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# A comparative assessment of antifungal activity of essential oils of five medicinal plants from Tunisia

Taycir Grati Affes[a,c], Salma Lasram[b], Majdi Hammami[a], Walid Yeddes[a], Wissem Aidi Wannes[a,*], Saber Khammassi[a], Bouzid Nasraoui[c], Moufida Saidani Tounsi[a], Nehla Labidi Ben Hmida[a]

[a] *Borj-Cedria Biotechnology Center, Laboratory of Aromatic and Medicinal Plants, BP901, Hammam-Lif 2050, Tunisia*
[b] *Borj-Cedria Biotechnology Center, Laboratory of Molecular Physiology of Plants, BP901, Hammam-Lif 2050, Tunisia*
[c] *Carthage University, INAT, LR / Bioaggressors and Integrated Pest Management in Agriculture, LR14AGR02, Tunis, Tunisia*

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ABSTRACT

The leaf essential oil yields of clementine, cypress, rosemary, tea, and thyme were 0.22, 0.87, 1.46, 1.20, and 0.72%, respectively, based on the dry weight of the plant material. The leaf essential oils of rosemary, tea, and thyme contained the highest levels of oxygenated monoterpenes (60.14-91.70%). Rosemary and tea leaf essential oils were rich in 1,8-cineole (49.98% and 57.55%, respectively), and they have potent antifungal activity against *Alternaria alternata* strain (MIC = 5000 µg/ml). Thyme was rich in carvacrol (78.54%) and had a MIC of 6000 µg/ml against *A. alternata* strain. Clementine leaf essential oil was characterized by the predominance of monoterpene hydrocarbons (88.65%), and it possessed a weak antifungal activity against *A. alternata* (MIC = 8000 µg/ml). Cypress leaf essential oil was characterized by the predominance of oxygenated sesquiterpenes (60.67%), having an antifungal activity of 8000 µg/ml.

## 1. Introduction

Fungal infections are not just a human health problem but also greatly impact the field of agriculture (Mohammadi and Bahramikia, 2019). The emergence of resistant fungus strains may limit the use of synthetic fungicides, and some fungicides possess considerable toxicity. Hence, there is a growing public concern over synthetic molecules' increased health and environmental hazards. For this reason, alternative, safe, and natural methods that develop new antifungal agents are actively studied (Lopez-Reyes et al., 2013). Recently, there has been a great interest in using essential oils as possible natural substitutes for conventional synthetic fungicides (Elshafie and Camele, 2015). Essential oils can represent one of the most promising natural products for fungal inhibition (D'agostino et al., 2019). The essential oil preparations that possess antimicrobial activities have been the subject of many investigations resulting in screening a wide variety of plant species and have revealed structurally unique biologically active compounds (Matasyohet al., 2007). Again, the essential oils of some plants have recently been proven to be a successful eco-friendly bio-control agent (Raveau et al., 2020). Many authors have reported antimicrobial, antifungal, antioxidant, and radical-scavenging properties of essential oils (Chouhan et al., 2017) and, in some cases, a direct food-related application (Bhavaniramya et al., 2019). In recent years, plant essential oils have been widely studied as an emerging, environmentally-friendly, antibacterial substance. The main essential oils studied for their antifungal activity are thyme (*Thymus vulgaris* L.) essential oil, rich in thymol and carvacrol. It is already known to be effective against fungi infecting humans. Its antifungal activity is due to its high concentration of thymol and carvacrol (Yeddes et al., 2018). Cypress (*Cupressus sempervirens* L.) is known for its leaf essential oil, which is used to protect stored grains from

* Corresponding author:
E-mail address: aidiwissem@yahoo.fr (W.Aidi Wannes)
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insect infestation (Elansary et al., 2012), and for its antimicrobial properties (Yan et al., 2009; González and Marioli, 2010). Rosemary (*Rosmarinus officinalis* L.) essential oil is used for its antioxidant, anti-inflammatory, anti-diabetic, and anti-cancer therapeutic properties. This essential oil also showed antimicrobial activity against various microorganisms, including pathogenic fungi (Moore et al., 2016). Already known for its antibacterial action (Golab and Skwarlo-Sonta, 2007), tea tree essential oil (*Melaleuca alternifolia* Sm.) also has an antifungal effect that was successfully evaluated in dermatomycological infections (Carson et al., 2006). Citrus leaf essential oils showed an important antimicrobial activity against some tested organisms. All citrus leaf essential oils were more effective against the fungus *Kluyveromyces fragilis* than the other microorganisms (Kasali et al., 2011).

Although a large variety of plants have been studied for their antimicrobial activities worldwide, the antifungal activities of leaf essential oils from these species are still scarce against *Alternaria alternata*. *A. alternata* is one of the most common saprophytes worldwide (Mohammadi and Bahramikia, 2019). *A. alternata* causes various diseases with an economic impact on a large range of crops, such as potato, pomegranate, almond, kiwi, cactus, tomato, ginseng, citrus, banana, and pepper water hyacinth (Dube, 2014). Therefore, this study aimed to screen the antifungal activities of the leaf essential oils from clementine, cypress, rosemary, tea, and thyme against *A. alternata* strain.

## 2. Materials and methods

### *2.1. Plant material*

The samples were taken from clementine (*Citrus clementina* Hort.) at Beni Khaled (Cap Bon region, North-East Tunisia), cypress (*C. semperviens* L.) at Bir Hlima (Zaghouan region-North-West Tunisia), rosemary (*R. officinalis* L.) at Djebel Zaghouan (Zaghouan region, North-West Tunisia), tea (*M. linariifolia* Sm.) in Tunis botanical park (Tunis region-North-East Tunisia), and thyme (*T. vulgaris* L.) at Djebel Zaghouan (Zaghouan region-North-West Tunisia) during spring-summer 2019.

### *2.2. Essential oil extraction and analysis*

The fresh leaves of each species (100 g) were submitted to hydrodistillation for 180 min using a Clevenger-type apparatus. The essential oils obtained were dried over anhydrous sodium sulfate and stored at -20 °C in darkness until analyzed (Yeddes et al., 2022a).

Analysis of volatile compounds by gas chromatography (GC) was carried out on a Hewlett-Packard 6890 GC (Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control injector. A polar polyethylene glycol HP Innowax, a 5% diphenyl, and 95% dimethylpolysiloxane apolar HP-5 capillary columns were used.

Volatile compounds analysis by gas chromatography/mass spectrometry (GC/MS) was performed on a gas chromatograph HP 5890 (II) interfaced with an HP 5972 mass spectrometer (Palo Alto, CA, USA) with electron impact ionization (70 eV).

