Chemical composition, cytotoxicity, antimicrobial and antifungal activity of several essential oils

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Essential oils (EOs) are known and used for their biological, antibacterial, antifungal and antioxidant properties. Numerous studies have shown that EOs exhibit a large spectrum of biological activities in vitro. The incidence of drug-resistant pathogens and the toxicity of antibiotics have drawn attention to the antimicrobial activity of natural products, encouraging the development of alternative treatments. The aim of this study was to analyse the phytochemical and the cytotoxic characteristic of 36 EOs; we then evaluated the antimicrobial activity of the less-toxic EOs on Gram-positive, Gram-negative and fungi strains. The results showed low cytotoxicity in seven EOs and good activity against Gram-negative and Candida spp. strains. Based on our results, EOs could be proposed as a novel group of therapeutic agents. Further experiments are necessary to confirm their pharmacological effectiveness, and to determine potential toxic effects and the mechanism of their activity in in vivo models.

Keywords: essential oil; cytotoxicity; antimicrobial activity; antifungal activity; chemical composition

Abbreviations: GC–MS, gas chromatography/mass spectrometry; GC–FID, gas chromatography/flame ionisation detector; MBC, minimum bactericidal concentration; ATCC, American type culture collection; IC_{50}, half maximal inhibitory concentration

1. Introduction

Medicinal plants have been used for centuries in traditional medicine because of their therapeutic usefulness; they are extensively used in folk medicine, as sources of natural products because they represent an economic alternative, are easily accessible and can be applicable to

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various pathologies (Prabuseenivasan et al. 2006; Cruz et al. 2007; Sardi et al. 2011). EOs, also called volatile oils, are obtained from flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots. An estimated 3000 EOs are known; 300 are commercially important in the fragrance market (Van de Braak & Leijten 1999) and are known and primarily used for their biological, antibacterial, antifungal and antioxidant properties (Deans & Waterman 1993). In addition, there is currently great interest by the pharmaceutical, food and cosmetics industries in the use of new volatile oils; despite the large progress in medicine and pharmacy in the last few decades, the traditional treatment of bacterial or viral diseases is frequently ineffective and has some side effects. Terpenes and their derivatives are a large class of natural organic components of EOs and are widespread in the plant kingdom. The antimicrobial activity of EOs is due to a number of small terpenoids and phenolic compounds (Cannas et al. 2014); several of these are generally recognised as safe (Sharififar et al. 2007). The spread of drug-resistant pathogens is one of the most serious threats to the successful treatment of microbial diseases. Therefore, researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against current antibiotics. In addition to the specific chemical composition of these EOs, it is very important to understand their cytotoxicity before using them in humans.

Further studies are needed to determine any potential toxic effects, their pharmacological effectiveness and the mechanism of their activity in *in vitro* and *in vivo* models.

