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Original Research Article

In vitro antifungal potential of peel essential oils from different *Citrus* species on *Alternaria alternata*

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ABSTRACT

The essential oil extraction from *Citrus* peels constitutes an effectual approach for the valorization of *Citrus* fruit waste. *Citrus* peel essential oils can have an antifungal activity. The peel essential oils of seven species of *Citrus*, namely clementine, pummelo, tangelo, sweet orange, sour orange, tangerine and lemon, were extracted by steam distillation and subjected to chemical analysis to identify and evaluate the chemical constituents. Tangerine exhibited the highest yield (1.23%) followed by lemon (0.66%), clementine (0.56%), sweet orange (0.55%), tangelo (0.50%) and sour orange (0.47%). The lowest yield was obtained in pummelo (0.16%). The main compound in all *Citrus* essential oils was limonene with 62.1% for tangerine, 69.7% for lemon, 72.2% for tangelo, 76.4% for clementine, 89.8% for pummelo, 92.8% for sour orange and 94.1% for sweet orange. All these *Citrus* essential oils had a weak antifungal activity (MIC = 8000-12000 µg/mL) against *Alternaria alternata* strain.

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1. Introduction

In addition to the presence of many bioactive components, *e.g.*, alkaloids, coumarins and flavonoids, in Rutaceae species, they are also characterized by their richness in essential oils which contribute to their aromatic, agrochemical and medicinal properties (Nahar et al., 2021). The genus of *Citrus* belongs to the family of Rutaceae which has about 150 genera and 1600 species that are broadly distributed in tropical, subtropical and temperate zones around the world (Lemes et al., 2018). *Citrus* genus includes various important fruits as pummelo, orange, mandarin, lime and lemon (Bourgou et al., 2012). The worldwide production of *Citrus* reached more than 158 million tons in 2020 (FAO, 2021). Tunisian production has varied between 360 to 460 million tons over the last decade. In Tunisia, *Citrus* orchards currently cover an area of 27,000 ha with a total of 7 million trees (Haas et al., 2017). *Citrus* cultivation mainly developed in Tunisian Cap Bon regions benefiting from good soil, water resources, and climate, where winter frosts are rare (Haas et al., 2017).

Essential oils are well-known for their bioactivities, *e.g.*, antimicrobial and antioxidant, and have long been used in different traditional medicine systems as well as in food, modern medicine, cosmetics, and pharmaceutical products (Mohammadhosseini, 2015a; Mohammadhosseini, 2015b; Karan et al., 2018; Erenler et al., 2018; Barbeche et al., 2021; Nahar et al., 2021). Essential oils, also known as volatile oils, are complex mixtures of volatile constituents that are being released upon applying heat and could be considered as being used in the classical hydrodistillation, steam distillation, and those based on the application of microwave beams, *e.g.*, microwave-assisted hydrodistillation or solvent-free microwave extraction (Mohammadhosseini, 2017; Mohammadhosseini et al., 2017; Oloyede et al., 2021;

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Yousefi et al., 2021). Essential oils, or plant essences, are volatile and fragrant substances with an oily consistency typically produced by plants. They are synthesized by all plant organs, namely buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretary cells, cavities, canals, epidermic cells or glandular trichomes (Bakkali et al., 2008; Yeddes et al., 2022).