Identification of essential oil volatile compounds was based on calculating their retention indices (RI) relative to ($C_8$-$C_{22}$) *n*-alkanes with those of authentic compounds available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the GC-MS data systems' Wiley/NBS mass spectral library and other published mass spectra.

### *2.3. Fungal strain*

*A. alternata* strain was an isolate of 7025 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5, 99.4% (*C. reticulata* - Fortune variety, Beni Khaled) GenBank accession number for nucleotide sequence: (T1): OK448177 (Grati Affes et al., 2022).

### *2.4. Minimum inhibitory concentration of essential oils*

A portion of *A. alternata* culture stored at -20 °C in a 20% glycerol solution was taken and cultured on potato dextrose agar (PDA) in the dark at 27 °C. The method of Zerigui and Mouzaoui (2018) was applied to stimulate sporulation. After 10 days of incubation, the petri dishes were put at 4 °C for one hour. Then, they were exposed to direct light for 3 hours and returned to room temperature for 24 hours in the dark. In the end, the spore suspension was recovered after scraping the culture of *A. alternata*.

The spore suspension of *A. alternata* in a .01% Tween 80 medium was prepared from an 8-day-old single-spherical culture of several eppendorf tubes with different doses of essential oils from 0 to 12 mg/ml (diluted with a 0.5% Tween 20 solution) in potato dextrose broth (PDB) medium. Then, 20 µl of spore suspension ($10^{-5}$) was added. Finally, the tubes were incubated with shaking in the dark at 25 °C. The MIC was defined as the lowest concentration of the essential oil required to completely prevent visible fungal growth (Grati Affes et al., 2022).

### *2.5. Statistical analysis*

All experiments were conducted in triplicates, and the results were expressed as mean values of standard deviation (SD). Data were subjected to statistical analysis using SAS (V.9.1). One-way analysis of variance (ANOVA) followed by Student Newman Keulstests at the significance level of 5% was used to compare means. The principal component analysis (realized by XLSTAT-2017) was used to comprehend the similarity among citrus essential oils and their antifungal activities.

## 3. Results and discussion

### *3.1. Essential oil analysis*

The leaf essential oil yields of clementine, cypress, rosemary, tea, and thyme leaves were 0.22, 0.87, 1.46, 1.20, and 0.72%, respectively, based on the dry weight of the plant material. GC-MS analysis of citrus, cypress, rosemary, tea, and thyme essential oils are given in Figure 1.

As shown in Table 1, nineteen volatile compounds were identified in clementine leaf representing 94.80% of essential oil, twenty-four compounds in cypress representing 98.96% of essential oil, twenty-two compounds in rosemary representing 99.97% of essential oil, eighteen compounds in tea representing 99.92% of essential oil, and sixteen compounds in thyme representing 99.95% of essential oil. Clementine leaf essential oil was characterized by the predominance of sabinene (29.90%), limonene (28.73%), linalool (14.62%), and Δ-3-carene (7.70%). Cypress leaf essential oil was rich in germacrene-D (34.26%) and α-pinene (22.71%), while tea essential oil mainly contained 1,8-cineole (49.98%) and α-terpineol (35.92%). 1,8-

Cineole (57.55%) was the main component in rosemary essential oil and thymol (78.54%) in thyme essential oil.

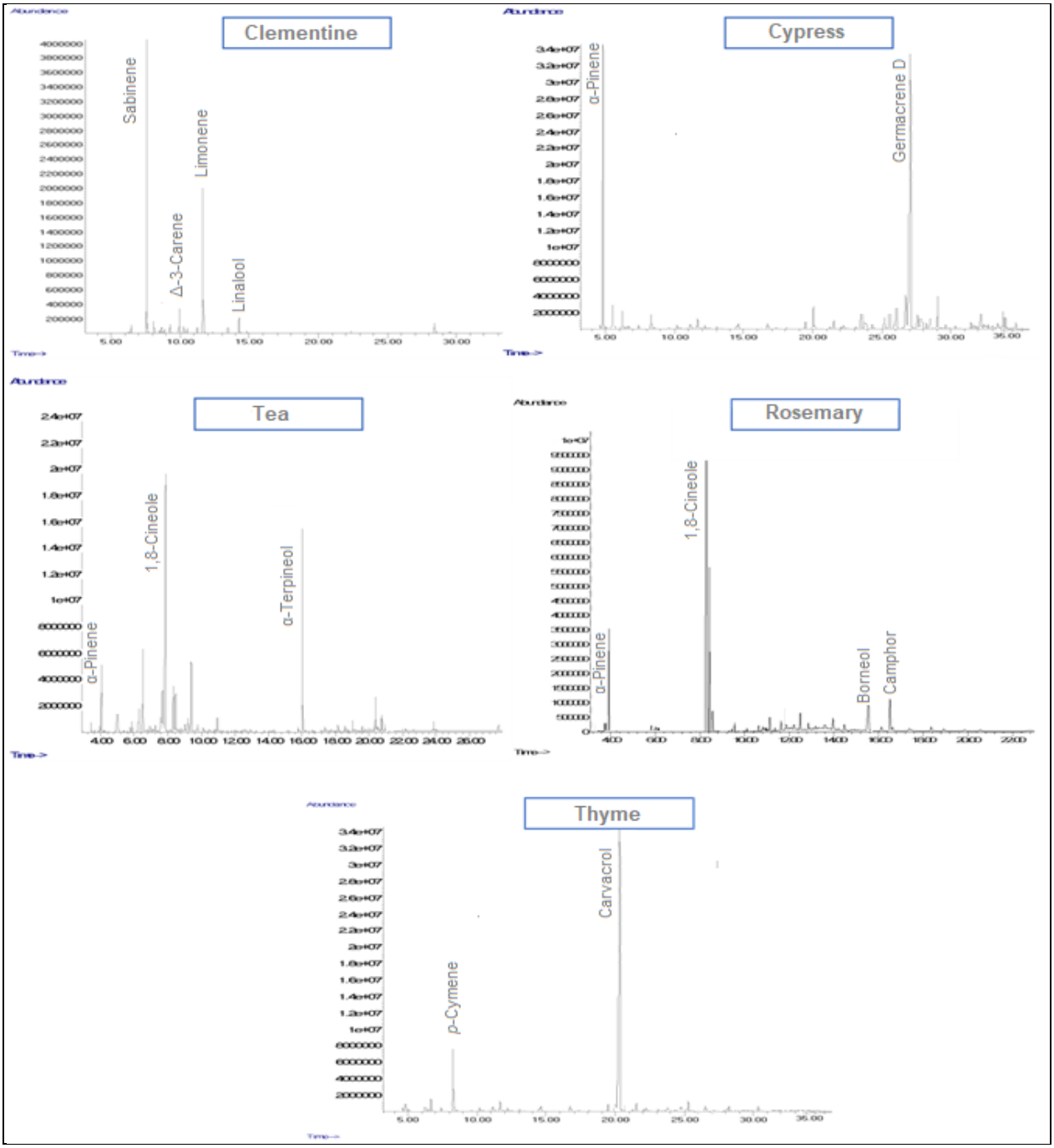


**Figure 1.** Gas chromatography chromatograms of the leaf essential oils from clementine, cypress, rosemary, tea, and thyme and their main volatile compounds

