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

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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 (Bhavaniramy 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

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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. sempervivens* 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 Innnowax, 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 (C₈-C₂₂) *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.

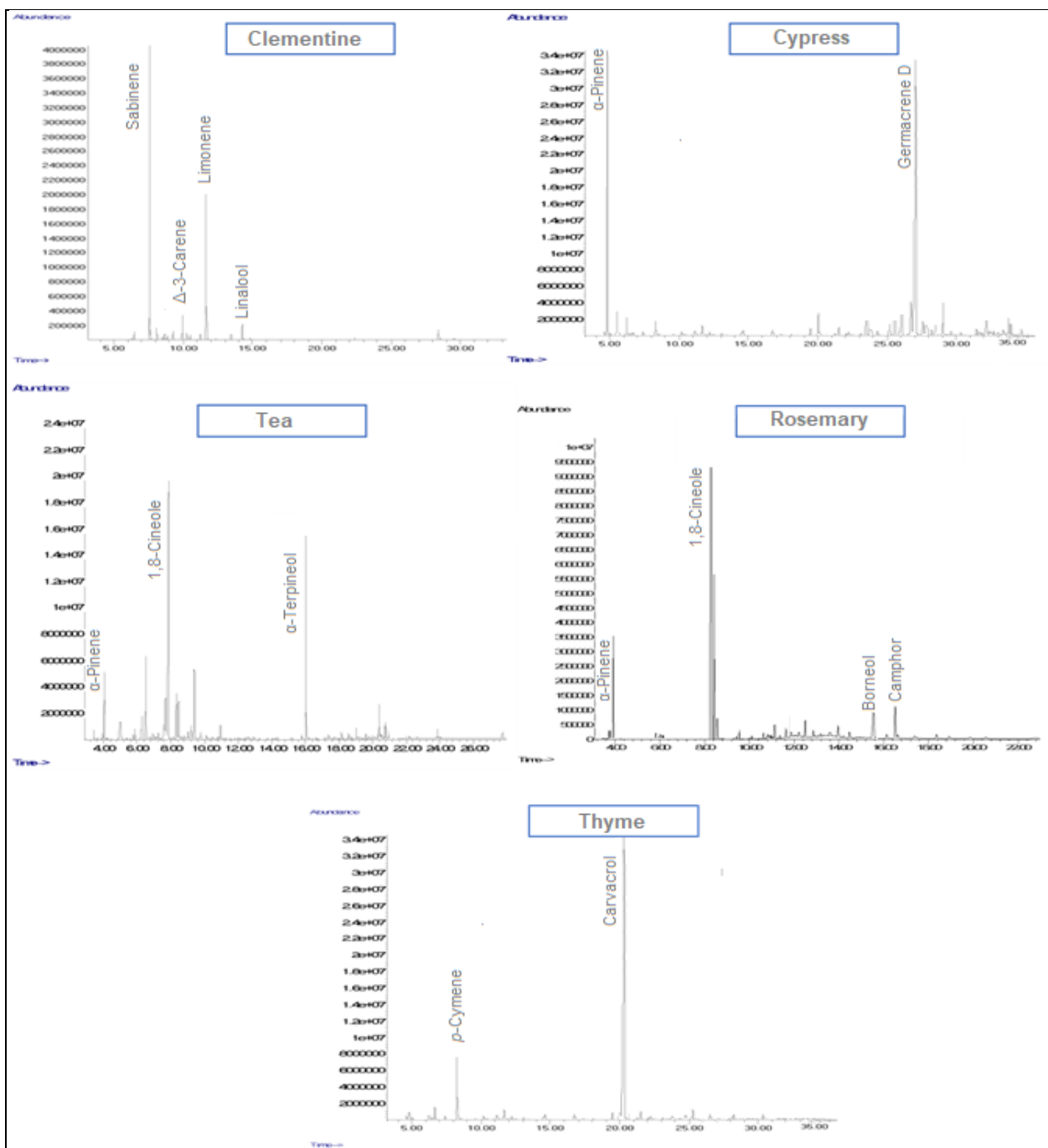


Figure 1. Gas chromatography chromatograms of the leaf essential oils from clementine, cypress, rosemary, tea, and thyme and their main volatile compounds

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 ^a	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
Isolatedene	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 ± 0.09 ^b	0.06 ± 0.03 ^c	1.58 ± 0.33 ^c
cis-muurolo-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-Muurolole	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 ^c	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.

