs. 0.53 mg mL⁻¹) [69] or, even, almost no antioxidant activity [19]. Clove EOs isolated by hydro- or steam-distillation and coming from Egypt, Spain, and Indonesia reported a higher antioxidant activity (IC₅₀ = 0.015–0.54 mg mL⁻¹) [69–71] than the counterpart of this study. Tunisian sage EOs extracted by hydrodistillation revealed IC₅₀ values between 0.0067 and 0.021 mg mL⁻¹, demonstrating to have an antioxidant activity similar to that from this study, when considering the upper part of the range [72, 73]. Similar antioxidant activities may be explained by highly comparable percent contents of the main volatile constituents (i.e., α-thujone, 1,8-cineol, β-thujone, α-humulene, and borneol) (Table 1) [72, 73].

On the other hand, Algerian and Spanish sage EOs had a lower antioxidant potential, with IC₅₀ of 1.99 mg mL⁻¹ and 4.20 mg mL⁻¹ [69, 74]. Thyme EOs with various origins (e.g., Slovakia, Bosnia, Spain, Germany and

Mexico) inhibited 50% of the DHHP radical at concentrations between 0.009 mg mL⁻¹ and 0.414 mg mL⁻¹ [19, 69, 75, 76], being the German EO more similar to that from this study, in terms of antioxidant capacity; whereas ginger EO had a IC₅₀ variability spanning from 0.065 mg mL⁻¹ to 11.68 mg mL⁻¹ [77–79], being the EO of Ecuadorian origin characterized by a DPPH activity comparable to that observed in this study (IC₅₀ = 0.675 mg mL⁻¹) [79]. This may be due to similar contents of certain antioxidant constituents, such as camphene and geraniol (Table 1) [63].

Concerning the EOs from mint species, such as *M. spicata* (spearmint), *M. pulegium* (pennyroyal mint), and *M. × piperita* (peppermint), a dramatic variability in the radical scavenging activity was reported in literature. The spearmint EO originating from different areas (i.e., Algeria, Greece, Pakistan Brazil, and US Midwest) and obtained by hydrodistillation, showed IC₅₀ values in the range of 0.007–86.5 mg mL⁻¹ [80–83] or, even, no antioxidant activity [84]. A great variability was observed also within the Tunisian country, as spearmint EOs extracted by hydrodistillation were marked by a stronger antioxidant capacity than BHT (IC₅₀ = 0.003 mg mL⁻¹ and 0.011 mg mL⁻¹, respectively) [66], as well as by a weak antioxidant activity (IC₅₀ = 3.0 mg mL⁻¹) [85]. In this respect, results from our study highlighted an intermediate antioxidant activity (IC₅₀ = 0.024 mg mL⁻¹) for the corresponding EO. Pennyroyal mint EOs with diverse provenance (e.g., Morocco, Algeria, Iran, and Portugal) and isolated by hydro- or

steam-distillation were characterized by IC_{50} values in the range 0.069–16.03 mg mL⁻¹. Interestingly, the same EO from Tunisia reported a comparable antioxidant activity to that from this study, being its IC_{50} equal to 3.9 mg mL⁻¹ [85]. This could be due to the paragonable percent composition of main constituents such as pulegone, neo-menthol, iso-menthone and menthone (Table 1).

Similarly to the other mint EOs, a highly varying DPPH activity (IC_{50} = 0.003–70 mg mL⁻¹) was observed in peppermint EOs produced by hydro- or steam-distillation and coming from Egypt, Taiwan, Korea, and US Midwest [65, 68, 83, 86].

Element profile

As matter of fact, the inorganic elements characterized by an appreciable vapor pressure at technically practicable temperatures, can be transferred into the vapor phase, and, thus, in the distillate by heating the matrix containing metals at elevated temperatures under normal pressure or, to lower the distillation temperature, under reduced pressure [87]. Based on this principle, the vaporization of inorganic molecules and potential metal-volatile complexes would be responsible of the presence of such microconstituents in EO [88, 89].

Results from the screening of inorganic elements conducted on different Tunisian EOs, are outlined in Table 3. For the majority of investigated EOs, minerals and essential trace elements were present in the following concentration order: Mg ≥ Na > K > Cu > Fe > Zn > Mn; whereas potentially toxic elements were found according to the sequence: Al > Pb > Cr ≥ Ni > Hg > Cd > As.