Fewer studies have determined the essential oil composition of clementine leaf essential oil, which was dominated by sabinene up to 36.80% (Dugo et al., 2011; Germanà et al., 2013; Thi Nguyen et al., 2016). The major component of cypress leaf essential oil was usually considered α-pinene (37.14-73.75%), as reported by Khadidja et al. (2010), Boukhris et al. (2012), Amri et al. (2013) and Hosni et al. (2019). As mentioned in the literature, rosemary leaf essential oil revealed the existence of several chemotypes as 1,8-cineole (Napoli et al., 2010; Guetat et al., 2014; Yeddes et al., 2018; Yeddes et al., 2022a), camphor (Celiktas et al., 2007; Zaouali and Boussaid, 2008; Lakušić et al., 2012), verbenone (Mata et al., 2007; Papageorgiou et al., 2008; Varela et al., 2007), α-pinene (Angioni et al., 2004; Napoli et al., 2010), linalool (Varela et al., 2007), and *p*-cymene (Özcan and Chalchat, 2008) chemotypes. Tea leaf essential oil was characterized by its richness in 1,8-cineole (61.10-77.40%) and α-terpineol (7.72-12.30%) in India (Padalia et al., 2015; Joshi et al., 2022). However, Australian tea leaf essential oil showed α-terpineol (30.18%) and γ-terpinene (15.20%) chemotype (Park et al., 2011). The essential oil of tea leaf from Brazil was shown to be characterized by methyl eugenol (86.8%) and *(E)*-methyl isoeugenol (1.4%) (Silva et al., 2010). Thyme leaf essential oil was rich in carvacrol ranging from 20 to 71% (Porte and Godoy, 2008; Aslam et

al., 2022; Yeddes et al., 2022b). Numerous studies also reported the essential oil composition of thyme with thymol as the main constituent ranging from 22 to 71% (Soković et al., 2009; Shabnum and Wagay, 2011; Kowalczyk et al., 2020). These variations in the essential oil compositions between the same species could be attributed to many factors such as genetic dissimilarity within accessions, climate variability, plant origin, sample drying, storage, and extraction processes (Sadeh et al., 2019).