Essential oil is mainly founded in the peel part of *Citrus* fruit. *Citrus* peel (Singh et al., 2021), a by-product of *Citrus* fruit processing, accounts for approximately 45% of the total fruit portion. The essential oil extraction from *Citrus* peels constitutes an effectual approach for the valorization of *Citrus* fruit waste (Köse and Bayraktar, 2018). The essential oils have been used commonly in food and pharmaceutical industries (Karan et al., 2018; Erenler et al., 2018; Barbeche et al., 2021). The main classes of *Citrus* peel essential oil include monoterpenes, sesquiterpenes and their oxygenated derivatives (Singh et al., 2021). Limonene is the major volatile component identified in the peel of different *Citrus* species. *Citrus* peel essential oils have several biological activities as antioxidant, anti-inflammatory, analgesic, antimicrobial and anticancer activities (Singh et al., 2021). *Citrus* peel essential oils can have an antimicrobial effect against both bacteria (*i.e.*, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 24682, and *Staphylococcus aureus* ATCC 25923) and fungi (i.e., *Aspergellus flavus* ATCC 204304, *Candida albicans* ATCC 10231 and *Penicillium chrysogenum* ATCC 10106) (Chanthaphon et al., 2008; Jafari et al., 2011; Bourgou et al., 2012; Jing and al., 2014; Ngele et al., 2014; Vasek et al., 2015; Sajid et al., 2016; Kademi and Garba, 2017). The antifungal activity of from different *Citrus* peel essential oils species against *Alternaria alternata* has been also studied (Sharma and Tripathi, 2006; Phillips et al., 2012; Bozkurt et al., 2017; Ajayi-Moses et al., 2019; Sedeek et al., 2021). Aslam et al. (2022) investigated only the antifungal activity of lemon peel essential oil against *Alternaria alternata*. Lu et al. (2019) determined only the effect of tangerine peel essential oil on *Alternaria alternata*. However, there are no studies on the antifungal activity of Tunisian citrus peel essential oils against *Alternaria alternata*.

This paper provides an in-depth knowledge about the *in vitro* antifungal potential of peel essential oils from seven Tunisian *Citrus* species, *e.g.*, pummelo, clementine, sweet orange, tangerine, sour orange, tangelo and lemon on *Alternaria alternata* strain.

2. Experimental

2.1. Sampling locations of *Citrus* species

In this study, seven *Citrus* species were investigated, namely clementine (*Citrus clementina* Hort. synonym *Citrus reticulata* Blanco x *Citrus sinensis* (L.) Osbeck variety MA3), sweet orange (*Citrus sinensis* Osbeck variety Meski), tangerine (*Citrus reticulata* (L.) Osbeck synonym *Citrus tangerina* Hort. variety *Fortune*), tangelo (*Citrus tangelo* Ingram & Moore synonym *Citrus aurantium* L. variety *Minneola*), sour orange (*Citrus aurantium* (L.) Osbeck), pummelo (*Citrus grandis* (L.) Osbeck synonym *Citrus maxima* (Burm.) Merr.) and lemon (*Citrus limon* (L.) Osbeck variety Eureka).

As shown in Fig. 1, clementine MA3, tangerine, tangelo, sweet orange and sour orange fruits were gathered in June 2019 at agriculture land in Beni khled, Cap Bon region (North-East Tunisia; Altitude: 51 m; Latitude: 36°39′01″ North; Longitude: 10°35′24″ East). Pummelo and lemon were collected in June 2019 at agriculture land in Mateur, Bizerte region (North Tunisia; Altitude: 36 m; Latitude: 37°02′25″ North; Longitude: 9°39′56″ East). The identification of *Citrus* species was carried out by the botanist Abderrazzak Smaoui in Biotechnologic Center of Borj-Cedria (Tunisia). Voucher specimens were deposited in the herbarium of our laboratory (*Citrus clementina* Cc-LPAM-2019; *Citrus sinensis* Cs-LPAM-2019; *Citrus reticulata* Cr-LPAM-2019; *Citrus aurantium* Ca-LPAM-2019; *Citrus grandis* Cg-LPAM-2019; *Citrus. limon* Cl-LPAM-2019).

2.2. Essential oil extraction

The fresh peels (100 g) were submitted to hydrodistillation for 120 min using a Clevenger type apparatus. This time was fixed after a kinetic survey during 30, 60, 90, 120, 150, 180, and 210 min. The essential oils obtained were dried over anhydrous sodium sulphate and stored at -20 °C in darkness until analyzed.