Concerning the levels of mineral and trace essential metals, a tangible uniformity was observed among the greater part of investigated samples. In fact, just few EOs were marked by higher and significantly different contents of Na, Mg, K, and Fe. For example, bigroot geranium and Scots pine EOs revealed high concentrations of Na (18.37 and 15.15 mg Kg⁻¹, $p > 0.05$), Mg (13.24 and 13.40 mg Kg⁻¹, $p > 0.05$), K (4.84 and 20.90 mg Kg⁻¹, $p < 0.05$), Fe (0.74 and 1.02 mg Kg⁻¹, $p > 0.05$), and Cu (2.98 and 2.31 mg Kg⁻¹, $p > 0.05$). To follow, the desert wormwood EO showed Na and K at high and comparable levels (16.58 and 4.84 mg Kg⁻¹, $p > 0.05$), and Mg even at a higher, but not significantly different, content (14.12 mg Kg⁻¹, $p > 0.05$). However, lavender EO was marked by a high and significantly different content of K (9.56 mg Kg⁻¹, $p < 0.05$); whereas laurel and thyme EOs were marked by the highest level of Zn (0.90 mg Kg⁻¹ and 0.70 mg Kg⁻¹, $p < 0.05$). On the other hand, juniper EO was marked by the lowest amounts of Na (1.75 mg Kg⁻¹, $p < 0.05$), Mg (1.72 mg Kg⁻¹, $p < 0.05$), K (<LoD), Mn (0.011 mg Kg⁻¹, $p > 0.05$), Fe (0.070 mg Kg⁻¹, $p < 0.05$), and Cu (0.20 mg Kg⁻¹, $p > 0.05$). Overall, excluding these outliers, Na, Mg

and K varied not significantly among EOs in the ranges of 2.73–7.44 mg Kg⁻¹, 2.56–8.11 mg Kg⁻¹, 0.57–4.84 mg Kg⁻¹, $p > 0.05$), respectively. Mn was equally present in all the investigated EOs (from 0.011 to 0.093 mg Kg⁻¹, $p > 0.05$); whereas Fe, Cu, and Zn were respectively 0.16–0.66 mg Kg⁻¹ ($p > 0.05$), 0.26–2.09 mg Kg⁻¹ ($p > 0.05$), and 0.046–0.32 mg Kg⁻¹ ($p > 0.05$, Table 3).

Concerning the concentration of potentially toxic elements, the greatest variability was observed for Al (106.74–978.90 µg Kg⁻¹), Ni (<LoD–32.53 µg Kg⁻¹), and Cr (3.30–23.43 µg Kg⁻¹). In particular, the highest and significantly different contents ($p < 0.05$) of Al, Ni and Cr were respectively found in Scots pine (978.90 µg Kg⁻¹), cypress (32.53 µg Kg⁻¹) and desert wormwood 23.43 µg Kg⁻¹ EOs. Conversely, As, Cd, Pb and Hg were found at comparable and not significantly different levels ($p > 0.05$) in all EO types (<LoD–1.89 µg Kg⁻¹, <LoD–1.04 µg Kg⁻¹, 22.04–55.04 µg Kg⁻¹, and <LoD–5.22 µg Kg⁻¹, respectively). In particular, As was the heavy metal detected in the lowest number of EOs, being <LoD in 11 out of 18 EOs (Table 3).

The Commission Regulation (EU) No. 231/2012 [90] lays down specifications, including the content of heavy metals, for those food additives listed in Annexes II and III of Regulation (EC) No. 1333/2008. Although EOs have not yet been considered in the Regulation, they may reasonably be referred to the natural additive “extract of rosemary” (E392), which the following limits have been fixed for: As = 3 ppm; Pb = 2 ppm; Cd = 1 ppm and Hg 1 = ppm. As a result, the Tunisian EOs may be safely employed as food additives, as they are characterized by heavy metal contents well below the regulatory limits.