**Table 1.** Essential oil compositions of clementine, cypress, rosemary, tea, and thyme leaves*

| Volatile compounds | RI[a] | RI[b] | Clementine | Cypress | Rosemary | Tea | Thyme |
|---|---|---|---|---|---|---|---|
| α-Thujene | 923 | 836 | 0.17 ± 0.08[a] | 0.08 ± 0.04[c] | 0.11 ± 0.12[b] | 0.08 ± 0.04[c] | - |
| α-Pinene | 934 | 982 | 0.65 ± 0.22[e] | 22.71 ± 0.04[a] | 8.83 ± 0.14[b] | 2.96 ± 0.79[c] | 1.07 ± 0.22[d] |
| β-Pinene | 937 | 1113 | - | 0.08 ± 0.04[c] | 2.82 ± 0.36[a] | 0.30 ± 0.14[b] | - |
| Camphene | 952 | 1077 | - | - | 2.36 ± 0.19[a] | 0.13 ± 0.06[c] | 0.31 ± 0.06[b] |
| Sabinene | 983 | 1111 | 29.90 ± 0.22[a] | - | 0.04 ± 0.02[c] | - | 0.16 ± 0.03[b] |
| β-Myrcene | 991 | 1168 | 1.58 ± 0.36[b] | 0.18 ± 0.08[e] | 0.89 ± 0.36[c] | 1.65 ± 0.56[a] | 0.58 ± 0.12[d] |
| α-Phellandrene | 1005 | 1025 | 0.23 ± 0.10[a] | - | 0.19 ± 0.20[b] | - | - |
| Δ-3-Carene | 1011 | 1159 | 7.70 ± 0.23[a] | 2.05 ± 0.36[b] | 0.08 ± 0.04[c] | - | - |
| α-Terpinyl acetate | 1014 | 1340 | - | 2.61 ± 0.48[a] | - | - | - |
| α-Terpinene | 1018 | 1255 | - | - | 0.62 ± 0.16[b] | - | 0.91 ± 0.19[a] |
| α-Cubebene | 1352 | 1460 | - | 0.76 ± 0.23[a] | - | - | - |
| *p*-Cymene | 1026 | 1277 | - | - | 1.01 ± 0.36[b] | 0.07 ± 0.04[c] | 7.13 ± 1.49[a] |
| Limonene | 1030 | 1031 | 28.73 ± 0.67[a] | - | - | 0.04 ± 0.02[b] | - |
| 1,8-cineole | 1033 | 1029 | - | - | 57.55 ± 1.54[a] | 49.98 ± 3.27[b] | 3.50 ± 0.73[c] |
| (*E*)-β-Ocimene | 1052 | 1022 | 4.20 ± 0.43[a] | - | - | - | - |
| γ-Terpinene | 1059 | 1262 | 0.89 ± 0.27[d] | - | 1.00 ± 0.09[c] | 1.56 ± 0.54[b] | 3.44 ± 0.72[a] |
| Terpinolene | 1083 | 1086 | 1.52 ± 0.36[a] | 0.41 ± 0.15[b] | 0.34 ± 0.05[c] | - | - |
| Fenchol | 1121 | 1126 | - | - | - | 0.41 ± 0.19[a] | - |
| Linalool | 1098 | 1551 | 14.62 ± 0.53[a] | - | 0.57 ± 0.09[b] | - | 0.39 ± 0.08[c] |
| Citronellal | 1151 | 1542 | 3.72 ± 0.42[a] | - | - | - | - |
| Borneol | 1165 | 1642 | - | - | 5.54 ± 1.12[a] | - | 0.85 ± 0.18[b] |
| Terpinen-4-ol | 1178 | 1593 | 2.68 ± 0.43[a] | - | 1.02 ± 0.31[b] | 0.50 ± 0.22[d] | 0.64 ± 0.13[c] |
| α-Terpineol | 1185 | 1772 | 0.41 ± 0.16[c] | - | 3.62 ± 0.68[b] | 35.93 ± 0.92[a] | 0.14 ± 0.03[d] |
| Citronellol | 1224 | 1624 | 0.69 ± 0.23[a] | - | - | - | - |
| Geraniol | 1271 | 1856 | 0.95 ± 0.25[a] | - | - | - | - |
| Eugenol | 1344 | 1752 | - | - | - | 0.20 ± 0.09[a] | - |
| Camphor | 1192 | 1498 | - | - | 8.82 ± 1.09[a] | - | - |
| Bornyl acetate | 1285 | 1291 | - | - | 0.82 ± 0.08[a] | - | - |
| Thymol | 1296 | 2198 | - | - | - | - | 0.18 ± 0.04[a] |
| Carvacrol | 1306 | 2239 | - | - | - | - | 78.54 ± 4.50[a] |
| Isoledene | 1376 | 1687 | - | 0.56 ± 0.19[a] | - | - | - |
| α-Copaene | 1395 | 1391 | - | 0.28 ± 0.12[a] | - | 0.09 ± 0.04[b] | - |
| Methyl eugenol | 1401 | 1405 | - | - | - | 0.49 ± 0.22[a] | - |
| α-Cedrene | 1410 | 1577 | - | 1.30 ± 0.31[a] | - | - | - |
| (*E*)-Caryophyllene | 1446 | 1608 | 0.69 ± 0.23[d] | 3.94 ± 0.37[a] | 1.99 ± 009[b] | 0.06 ± 0.03[e] | 1.58 ± 0.33[c] |
| *cis*-muurola-3.5-diene | 1465 | 1699 | - | 0.84 ± 0.25[a] | - | - | - |
| Germacrene D | 1480 | 1685 | - | 34.26 ± 3.89[a] | - | - | - |
| α-Humulene | 1485 | 1691 | 0.08 ± 0.04[b] | - | 0.29 ± 0.02[a] | 0.04 ± 0.02[c] | - |
| Longipinene | 1489 | 1692 | - | 0.81 ± 0.24[a] | - | - | - |
| Bicyclogermacrène | 1490 | 1757 | 0.18 ± 0.17 | - | - | - | - |
| *epi*-Bicyclosesquiphellandrene | 1492 | 1702 | - | 2.67 ± 0.38[a] | - | - | - |
| γ-Muurolene | 1502 | 1704 | - | 2.80 ± 0.38[a] | - | - | - |
| β-Cadinene | 1525 | 1776 | - | 6.66 ± 0.21[a] | - | - | - |
| γ-Cadinene | 1532 | 1530 | - | 1.60 ± 0.34[a] | - | - | - |
| α-Caryophyllene | 1564 | 1677 | - | 2.98 ± 0.38[a] | - | - | - |
| Caryophyllene oxide | 1578 | 1582 | - | 0.18 ± 0.06[c] | 0.20 ± 0.10[c] | 0.49 ± 0.22[b] | 0.57 ± 0.12[a] |
| α-Cedrol | 1608 | 1616 | - | 9.17 ± 0.03[a] | - | - | - |
| α-Cadinol | 1654 | 1546 | - | 0.45 ± 0.16[a] | - | - | - |
| Sclareol | 1659 | 1555 | - | 0.39 ± 0.15[a] | - | - | - |
| Monoterpene hydrocarbons | | | 88.65 ± 1.12[a] | 25.51 ± 2.38[c] | 26.99 ± 0.73[b] | 6.79 ± 0.72[e] | 13.60 ± 0.25[d] |
| Oxygenated monoterpenes | | | 4.27 ± 0.75[d] | 2.61 ± 0.22[e] | 60.14 ± 0.66[c] | 91.70 ± 2.15[a] | 84.20 ± 1.75[b] |
| Sesquiterpene hydrocarbons | | | 1.88 ± 0.09[b] | 10.17 ± 0.10[a] | 0.56 ± 0.04[c] | 0.49 ± 0.10[d] | 0.57 ± 0.05[c] |
| Oxygenated sesquiterpenes | | | - | 60.67 ± 0.90[a] | 12.28 ± 0.06[b] | 0.94 ± 0.16[d] | 1.58 ± 0.09[c] |
| Total | | | 94.80 ± 1.32[c] | 98.96 ± 2.30[b] | 99.97 ± 1.12[a] | 99.92 ± 0.99[a] | 99.95 ± 1.32[a] |

*Compounds in order of elution on HP-5 MS.
[a]RI: Retention index calculated on HP-5 MS column.
[b]RI: Retention index calculated on HP Innowax column.
Means of three replicates (Values with different superscripts are significantly different at $p < 0.05$).

### *3.2. In vitro antifungal activity*

The antifungal activities of leaf essential oils from clementine, cypress, rosemary, tea, and thyme species were tested against *A. alternata* strain. The minimal concentration of these essential oils ranged between 5000 and 8000 µg/ml, completely inhibited the growth of *A. alternata*. The results presented in Table 2 showed similar activity between clementine and cypress essential oils with MIC = 8000 µg/ml. A similar result was obtained between rosemary and tea essential oils with MIC = 5000 µg/ml. The MIC of thyme essential oil was 6000 µg/ml. So, rosemary and tea essential oils had the best antifungal activity against *A. alternata* strain. Contrarily to our results, Shaban (2014) reported that the highest degree of antifungal activity against *A. alternata* was caused by thyme essential oil and followed by rosemary essential oil. Amri et al. (2013) found that cypress leaf essential oil had potent antifungal activity against *Alternaria* spp. (75.21%). Alves et al. (2019) detected that tea (*M. alternifolia*) essential oil had a strong antifungal activity

(MIC = 14.49 µg/ml) against *A. alternata*. Hamdani and Allem (2015) determined the antifungal activity of the leaf essential oil from citrus (*C. limon*, *C. sinensis*, and *C. reticulata*) and found that the concentration of 1000 µg/ml was sufficient to inhibit the development of *A. alternata*. This difference in results could be due to the existent differences in components and the concentrations of the active compounds in the essential oils of each species.