2. Results and discussion

Different kinds of EOs were analysed, from those with a typical monoterpene hydrocarbon pattern such as *C. aurantium* var. *dulcis* and *J. communis*, to those characterised by the presence of monoterpene alcohols (*C. martini*, *L. angustifolia*, *M. alternifolia*, *M. piperita* and *P. asperum*), phenolics (*E. caryophyllata*, *O. vulgare*), aldehydes (*C. citratus*, *C. citriodora* and *M. officinalis*), esters (*S. sclarea*) and epoxides (*C. fragrans*, *E. globulus*, *M. viridiflora*, *M. communis* and *R. officinalis*). Moreover, a peculiar highly sesquiterpene rich EO such as *C. grevei* was investigated. Due to the typical variability in *T. vulgaris* EO composition, we analysed 15 different samples, which belong to the most famous both phenolic and non-phenolic, chemotypes, such as: thymol, linalool, terpinen-4-ol, geraniol, carvacrol, 1,8 cineole and sabinene hydrate. Moreover, a sample of *T. hyemalis* typical of Spanish regions was investigated. Some of the EOs – *C. aurantium* var. *dulcis*, *E. caryophyllata*, *J. communis*, *L. angustifolia*, *M. alternifolia*, *M. piperita*, *M. viridiflora*, and *S. sclarea* – proved to be in accordance with the data reported in Ph. Eur. 8th Ed. (Council of Europe 2014) and only minor differences were observed. In *M. viridiflora* cineole chemotype and *E. globulus*, all the constituents were in ranges present in the Eur. Ph. except for limonene, which was not found in our samples. The sample of *R. officinalis*, coming from France, that was rich in 1,8 cineole (32.2%), α-pinene (22.7%) and camphor (17.9%) showed an intermediate composition when compared to the two types present in the Ph. Eur. 8th Ed (Spanish type, Moroccan and Tunisian type) (Council of Europe 2014). In *C. camphora*, the major compounds were found to be 1,8 cineole (54.2%), sabinene (16.5%) and terpinen-4-ol (7.7%) a typical cineole-type composition (Stubbs & Specht 2004; Juliani et al. 2006). The principal constituents of *M. communis*, which comes from Morocco, were 1,8 cineole (45.2%), α-pinene (30.7%) and myrtanyl acetate (6.1%), as described in the literature for EOs of the same area (Chalchat et al. 1998). *O. vulgare* was shown to be rich in carvacrol (62.6%) and its precursor *p*-cymene (12.4%) (Russo et al. 1998; Council of Europe 2014). *P. asperum* EO showed a composition that was rich in monoterpene alcohols, such as citronellol (25.5%), geraniol (16.2%) linalool (10.9%) and a peculiar content in citronellyl formate (5.1%) in agreement with Hassane et al. (2012). *C. citratus*, *C. fragrans*,
C. martini, E. citriodora and M. officinalis showed the representative phytochemical profile present in the literature (Kasali et al. 2001; Rao et al. 2005; Nirmal et al. 2007; Romeo et al. 2008; Tucker et al. 2008; Randrianarivelo et al. 2009; Traoré et al. 2010; Piardu et al. 2012; Singh et al. 2012). The poorly known composition of C. grevei bark EO was investigated and 36 components were identified. It shows a peculiar composition that is very rich in sesquiterpenes, and the main compounds are ishwarane (26.6%), β-elemene (10%) and α-copaene (8.1%) (Cavalli et al. 2003; Gauvin et al. 2004). Among the 16 thyme samples, T. hyemalis was shown to be rich in 1,8 cineole (32.5%), camphor (15%) and borneol (5.3%). The main components of T. vulgaris samples 20 were linalool (43.7%) and terpinen-4-ol (13.8%); in T. vulgaris 21 terpinen-4-ol (48.2%) and p-cymene (10.1%). T. vulgaris 22 belongs to the carvacrol chemotype with a 27.3% of carvacrol and 29.3% of its precursor p-cymene. Samples 24 and 25 with geranyl acetate (23.6% and 38.2%, respectively) and geraniol (17.9%, 32.8%), representing more than 60% of the total composition, belong to the geraniol chemotype. Red thyme 26 and 27, linalool chemotype, contain about 75% linalool and 5% linalyl acetate. Sample 26 obtained from selected conventional crops (sel.) is slightly more enriched than sample 27 which was obtained from plants grown by organic farming (bio). Samples 28, 30, 33 and 34 belong to the thymol chemotype, the amount of thymol ranging from a minimum of 21.4% (sample 28) to a maximum 63% (sample 33) and its precursor p-cymene from 11.7% (sample 33) to 30.5% (sample 30). Red thyme 29, 31 and 32 as major constituents contain sabine hydrate and terpinen-4-ol ranging from 26.5% to 14% and from 26.5% to 16.3%, respectively so they belong to the thujanol-4/terpinen-4-ol chemotype. Finally, the major constituents of citrus thyme sample 35 are 1,8 cineole (47.7%), camphor (11.7%) and trans-sabinene hydrate (9.3%) (Thompson et al. 2003; Giordani et al. 2004; Council of Europe 2014).