Few literature data are available on the profile of inorganic elements in EOs. La Pera and colleagues [24] elucidated the elemental profiles of diverse Italian citrus EOs produced between 2003 and 2004. Among them, the lemon EO had lower levels of Cu (0.060–0.145 mg Kg) and higher contents of Zn and Mn (respectively, 0.300–0.790 and 0.260–1.403 mg Kg⁻¹) than the Tunisian counterpart. Additionally, in these oils, Cd was <LOD (0.6 µg Kg⁻¹) and Pb amounted to 70.2–135 µg Kg⁻¹. A recent work investigated the safety of EOs from *Zanthoxylum bungeanum* from different Chinese areas by exploring the profile of potentially toxic elements [32]. Overall, much lower contents of Cr (0.72–6.02 µg Kg⁻¹), Ni (0.09–2.87 µg Kg⁻¹), Pb (0.17–0.73 µg Kg⁻¹) and Hg (0.13–0.92 µg Kg⁻¹) were described. However, As (0.21–5.84 µg Kg⁻¹) and Cd (0.16–2.15 µg Kg⁻¹) resulted at higher levels than those reported for the Tunisian EOs [30]. Similarly to the antioxidant and antibacterial activities, the variability of element profile could be ascribed to many intrinsic and extrinsic variables [24, 30, 33].

Table 3 Profile of minerals (mg Kg⁻¹), essential trace metals (mg Kg⁻¹), and potentially toxic elements (µg Kg⁻¹) of 18 Tunisian EOs