**Table 2.** Antifungal activity of leaf essential oils from clementine, cypress, rosemary, tea and thyme

| Species | Minimum inhibitory concentration (MIC, µg/ml) | | | | |
|---|---|---|---|---|---|
| | Clementine | Cypress | Rosemary | Tea | Thyme |
| *A. alternata* | 8000 ± 0.00$^{a}$ | 8000 ± 0.00$^{a}$ | 5000 ± 0.00$^{c}$ | 5000 ± 0.00$^{c}$ | 6000 ± 0.00$^{b}$ |

Values with different superscripts are significantly different at $p < 0.05$.

**Table 3.** Correlation between the main essential oil compounds, essential oil yield, and antifungal activity

| | α-Pinene | Sabinene | Δ-3-Carene | *p*-Cymene | Limonene | 1,8-Cineole | Linalool | α-Terpineol | Carvacrol | EO yield | Strain |
|---|---|---|---|---|---|---|---|---|---|---|---|
| α-Pinene | 11 | | | | | | | | | | |
| Sabinene | 0.095 | 1 | | | | | | | | | |
| Δ-3-Carene | 0.809 | 0.453 | 1 | | | | | | | | |
| *p*-Cymene | -0.073 | -0.309 | -0.405 | 1 | | | | | | | |
| Limonene | -0.796 | 0.000 | -0.348 | -0.296 | 1 | | | | | | |
| 1,8-Cineole | 0.747 | -0.309 | 0.704 | -0.250 | -0.297 | 1 | | | | | |
| Linalool | -0.073 | -0.237 | -0.405 | -0.250 | -0.284 | -0.249 | 1 | | | | |
| α-Terpineol | 0.723 | -0.010 | 0.850 | -0.528 | -0.183 | 0.934 | -0.255 | 1 | | | |
| Carvacrol | -0.801 | -0.245 | -0.405 | -0.250 | 0.990 | -0.250 | -0.250 | -0.179 | 1 | | |
| EO yield | -0.256 | 0.687 | -0.216 | -0.028 | -0.110 | 0.794 | 0.361 | -0.611 | -0.205 | 1 | |
| Strain | 0.560 | 0.587 | 0.145 | 0.590 | -0.232 | -0.589 | -0.516 | 0.306 | -0.547 | -0.709 | 1 |

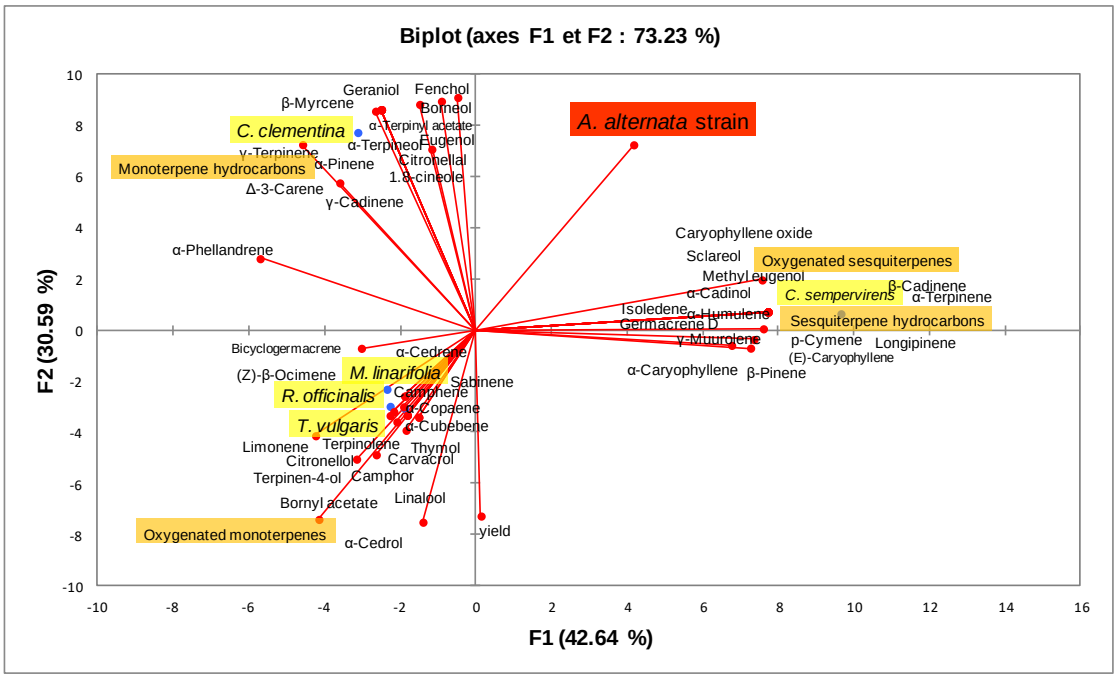


**Figure 2.** Biplot obtained from principal component analysis of variables comprising essential oil components, chemical classes, and antifungal activities

### *3.3. Relation between essential oil compounds and antifungal activity*

The analysis of Pearson's correlation coefficients between the main essential oil compounds and antifungal activity (Table 3) showed a positive correlation between *A. alternata* strain and the volatile compounds α-pinene ($r$ = 0.560), sabinene ($r$ = 0.587), and *p*-cymene ($r$ = 0.590). This could be explicated by the fact that the high proportions of α-pinene in cypress and sabinene in clementine contributed to the high MIC values of these two species (MIC = 8000 µg/ml). However, there was a negative correlation between *A. alternata* strain and the volatile compounds 1,8-cineole ($r$ = -0.589), linalool ($r$ = -0.516) and carvacrol ($r$ = -0.547). So, the high proportions of carvacrol in thyme and 1,8-cineole in rosemary and tea contributed to the low MIC values of these species (MIC = 6000 µg/ml for thyme and 5000 µg/ml for rosemary and tea). On the other hand, there was a strong negative correlation between the essential oil yield and *A. alternata* strain ($r$ = -0.709), explicating that the antifungal activity depended on the yield of these essential oils. On the other hand, there was a strong correlation between these volatile compounds as between α-pinene/Δ-3-carene (0.809), α-pinene/limonene ($r$ = -0.796), α-pinene/1,8-cineole ($r$ = 0.747), α-pinene/α-terpineol ($r$ = 0.723), α-pinene/carvacrol ($r$ = -0.801), Δ-3-carene/1,8-cineole ($r$ = 0.704), Δ-3-carene/α-terpineol ($r$ = 0.850), *p*-cymene/α-terpineol ($r$ = -0.528), limonene/carvacrol ($r$ = -0.990), and 1,8-cineole/α-terpineol ($r$ = 0.934). So, there is a relationship in the biosynthesis of these compounds, known as monoterpenes, via

a universal precursor of monoterpenes is geranyl pyrophosphate, combining two C5 units, which is then further processed by monoterpene synthases/cyclases to produce a vast array of chemical structures (Reiling et al., 2004).