The results of the cytotoxicity test revealed that 29 EOs showed high cytotoxicity and only 7 were slightly toxic: S. sclarea, M. alternifolia, T. hyemalis, T. vulgaris sample 24 (red thyme geraniol bio.), T. vulgaris sample 25 (red thyme geraniol sel.), T. vulgaris sample 26 (red thyme bio. France) and T. vulgaris sample 27 (red thyme sel. France). In particular, it is considered to be the concentration of the EO that allowed the 60% and 50%, respectively, in cell viability WKD, Caco-2 and Hep-2; all the results are summarised in Table 1. The toxicity test carried out on the selected EOs showed an IC60 for VKD in a range of 0.019–0.039% (v/v) while the CaCo-2 and Hep 2, IC50 value ranges were between 0.0039% and 0.062% (v/v). The seven EOs with the lowest cytotoxicity showed a very high concentration of monoterpene alcohols (in particular linalool and/or geraniol); in the case of a low percentage of these alcohols, however, their related esters were present at high levels.

These seven EOs were chosen for the subsequent investigations on the antimicrobial and antifungal activities. The MBC data for Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus for the aforementioned EOs are summarised in Table 2. The results obtained highlight good activity of the M. alternifolia EO. In particular, the P. aeruginosa ATCC strain showed an MBC of 1% and 4% (v/v). The clinical strain, T. hyemalis showed an MBC of 4% and 8% (v/v) (ATCC and clinical strains, respectively). In contrast to antimicrobial drugs, EOs were effective at inhibiting the growth of bacteria isolated from eye swabs. With regard to the S. aureus strains, the best result was obtained with T. hyemalis EO, while the worst result was obtained with S. sclarea, in which the MBC is >16% (v/v). Considering the results obtained with the E. coli strain ATCC, the best results, with an MBC <0.125% (v/v), was achieved with T. vulgaris sample 26, while the worst performance was highlighted with S. sclarea. The MBC of T. vulgaris 24, 25, 26 and 27 and T. hyemalis 23 observed showed good activity against E. coli ATCC, both clinical. The effectiveness of these EOs against bacteria is controversial, considering the concentration of the MBC in relation to the cytotoxicity. The MBC optimal value for both bacteria and fungi is <0.125% (v/v), which is still compatible with cell viability and shows good antibacterial activity. S. sclarea and M. alternifolia show MBCs
ranging from 0.5 to >16% (v/v), which are lower concentrations than those in the literature. In Table 3, the MBC results on 11 clinical Candida spp. strains are given. In C. albicans strains, the MBC range varies from <0.125% to 0.5% (v/v); the worst results were obtained with M. alternifolia and S. sclarea. The MCB of C. parapsilosis varies in the range from <0.125% to 1% (v/v) (only one strain has an MCB equal to 4) and also in this case the worst results were found with the S. sclarea and M. alternifolia EOs. The EOs in C. tropicalis show a range from <0.125% to 1% (v/v) and the worst results 16% and >16% (v/v) were obtained again with S. sclarea and M. alternifolia. The MBCs of C. glabrata show a range between <0.125% and 1% (v/v) and the less interesting data were achieved with M. alternifolia. Finally, C. krusei shows results that were fully comparable with those obtained with C. glabrata. The association