| EO sample | Major and essential trace elements (mg Kg ⁻¹) | | | | | | |
|--------------------|---|------------------------------|--------------------------------|-----------------------------|---------------------------------|----------------------------------|-----------------------------|
| | Na | Mg | K | Mn | Fe | Cu | Zn |
| Cypress | 6.48 ± 0.74 ^a | 8.11 ± 0.76 ^a | 4.43 ± 1.14 ^a | 0.058 ± 0.032 ^a | 0.66 ± 0.29 ^a | 0.93 ± 0.092 ^a | 0.23 ± 0.10 ^a |
| Tasmanian Blue Gum | 7.44 ± 2.09 ^{a,d} | 6.43 ± 2.12 ^{a,b} | 1.67 ± 0.92 ^{a,b} | 0.025 ± 0.017 ^a | 0.47 ± 0.10 ^{a,b} | 1.22 ± 0.40 ^a | 0.24 ± 0.14 ^a |
| Spearmint | 4.85 ± 0.87 ^a | 5.59 ± 1.47 ^{a,b} | 2.30 ± 0.68 ^{a,e} | 0.028 ± 0.019 ^a | 0.26 ± 0.052 ^{a,b} | 1.95 ± 0.86 ^{a,c} | 0.20 ± 0.010 ^a |
| Pennyroyal Mint | 2.73 ± 0.81 ^{a,e} | 6.52 ± 1.13 ^{a,b} | 0.86 ± 0.63 ^{b,e,f} | 0.022 ± 0.011 ^a | 0.17 ± 0.034 ^b | 1.24 ± 0.47 ^a | 0.046 ± 0.012 ^b |
| Peppermint | 6.51 ± 1.56 ^a | 5.26 ± 1.82 ^{a,b} | 1.77 ± 0.30 ^{a,f} | 0.024 ± 0.010 ^a | 0.18 ± 0.040 ^b | 0.63 ± 0.08 ^{a,d} | 0.32 ± 0.10 ^a |
| Laurel | 6.96 ± 1.74 ^a | 8.69 ± 1.92 ^a | 1.94 ± 0.21 ^{a,f} | 0.039 ± 0.015 ^a | 0.29 ± 0.091 ^{a,b} | 0.65 ± 0.11 ^{a,d} | 0.90 ± 0.08 ^c |
| Bigroot geranium | 18.37 ± 2.02 ^b | 13.24 ± 2.81 ^{a,d} | 4.84 ± 1.32 ^{a,g} | 0.026 ± 0.0042 ^a | 0.74 ± 0.20 ^{a,f} | 2.98 ± 0.80 ^b | 0.053 ± 0.014 ^b |
| Scots pine | 15.15 ± 1.11 ^{b,f} | 13.40 ± 3.62 ^{a,d} | 20.90 ± 2.62 ^c | 0.087 ± 0.022 ^a | 1.02 ± 0.18 ^{c,f,m} | 2.31 ± 0.67 ^{b,f} | 0.17 ± 0.055 ^{a,b} |
| Sage | 4.78 ± 0.91 ^a | 5.65 ± 1.98 ^{a,e} | 0.92 ± 0.56 ^{b,e,f} | 0.019 ± 0.0027 ^a | 0.18 ± 0.043 ^{a,b} | 0.29 ± 0.08 ^{a,d,e} | 0.070 ± 0.020 ^b |
| Thyme | 5.49 ± 1.49 ^a | 2.56 ± 0.57 ^b | 1.16 ± 0.35 ^{b,c,f,g} | 0.018 ± 0.0018 ^a | 0.25 ± 0.082 ^{a,b} | 0.30 ± 0.10 ^{a,d,e} | 0.70 ± 0.064 ^d |
| Desert wormwood | 16.58 ± 0.93 ^{b,f} | 14.12 ± 4.15 ^{a,d} | 4.81 ± 1.21 ^a | 0.023 ± 0.0015 ^a | 0.41 ± 0.20 ^{a,b,f} | 0.71 ± 0.14 ^{a,d,e} | 0.056 ± 0.018 ^b |
| Rosemary | 5.37 ± 2.18 ^a | 3.41 ± 0.62 ^a | 0.84 ± 0.10 ^{b,e,f} | 0.022 ± 0.0031 ^a | 0.66 ± 0.19 ^{a,b,m} | 1.54 ± 0.72 ^{a,d,e,f,g} | 0.14 ± 0.025 ^{a,b} |
| Grapefruit | 6.50 ± 1.51 ^a | 7.72 ± 1.12 ^{a,d,f} | 4.16 ± 0.72 ^a | 0.015 ± 0.0056 ^a | 0.21 ± 0.097 ^{a,b,g,m} | 0.30 ± 0.077 ^{a,d,e} | 0.16 ± 0.027 ^{a,b} |
| Lemon | 5.61 ± 1.10 ^a | 8.17 ± 1.49 ^{a,d,f} | 2.58 ± 1.13 ^{a,f} | 0.027 ± 0.010 ^a | 0.20 ± 0.048 ^{a,b,h,m} | 0.72 ± 0.032 ^{a,d,e} | 0.16 ± 0.021 ^{a,b} |
| Juniper | 1.75 ± 0.75 ^{c,e} | 1.72 ± 0.84 ^c | < LoD | 0.011 ± 0.0031 ^a | 0.07 ± 0.012 ⁱ | 0.20 ± 0.10 ^{a,d,e} | 0.31 ± 0.062 ^a |
| Clove | 4.64 ± 0.94 ^a | 3.55 ± 1.48 ^{a,f} | 0.57 ± 0.20 ^{b,e,f} | 0.013 ± 0.0022 ^a | 0.16 ± 0.074 ^{b,e} | 0.26 ± 0.038 ^{a,d,e} | 0.28 ± 0.084 ^a |