### *3.4. Principal component analysis*

The principal component analysis (PCA) was applied to assess the chemical composition and the antifungal activity of leaf essential oils from clementine, cypress, tea, rosemary, and thyme species (Figure 2). So, PCA greatly helps interpret results from the experiments, the two-dimensional axial systems generated from PCA of these essential oils showed that there were three main groups, as indicated in Figure 2. In fact, rosemary, tea, and thyme were closer due to the similarities of the highest levels of oxygenated monoterpenes (60.14-91.70%). Rosemary and tea were rich in 1,8-cineole (49.98% and 57.55%, respectively), and they have potent antifungal activity against *A. alternata* strain (MIC = 5000 µg/ml). Thyme was rich in carvacrol (78.54%) and had a MIC of 6000 µg/ml against *A. alternata* strain. Clementine leaf essential oil was characterized by the predominance of monoterpene hydrocarbons (88.65%), and it possessed a weak antifungal activity against *A. alternata* (MIC = 8000 µg/ml), constituting the second cluster. Finally, cypress leaf essential oil formed the third cluster characterized by the predominance of oxygenated sesquiterpenes (60.67%), having an antifungal activity of 8000 µg/ml. Accordingly to Bassolé et al. (2010), phenolic monoterpenes (thymol and carvacrol) and phenylpropanoids (eugenol) in combination with other components were found to increase the bioactivities of essential oils. Most studies have focused on the interaction of phenolic monoterpenes and phenylpropanoids with other groups of components, particularly with other phenols, phenylpropanoids, and monoterpenes alcohols, while monoterpenes and sesquiterpenes hydrocarbons were used to a lesser extent. Socović et al. (2009) found that thyme essential oil and its phenolic components (carvacrol and thymol) had very high antifungal activities, even higher than the commercial fungicide bifonazole. These authors deduced a relationship between the high activity of thyme essential oil and the presence of phenol components, such as thymol and carvacrol. It seems possible that phenol components may interfere with cell wall enzymes like chitin synthase/chitinase as well as with the α- and β-glucanases of the fungus (Adams et al., 1996).

## 4. Conclusions

In summary, the leaf essential oils of rosemary and tea had the highest antifungal activity, followed by thyme, cypress, and clementine essential oils. Rosemary and tea leaf essential oils were rich in 1,8-cineole. Carvacrol was the main component of thyme essential oil, while cypress leaf essential oil mainly contained germacrene-D and α-pinene. Clementine leaf essential oil was characterized by the predominance of sabinene, limonene, linalool, and Δ-3-carene. It was difficult to attribute the antifungal activity of essential oils to a single component. Possible combinations between these essential oils could be conducted in further investigations to determine these bioactive compounds' synergistic and antagonistic effects. These investigations could lead to the development of a new treatment based on combining these essential oils as natural bioactive substances against *A. alternata*.

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## Conflict of interest

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## CRediT authorship contribution statement

**Taycir Grati Affes:** Conceptualization, Writing, Methodology, Project administration, Investigation, Formal analysis, Resources
**Salma Lasram:** Conceptualization, Methodology, Writing, Resources, Investigation
**Majdi Hammami:** Methodology, Data curation
**Walid Yeddes:** Writing
**Wissem Aidi Wannes:** Writing, Data curation, Resources, Writing, Review & Editing
**Saber Khammassi:** Methodology
**Bouzid Nasraoui:** Project administration, Data curation, Supervision
**Moufida Saidani Tounsi:** Project administration, Investigation, Formal analysis, Supervision, Validation
**Nehla labidi Ben Hmida:** Investigation, Formal analysis, Supervision

## ORCID Numbers of the Authors

**T. Grati Affes:** 0000-0003-2627-3106
**S. Lasram:** 0000-0003-2257-7743
**M. Hammami:** 0000-0002-4376-0638
**W. Yeddes:** 0000-0002-1439-7956
**W. Aidi Wannes:** 0000-0003-3134-0457
**S. Khammassi:** 0000-0003-3223-906X
**B. Nasraoui:** 0000-0002-1500-3701
**M. Saidani Tounsi:** 0000-0001-6052-1367
**N. Labidi Ben Hmida:** 0000-0002-7217-3475

## Supplementary File

None.

## References

Adams, S., Kunz, B., Weidenbörner, M., 1996. Mycelial deformations of *Cladosporium herbarum* due to the application of eugenol or carvacrol. *Journal of Essential Oil Research*, 8(5), 535-540.

Alves, F.M.F., França, K.R.S., de Araújo, I.G., da Nóbrega, L.P., Xavier A.L.S., Lima T.S., Rodrigues, A.P.M.S., Júnior, A.F.M., Cardoso, T.A.L., 2019. Control of *Alternaria alternata* using Melaleuca essential oil (*Melaleuca alternifolia*). *Journal of Experimental Agriculture International*, 40(3), 1-10.

Amri, I., Hamrouni, L., Hanana, M., Gargouri, S., Jamoussi, B., 2013. Chemical composition, bio-herbicidal and antifungal activities of essential oils isolated from Tunisian common cypress (*Cupressus sempervirens* L.). *Journal of Medicinal Plants Research*, 7(16), 1070-1080.

Angioni, A., Barra, A., Cereti, E., Barile, D., Coïsson, J.D., Arlorio, M., Cabras, P., 2004. Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. *Journal of Agricultural and Food Chemistry*, 52(11), 3530-3535.

Aslam, M.F., Irshad, G., Naz, F., Khan, M.A., 2022. Evaluation of the antifungal activity of essential oils against *Alternaria alternata* causing fruit rot of *Eriobotrya japonica*. *Turkish Journal of Biochemistry*, https://doi.org/10.1515/tjb-2021-0225.

Bassolé, I.H.N., Lamien-Meda, A., Bayala, B., Tirogo, S., Franz, C., Novak, J., Dicko, M.H., 2010. Composition and antimicrobial activities of *Lippia multiflora* Moldenke, *Mentha x piperita* L. and *Ocimum basilicum* L. essential oils and their major monoterpene alcohols alone and in combination. *Molecules*, 15(11), 7825-7839.

Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M.S., Vijayakumar, R., Baskaran, D., 2019. Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain & Oil Science and Technology*, 2(2), 49-55.

Boukhris, M., Regane, G., Yangui, T., Sayadi, S., Bouaziz, M., 2012. Chemical composition and biological potential of essential oil from Tunisian *Cupressus sempervirens* L. *Journal of Arid Land Studies*, 22(1), 329-332.

Carson, C.F., Hammer, K.A., Riley, T.V., 2006. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clinical Microbiology Reviews*, 19(1), 50-62.

Celiktas, O.Y., Kocabas, E.H., Bedir, E., Sukan, F.V., Ozek, T., Baser, K.H.C., 2007. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chemistry*, 100(2), 553-559.

Chouhan, S., Sharma, K., Guleria, S., 2017. Antimicrobial activity of some essential oils—present status and future perspectives. *Medicines*, 4(3), 58.

D'agostino, M., Tesse, N., Frippiat, J.P., Machouart, M., Debourgogne, A., 2019. Essential oils and their natural active compounds presenting antifungal properties. *Molecules*, 24(20), 3713.

Dube, J.P., 2014. Characterization of *Alternaria alternata* isolates causing brown spot of potatoes in South Africa (Master Thesis in Science (Agriculture) Plant Pathology). University of Pretoria. (accessed 2014 February).

Dugo, G., Cotroneo, A., Bonaccorsi, I., Trozzi, A., 2011. Composition of the volatile fraction of citrus peel oils, Chapter 1, 1–161; Dugo, G., Cotroneo, A., Bonaccorsi, I. Composition of petitgrain oils, Chapter 5, 253-331. In citrus oils: Composition, advanced analytical techniques, contaminants, and biological activity. Edits. Dugo, Mondello, L., CRC Press - Taylor & Francis Group, Bacon Raton.

Elansary, H.O., Salem, M.Z., Ashmawy, N.A., Yacout, M.M., 2012. Chemical composition, antibacterial and antioxidant activities of leaves essential oils from *Syzygium cumini* L., *Cupressus sempervirens* L. and *Lantana camara* L. from Egypt. *Journal of Agricultural Science*, 4(10), 144-152.

Elshafie, H.S., Camele, I., 2017. An overview of the biological effects of some mediterranean essential oils on human health. *BioMed Research International*, 2017, 9268468.

Germanà, M.A., Palazzolo, E., Chiancone, B., Saiano, F., 2013. Characterization of leaf essential oil composition of homozygous and heterozygous *Citrus clementina* Hort. Extan. and its Ancestors. *Journal of Essential Oil Bearing Plants*, 16(1), 92-101.

Golab, M., Skwarlo-Sonta, K., 2007. Mechanisms involved in the anti-inflammatory action of inhaled tea tree oil in mice. *Experimental Biology and Medicine*, 232(3), 420-426.

González, M.J., Marioli, J.M., 2010. Antibacterial activity of water extracts and essential oils of various aromatic plants against *Paenibacillus* larvae, the causative agent of American Foulbrood. *Journal of Invertebrate Pathology*, 104(3), 209-213.

Grati Affes, T., Chenenaoui, S., Zemni, H., Hammami, M., Bachkouel, S., Aidi Wannes, W., Lasram, S., 2022. Biological control of *Citrus* brown spot pathogen,"*Alternaria alternata*" by different essential oils. *International Journal of Environmental Health Research*, 1-14.

Guetat, A., Al-Ghamdi, F.A., Osman, A.K., 2014. 1, 8-Cineole, α-Pinene and Verbenone chemotype of essential oil of species *Rosmarinus officinalis* L. from Saudi Arabia. *International Journal of Herbal Medicine*, 2, 137-141.

Hamdani, F.Z., Allem, R., 2015. Antifungal activity of the leaf essential oil of citrus against *Alternaria alternata in vivo*. Internati*onal Journal of Applied and Natural Sciences*, 4(4), 15-24.

Hosni, K., Hassen, I., M'Rabet, Y., Casabianca, H., 2019. Biochemical response of *Cupressus sempervirens* to cement dust: Yields and chemical composition of its essential oil. *Arabian Journal of Chemistry*, 12(7), 1308-1314.

Joshi, A., Prakash, O., Pant, A.K., Kumar, R., Szczepaniak, L., Kucharska-Ambrożej, K., 2022. Methyl eugenol, 1, 8-cineole and nerolidol rich essential oils with their biological activities from three melaleuca species growing in Tarai region of North India. Brazilian *Archives of Biology and Technology*, 64, e21210186.

Kasali, A.A., Lawal, O.A., Eshilokun, A.O., Olaniyan, A.A., Opoku, A.R., Setzer, W.N., 2011. *Citrus* essential oil of Nigeria part V: Volatile constituents of sweet orange leaf oil (*Citrus sinensis*). *Natural Product Communications*, 6(6), 875-878.

Khadidja, M., Nassima, B., Chahrazed, B., Xavier, F., 2010. Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L. *Journal of Medicinal Plants Research*, 4(10), 959-964.

Kowalczyk, A., Przychodna, M., Sopata, S., Bodalska, A., Fecka, I., 2020. Thymol and thyme essential oil—new insights into selected therapeutic applications. *Molecules*, 25(18), 4125.

Lakušić, D.V., Ristić, M.S., Slavkovska, V.N., Šinžar-Sekulić, J.B., Lakušić, B.S., 2012. Environment-Related Variations of the Composition of the Essential Oils of Rosemary (*Rosmarinus officinalis* L.) in the Balkan Penninsula. *Chemistry & Biodiversity*, 9(7), 1286-1302.

Lopez-Reyes, J.G., Spadaro, D., Preile, A., Garibaldi, A., Gullino, M.L., 2013. Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits *in vivo*. *Journal of Food Protection*, 76, 631-639.

Mata, A.T., Proença, C., Ferreira, A.R., Serralheiro, M.L M., Nogueira, J.M.F., Araújo, M.E.M., 2007. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. *Food Chemistry*, 103(3), 778-786.

Matasyoh, J.C., Kiplimo, J.J., Karubiu, N.M., Hailstorks, T.P., 2007. Chemical composition and antimicrobial activity of the essential oil of *Satureja biflora* (Lamiaceae). *Bulletin of the Chemical Society of Ethiopia*, 21(2), 249-254.

Mohammadi, A., Bahramikia, S., 2019. Molecular identification and genetic variation of *Alternaria* species isolated from tomatoes using ITS1 sequencing and inter simple sequence repeat methods. *Current Medical Mycology*, 5(2), 1-8.