| n  | EO                    | EO concentration for 60% cellular vitality: |  |  |  |
|----|-----------------------|---------------------------------------------|  |  |  |
|    | WKD %                 | Caco-2 %                                    |  |  |  |
|    | (v/v)                 | (v/v)                                       |  |  |  |
| 1  | C. aurantium          | <0.12                                       | <0.12 | <0.12 |  |
| 2  | E. cariophyllata      | <0.12                                       | <0.12 | <0.12 |  |
| 3  | E. globulus           | <0.12                                       | <0.12 | <0.12 |  |
| 4  | P. asperum            | <0.12                                       | <0.12 | <0.12 |  |
| 5  | L. angustifolia       | <0.12                                       | <0.12 | <0.12 |  |
| 6  | C. citratus           | <0.12                                       | <0.12 | <0.12 |  |
| 7  | M. piperita           | <0.12                                       | <0.12 | <0.12 |  |
| 8  | M. communis           | <0.12                                       | <0.12 | <0.12 |  |
| 9  | O. vulgare            | <0.12                                       | <0.12 | <0.12 |  |
| 10 | R. officinalis        | <0.12                                       | <0.12 | <0.12 |  |
| 11 | S. sclarea            | 0.031                                       | 0.031 | 0.031 |  |
| 12 | M. alternifolia       | 0.015                                       | 0.031 | 0.031 |  |
| 13 | C. grevei             | <0.12                                       | <0.12 | <0.12 |  |
| 14 | C. camphora           | <0.12                                       | <0.12 | <0.12 |  |
| 15 | C. fragrans           | <0.12                                       | <0.12 | <0.12 |  |
| 16 | E. citriodora         | <0.12                                       | <0.12 | <0.12 |  |
| 17 | M. viridiflora        | <0.12                                       | <0.12 | <0.12 |  |
| 18 | C. martini            | <0.12                                       | <0.12 | <0.12 |  |
| 19 | M. officinalis        | <0.12                                       | <0.12 | <0.12 |  |
| 20 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 21 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 22 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 23 | T. hyemalis           | 0.0039                                      | 0.0039 | 0.031 |  |
| 24 | T. vulgaris           | 0.031                                       | 0.062 | 0.031 |  |
| 25 | T. vulgaris           | 0.015                                       | 0.031 | 0.031 |  |
| 26 | T. vulgaris           | 0.015                                       | 0.031 | 0.031 |  |
| 27 | T. vulgaris           | 0.0019                                      | 0.062 | 0.031 |  |
| 28 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 29 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 30 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 31 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 32 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 33 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 34 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 35 | T. vulgaris           | <0.12                                       | <0.12 | <0.12 |  |
| 36 | J. communis           | <0.12                                       | <0.12 | <0.12 |  |
Table 2. Minimum bactericidal concentration (MBC) of gram-positive and gram-negative strains % (v/v).

| Bacterial strains | EOs            |
|-------------------|----------------|
|                   | S. sclarea 11  |
|                   | MIC % (v/v)    |
| S. aureus ATCC 43300 | >16            |
| S. aureus ATCC 29213 | >16            |
| E. coli ATCC 35281  | >16            |
| E. coli ATCC 25922  | >16            |
| E. coli           | >16            |
| P. aeruginosa ATCC 27853 | 16            |
| P. aeruginosa     | >16            |

|                   | M. alternifolia 12 |
|                   | MIC % (v/v)        |
|                   | 8                 |
|                   | >16               |
|                   | 4                 |
|                   | 1                 |
|                   | 0.5               |
|                   | <0.125            |
|                   | 16                |
|                   | 8                 |

|                   | T. hyemalis 23    |
|                   | MIC % (v/v)       |
|                   | 1                 |
|                   | 0.5               |
|                   | <0.125            |
|                   | 16                |
|                   | 16                |
|                   | >16               |

|                   | T. vulgaris 24    |
|                   | MIC % (v/v)       |
|                   | 1                 |
|                   | 0.25              |
|                   | <0.125            |
|                   | 16                |
|                   | 8                 |
|                   | >16               |

|                   | T. vulgaris 25    |
|                   | MIC % (v/v)       |
|                   | 0.5               |
|                   | <0.25             |
|                   | <0.125            |
|                   | 16                |
|                   | 16                |
|                   | >16               |

|                   | T. vulgaris 26    |
|                   | MIC % (v/v)       |
|                   | 0.5               |
|                   | <0.125            |
|                   | <0.125            |
|                   | 16                |
|                   | 16                |
|                   | >16               |

|                   | T. vulgaris 27    |
|                   | MIC % (v/v)       |
|                   | 0.25              |
|                   | <0.125            |
|                   | <0.125            |
|                   | 16                |
|                   | 16                |
|                   | >16               |
Table 3. MBC of *Candida* spp. strains % (v/v).