| Lavender | 5.42 ± 0.92 ^a | 6.38 ± 0.51 ^{a,f} | 9.56 ± 1.27 ^d | 0.093 ± 0.017 ^a | 0.53 ± 0.13 ^{a,b,c,m} | 1.71 ± 0.16 ^b | 0.058 ± 0.017 ^b |
| Ginger | 13.53 ± 2.58 ^{b,f} | 5.66 ± 1.09 ^{a,f} | 3.16 ± 0.45 ^{a,f} | 0.015 ± 0.0053 ^a | 0.21 ± 0.062 ^{a,b,l,m} | 2.09 ± 0.53 ^b | 0.25 ± 0.050 ^a |
| | Potentially toxic elements (µg Kg ⁻¹) | | | | | | |
| | Al | Ni | Cr | As | Cd | Pb | Hg |
| Cypress | 148.78 ± 22.16 ^a | 32.53 ± 3.49 ^a | 4.49 ± 1.24 ^a | 1.08 ± 0.33 ^a | 1.01 ± 0.01 ^a | 35.78 ± 7.03 ^a | 2.67 ± 1.25 ^a |
| Tasmanian Blue Gum | 223.53 ± 11.94 ^b | 9.70 ± 2.31 ^b | 22.17 ± 2.25 ^b | < LoD | 1.02 ± 0.01 ^a | 29.44 ± 7.61 ^a | 3.57 ± 1.50 ^a |
| Spearmint | 210.10 ± 10.51 ^{a,b} | 4.98 ± 0.88 ^{b,e} | 8.30 ± 0.98 ^{a,d} | < LoD | 1.01 ± 0.01 ^a | 44.10 ± 2.71 ^{a,d} | 2.83 ± 1.37 ^a |
| Pennyroyal Mint | 148.70 ± 22.72 ^a | 13.03 ± 2.84 ^{b,f} | 3.30 ± 0.90 ^{a,e} | < LoD | < LoD | 32.11 ± 5.65 ^a | 2.40 ± 0.76 ^a |
| Peppermint | 155.60 ± 19.40 ^a | 14.16 ± 1.61 ^{b,f} | 8.54 ± 1.12 ^{a,f} | < LoD | 1.01 ± 0.02 ^a | 32.49 ± 6.67 ^a | 1.68 ± 0.80 ^a |
| Laurel | 246.63 ± 24.18 ^b | 7.20 ± 1.65 ^b | 6.83 ± 1.45 ^a | 1.14 ± 0.57 ^a | 1.04 ± 0.02 ^a | 42.04 ± 7.86 ^{a,f} | 4.39 ± 0.64 ^a |
| Bigroot geranium | 200.13 ± 17.07 ^{a,b} | < LoD | 7.46 ± 2.11 ^a | 0.85 ± 0.18 ^a | 1.03 ± 0.05 ^a | 42.41 ± 1.87 ^{a,g} | 1.35 ± 0.38 ^a |
| Scots pine | 978.90 ± 20.13 ^c | 0.87 ± 0.19 ^c | 6.45 ± 1.83 ^a | 1.89 ± 0.88 ^a | 1.02 ± 0.01 ^a | 34.00 ± 3.10 ^a | 1.90 ± 0.23 ^a |
| Sage | 136.77 ± 10.52 ^a | < LoD | 13.52 ± 1.08 ^c | < LoD | 0.99 ± 0.11 ^a | 32.73 ± 2.53 ^a | 1.16 ± 0.15 ^a |
| Thyme | 370.19 ± 16.69 ^d | 13.20 ± 2.52 ^{b,f} | 3.58 ± 2.96 ^a | < LoD | 1.05 ± 0.01 ^a | 42.23 ± 6.90 ^{a,h} | 5.22 ± 4.18 ^a |
| Desert wormwood | 358.18 ± 11.73 ^d | 3.69 ± 0.46 ^{b,c} | 23.34 ± 1.48 ^b | 0.96 ± 0.06 ^a | 1.03 ± 0.01 ^a | 42.40 ± 1.68 ^a | 1.31 ± 0.24 ^a |
| Rosemary | 237.52 ± 19.66 ^{b,g} | 25.21 ± 2.43 ^d | 5.78 ± 1.29 ^a | < LoD | 1.02 ± 0.01 ^a | 45.31 ± 1.35 ^{a,c} | 3.47 ± 0.79 ^a |
| Grapefruit | 163.47 ± 18.05 ^{a,g} | < LoD | 3.43 ± 0.58 ^{a,e,f,g} | < LoD | 1.03 ± 0.03 ^a | 55.04 ± 4.58 ^{b,c} | 4.35 ± 1.39 ^a |
| Lemon | 208.38 ± 13.90 ^{a,e} | < LoD | 5.60 ± 1.13 ^a | < LoD | 1.02 ± 0.00 ^a | 41.31 ± 2.59 ^{a,c} | 1.45 ± 0.60 ^a |
| Juniper | 106.74 ± 15.32 ^{a,f} | < LoD | 4.66 ± 1.10 ^a | < LoD | 1.02 ± 0.01 ^a | 30.51 ± 1.52 ^{a,i} | 1.45 ± 0.33 ^a |
| Clove | 136.32 ± 11.07 ^a | < LoD | 4.16 ± 1.54 ^a | 0.97 ± 0.07 ^a | 1.03 ± 0.01 ^a | 31.22 ± 1.73 ^{a,i} | 1.00 ± 0.10 ^a |
| Lavender | 317.77 ± 21.37 ^d | 14.16 ± 3.45 ^{b,f} | 3.59 ± 1.09 ^{a,g} | < LoD | 1.02 ± 0.00 ^a | 22.04 ± 1.80 ^{a,e} | 2.88 ± 0.49 ^a |
| Ginger | 155.45 ± 12.34 ^{a,g} | 5.38 ± 0.82 ^{b,c} | 13.11 ± 2.03 ^c | 1.01 ± 0.12 ^a | 1.02 ± 0.00 ^a | 42.59 ± 2.29 ^a | < LoD |