Moore, J., Yousef, M., Tsiani, E., 2016. Anticancer effects of rosemary (*Rosmarinus officinalis* L.) extract and rosemary extract polyphenols. *Nutrients*, 8(11), 731.

Napoli, E.M., Curcuruto, G., Ruberto, G., 2010. Screening of the essential oil composition of wild Sicilian rosemary. *Biochemical Systematics and Ecology*, 38(4), 659-670.

Özcan, M.M., Chalchat, J.C., 2008. Chemical composition and antifungal activity of rosemary (*Rosmarinus officinalis* L.) oil from Turkey. *International Journal of Food Sciences and Nutrition*, 59(7-8), 691-698.

Padalia, R.C., Verma, R.S., Chauhan, A., Chanotiya, C.S., 2015. The essential oil composition of *Melaleuca leucadendra* L. grown in India: A novel source of *(E)*-nerolidol. *Industrial Crops and Products*, 69, 224-227.

Papageorgiou, V., Gardeli, C., Mallouchos, A., Papaioannou, M., Komaitis, M., 2008. Variation of the chemical profile and antioxidant behavior of *Rosmarinus officinalis* L. and *Salvia fruticosa* Miller grown in Greece. *Journal of Agricultural and Food Chemistry*, 56(16), 7254-7264.

Park, H.M., Kim, J., Chang, K.S., Kim, B.S., Yang, Y.J., Kim, G.H., Park, I.K., 2011. Larvicidal activity of Myrtaceae essential oils and their components against *Aedes aegypti*, acute toxicity on *Daphnia magna*, and aqueous residue. *Journal of Medical Entomology*, 48(2), 405-410.

Porte, A., Godoy, R.L., 2008. Chemical composition of *Thymus vulgaris* L.(Thyme) essential oil from the Rio de Janeiro state, Brazil. *Journal of the Serbian Chemical Society*, 73(3), 307-310.

Raveau, R., Fontaine, J., Lounès-Hadj Sahraoui, A., 2020. Essential oils as potential alternative biocontrol products against plant pathogens and weeds: A review. *Foods*, 9(3), 365.

Reiling, K.K., Yoshikuni, Y., Martin, V.J., Newman, J., Bohlmann, J., Keasling, J.D., 2004. Mono and diterpene production in *Escherichia coli*. *Biotechnology and Bioengineering*, 87(2), 200-212.

Sadeh, D., Nitzan, N., Chaimovitsh, D., Shachter, A., Ghanim, M., Dudai, N., 2019. Interactive effects of genotype, seasonality and extraction method on chemical compositions and yield of essential oil from rosemary (*Rosmarinus officinalis* L.). *Industrial Crops and Products*, 138, 111419.

Shaban, W., 2014. Evaluation of some fungicide alternatives against *Alternaria* fruit rot on tomato. *Egyptian Journal of Phytopathology*, 42(2), 91-104.

Shabnum, S., Wagay, M.G., 2011. Essential oil composition of *Thymus vulgaris* L. and their uses. *Journal of Research and Development*, 11, 83-94.

Silva, M.R., Ximenes, R.M., da Costa, J.G.M., Leal, L.K.A.M., de Lopes, A.A., de Barros Viana, G.S., 2010. Comparative anticonvulsant activities of the essential oils (EOs) from *Cymbopogon winterianus* Jowitt and *Cymbopogon citratus* (DC) Stapf. in mice. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 381(5), 415-426.

Soković, M.D., Vukojević, J., Marin, P.D., Brkić, D.D., Vajs, V., Van Griensven, L.J., 2009. Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. *Molecules*, 14(1), 238-249.

Thi Nguyen, T.T., Thi Tran, T.T., Hua, T.M., Diep, T.T., Chau, D.K.N., Duus, F., Le, T.N., 2016. Investigation of peel and leaf essential oils of *Citrus clementina* Hort. ex Tan. growing in the south of Vietnam. *Journal of Essential Oil Research*, 28(2), 96-103.

Varela, F., Navarrete, P., Cristobal, R., Fanlo, M., Melero, R., Sotomayor, J.A., Cases, A., 2007. Variability in the chemical composition of wild *Rosmarinus officinalis* L. *In I International Medicinal and Aromatic Plants Conference on Culinary Herbs*, 826, 167-174.

Yan, R., Yang, Y., Zeng, Y., Zou, G., 2009. Cytotoxicity and antibacterial activity of *Lindera strychnifolia* essential oils and extracts. *Journal of Ethnopharmacology*, 121(3), 451-455.

Yeddes, W., Aidi Wannes, W., Hammami, M., Smida, M., Chebbi, A., Marzouk, B., Saidani Tounsi, M., 2018. Effect of environmental conditions on the chemical composition and antioxidant activity of essential oils from *Rosmarinus officinalis* L. growing wild in Tunisia. *Journal of Essential Oil Bearing Plants*, 21(4), 972-986.

Yeddes, W., Hammami, M., Khammassi, S., Grati Affes, T., Aidi Wannes, W., Saidani-Tounsi, M., 2022a. Antibacterial activities of rosemary (*Rosmarinus officinalis* L.) essential oil andethanol extract. *Open Access Research Journal of Multidisciplinary Studies*, 3(1), 1-8.

Yeddes, W., Mejri, I., Grati Affes, T., Khammassi, S., Hammami, M., Aidi-Wannes, W., Saidani Tounsi, M., 2022b. Combined effect of essential oils from Clove (*Syzygium aromaticum* (L.) Merr. & LM Perry), Thyme (*Thymus vulgaris* L.) and Lemon peel (*Citrus limon* (L.) Osbeck) on anti-bacterial, cytotoxic and anti-inflammatory activities. *Trends in Phytochemical Research*, 6(1), 11-18.

Zaouali, Y., Boussaid, M., 2008. Isozyme markers and volatiles in Tunisian *Rosmarinus officinalis* L.(Lamiaceae): A comparative analysis of population structure. *Biochemical Systematics and Ecology*, 36(1), 11-21.

Zerigui, F., Mouzaoui, F., 2018.Contribution à l'étude d'*Alternaria* sp., agent causal de l'alternariose de la pomme de terre : Prospection, isolement et identification du pathogène. Master research, Abdelhamid Ben Badis-Mostagane Univresity, Algeria, 49pp.

## Reviewed by:

Yahyea Baktiar LASKAR: Assam University, Silchar, Assam, INDIA
Aysenur KAYA: Kilis 7 Aralik University, Kilis, TURKEY