| Candida spp.          | S. sclarea 11 MIC % (v/v) | M. alternifolia 12 MIC % (v/v) | T. hyemalis 23 MIC % (v/v) | T. vulgaris 24 MIC % (v/v) | T. vulgaris 25 MIC % (v/v) | T. vulgaris 26 MIC % (v/v) | T. vulgaris 27 MIC % (v/v) |
|-----------------------|---------------------------|-------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| *C. albicans* (three strains) | (1) > 16                  | (1) 1                          | (2) < 0.125               | (1) < 0.125               | (1) < 0.125               | (1) < 0.125               | (1) < 0.125               |
|                        | (1) 0.5                    |                               | (1) 0.5                   | (2) 0.25                  | (2) 0.5                   | (2) 0.5                   | (2) 0.5                   |
| *C. parapsilosis* (two strains) | (1) 4                      | (1) > 16                      | (1) 0.5                   | (1) 0.5                   | (1) 0.5                   | (1) 0.5                   | (1) 0.5                   |
|                        | (1) > 0.125                |                               | (1) < 0.125               | (1) < 0.125               | (1) < 0.125               | (1) < 0.125               | (1) < 0.125               |
| *C. tropicalis* (two strains) | (1) 16                     | (1) 0.5                        | (2) 0.5                   | (2) 0.25                  | (1) < 0.125               | (1) < 0.125               | (1) < 0.125               |
|                        | (1) 1                       |                              | (2) > 16                  | (1) 1                     | (1) 0.5                   | (1) 0.5                   | (1) 0.5                   |
| *C. glabrata* (two strains) | (1) 1                      | (1) 0.5                        | (1) 0.5                   | (1) 0.25                  | (1) < 0.125               | (1) < 0.125               | (1) < 0.125               |
|                        | (2) > 16                    |                              | (1) 0.25                  | (1) 0.25                  | (1) < 0.125               | (1) < 0.125               | (1) < 0.125               |
| *C. krusei* (two strains) | (1) 0.5                    | (2) > 16                       | (1) 0.5                   | (1) < 0.125               | (1) 0.5                   | (1) < 0.125               | (1) < 0.125               |
|                        | (1) 1                       |                              | (1) 0.25                  | (1) 0.25                  | (1) < 0.125               | (1) < 0.125               | (1) < 0.125               |
between the chemical fingerprinting of the EOs and the biological activity led to interesting composition–bioactivity correlations. In gas chromatographic analysis, we observed that all the samples of *Thymus* spp. showed a high concentration of hydrocarbon monoterpenes and oxygenated monoterpenes. What discriminated *T. vulgaris* 24, 25, 26, 27 and the *T. hyemalis* 23 from other the *Thymus* species analysed is the presence of esters and ketones that were absent in the others; *T. hyemalis* also has a high concentration of 1–8 cineole (32.5%) and a low concentration of linalool that was present in high concentration in species and 24, 25, 26 and 27. *S. sclarea* showed a high concentration of linalyl acetate (oxygenated monoterpen), whereas *M. alternifolia* contains high concentrations of monoterpenes both hydrocarbon and oxygenated. In conclusion, EOs are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In vitro* studies in this work showed that the EOs inhibited bacterial growth, but their effectiveness varied. Study of the EOs in their complexity and especially an understanding of the specific activity of each component will increase the appropriate uses of these natural products. Finally, the synergy between the components of EOs still requires more study before these complex matrices can be reliably used in antimicrobial applications.

### 3. Conclusion

In conclusion, EOs are potential sources of novel antimicrobial compounds, especially against bacterial pathogens. *In vitro* studies in this work showed that the EOs inhibited bacterial growth, but their effectiveness varied. Study of the EOs in their complexity and especially an understanding of the specific activity of each component will increase the appropriate uses of these natural products.

Finally, the synergy between the components of EOs still requires more study before these complex matrices can be reliably used in antimicrobial applications.

### Supplementary material

The underlying research materials for this article can be accessed at [http://dx.doi.org/10.1080/14786419.2015.1060592](http://dx.doi.org/10.1080/14786419.2015.1060592).

### Conflict of Interest

The authors declare no conflict of interest.

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