For each EO type, results are expressed as mean ± standard deviation of triplicate samples, where each sample was analyzed three times

LoD limit of detection

^{a-m}Different superscript letters in the same column indicate significantly different values ($p \leq 0.05$ by a post hoc Tukey's HSD test); same superscript letters in the same column indicate non-significantly different values ($p > 0.05$ by a post hoc Tukey's HSD test)

Table 4 Correlation between inorganic elements found in the Tunisian EOs and their antioxidant and antibacterial activities

| | Na | Mg | K | Mn | Fe | Cu | Zn |
|--|--------|---------|---------|--------|--------|---------|--------|
| Antioxidant activity (IC ₅₀) | - 0.23 | - 0.020 | - 0.30* | - 0.18 | - 0.18 | - 0.48* | 0.19 |
| <i>E. coli</i> O157:H7 | 0.33 | 0.0011 | 0.31 | 0.22 | 0.35* | 0.74** | - 0.25 |
| <i>K. pneumoniae</i> | 0.25 | - 0.076 | 0.26 | 0.20 | 0.36* | 0.67** | - 0.14 |

** , *Significant at the 0.01 and 0.05 probability levels, respectively

Table 4 reports the results from the correlation analysis conducted between the mean contents of minerals (Na, Mg, and K) and trace essential elements (Mn, Fe, Cu, Zn) revealed in Tunisian EOs and the relative mean antioxidant and antibacterial activities.

Overall, every inorganic element showed a weak or moderate negative correlation with the antioxidant activity of EOs, evaluated in terms of IC₅₀. Hence, the higher the element concentration, the lower the IC₅₀, which is reflected in a greater antioxidant activity. The highest correlation coefficients were observed for Cu (-0.48) and K (-0.30) at 5% probability level.

On the other hand, almost all elements were positively correlated with the antibacterial activity showed by Tunisian EOs toward *E. coli* O157:H7 and *K. pneumoniae*. The highest positive correlation coefficients were observed between Cu–*E. coli* O157:H7 (0.74) and Cu–*K. pneumoniae* (0.67) and they were significant at 1% probability level. Also, Fe significantly correlated with *E. coli* O157:H7 (0.35) and *K. pneumoniae* (0.36). Weak negative correlations were only observed between Mg–*K. pneumoniae* (- 0.076), Zn–*E. coli* O157:H7 (- 0.25) and Zn–*K. pneumoniae* (- 0.14). Additionally, there was no correlation between Mg–*E. coli* O157:H7 (0.0011).

These findings would support the already proven concept that the mineralization grade of a certain plant species—strictly correlated to the agronomic procedures employed—may affect the bioactivity of the derived EO. In this respect, different previous works have already demonstrated that fertilization protocols based on the application of high levels of K and Cu improved, respectively, the antioxidant activity and the antibacterial and antioxidant activities of EOs obtained from *M. piperita* and *Alpinia zerumbet* [81, 91].

Conclusion

The in vitro antibacterial and antioxidant activities of 18 Tunisian EOs have been reported and discussed in relation to their volatile and element profiles. Experimental data indicated that cypress, Tasmanian blue gum, spearmint, pennyroyal mint, peppermint, laurel, bigroot geranium, Scots pine,

sage, thyme, desert wormwood, rosemary, lemon, clove, lavender and ginger EOs showed a noteworthy antibacterial activity against *E. coli* O158:H7 and *K. pneumoniae*, whereas EOs from spearmint, bigroot geranium, and sage had antioxidant properties similar to that of the synthetic antioxidant BHT. As already widely reported in literature, several volatiles and their peculiar combinations as well may explain such bioactivities.

For the first time, it was also demonstrated that EOs were characterized by a variegated profile of minerals (Na, K, and Mg) and essential trace elements (Mn, Fe, Cu, and Zn) and by very low and safe levels of heavy metals (As, Cd, Pb, and Hg). In particular, a correlation analysis confirmed that K, Fe, and Cu could positively affect the antioxidant and antibacterial activities of such EOs.

Overall, the preliminary results obtained from this study pointed out that the selected Tunisian EOs were promising natural preservatives to be exploited in the food industry. Nevertheless, further bioactivity tests in simplified food system(s), will be very helpful in clarifying their effectiveness in food.

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