^aRI: Retention index calculated on HP-5 MS column.

^bRI: 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 $\mu\text{g/ml}$) 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 $\mu\text{g/ml}$ 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, $\mu\text{g/ml}$)				
	Clementine	Cypress	Rosemary	Tea	Thyme
<i>A. alternata</i>	8000 \pm 0.00 ^a	8000 \pm 0.00 ^a	5000 \pm 0.00 ^c	5000 \pm 0.00 ^c	6000 \pm 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

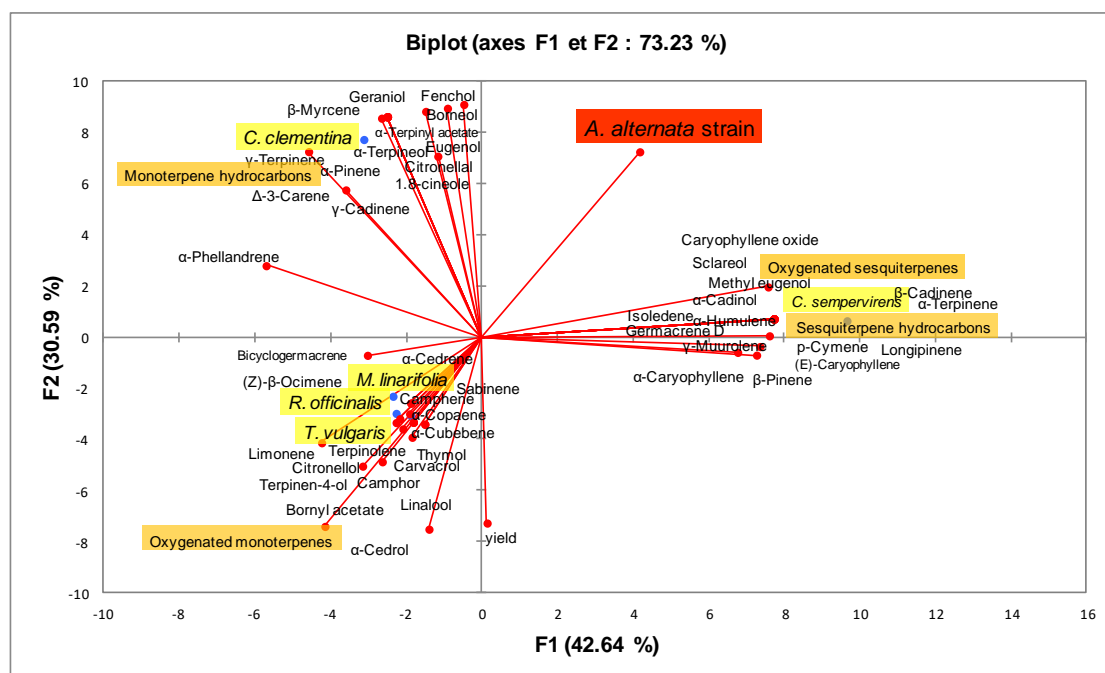


Figure 2. Biplot obtained from principal component analysis of variables comprising essential oil components, chemical classes, and antifungal activities

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 $\mu\text{g/ml}$). 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 $\mu\text{g/ml}$ for thyme and 5000 $\mu\text{g/ml}$ 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 ($r = 0.809$), 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

The authors confirm that there are no known conflicts of interest.

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None.

CRediT authorship contribution statement

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Supplementary File

None.

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