2.3. Essential oil analysis

The analysis of volatile compounds was carried out on Hewlett-Packard 6890 chromatograph equipped with an electronic pressure control injector, a flame ionization detector and an HP Innowax (polyethylene glycol capillary) column (30 m x 0.25 mm; 0.25 µm). The flow of the carrier gas (N_2) was 1.6 mL/min and the split ratio was 60:1. The analysis was carried out using the following temperature program: oven temperature; isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 2 °C/min and isotherm at 205 °C during 10 min. Injector and detector temperature were held, respectively, at 250 and 300 °C. The injection volume was 1 µL. The quantification of each essential oil was done by the co-injection of external standard method using calibration curves generated by running GC analysis of representative compounds.

The GC/MS was used to identify volatile compounds. The analysis was carried out by a chromatograph coupled to an Agilent mass spectrometer (5975C inert XL MSD) and electron impact ionization (70 eV). An HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) coated with 5% phenyl methyl silicone and 95 % dimethylpolysiloxane was used. The oven temperature was programmed at 40 °C for 1 min and then rise from 40 to 100 °C at a rate of 8 °C/min and kept constant at 100 °C for 5 min. After that, the temperature was heated to 200 °C with a rate of 10 °C/min and kept constant at 200 °C for 3 min and the final temperature was set up at 300 °C with a rate of 2 °C/min. Injector

temperature was set at 250 °C. The carrier gas was helium with a flow rate of 1 mL/min; the split ratio was 100:1. Scan time and mass ranges were 1 s and 50-550 *m/z*, respectively. Individual peaks corresponding to the volatile components were identified by comparison of their Kovats retention indexes (KRI) relative to (C_8-C_{40}) *n*-alkanes with those of literature or with those of authentic compounds available in the authors' laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley 09 NIST 2011 mass spectral library of the GC/MS data system (Yeddes et al., 2022).

2.4. Fungal strain

The strain *Alternaria alternata* was isolated from tangerine (*Citrus reticulata* variety Fortune, Beni Khaled) and it had a GenBank accession number for nucleotide sequence: (T1): OK448177 (Grati Affes et al., 2022).

2.5. Determination of the minimum inhibitory concentration of essential oils

A portion of *Alternaria 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. To stimulate sporulation, the method of Zerigui and Mouzaoui (2018) was applied. 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 h and returned to room temperature for 24 h in the dark. At the end the spore suspension was recovered after scraping the culture of *Alternaria alternata*.

The spore suspension of *Alternaria alternata* in a 0.01% tween 80 medium was prepared from an 8-day-old single-spherical culture 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}] were added. Finally, the tubes were incubated with shaking in the dark at 25 °C Daily. The MIC was defined as the lowest concentration of the essential oil required to completely prevent visible fungal growth. (Grati Affes et al., 2022). A reference fungicide (Melody duo) with an active ingredient (5.5% Iprovalicarb, 61.3% Propineb) was used as positive control.

2.6. Statistical analysis

All experiments were conducted in triplicates and the results were expressed as mean values 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 Components 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 yield

The essential oil yields of seven *Citrus* peels are given in Fig. 2. *Citrus* species had significant effect on essential oil yields. In fact, tangerine exhibited the highest yield (1.23%), followed by lemon (0.66%), clementine (0.56%), sweet orange (0.55%), tangelo (0.50%) and sour orange (0.47%). The lowest yield was obtained in pummelo (0.16%).

Similar results were obtained by Bourgou et al. (2012) concerning four *Citrus* species, mandarin (1.13%), lemon (0.62%), sweet orange (0.52%) and sour orange (0.46%). Hosni et al. (2010) found higher yields varying from 1.24 to 4.62%. These yields were higher than those obtained by Kamal et al. (2013) in the case of Pakistan sweet orange (0.24%) and mandarin (0.30%). Javed et al. (2011) also reported that the peel essential oil yields of Pakistan *Citrus reticulata* var. *mandarin*, *Citrus reticulata* var. *tangerine*, *Citrus sinensis* var. *malta*, *Citrus sinensis* var. *mousami*, *Citrus limon* and *Citrus paradisi* were 0.32, 0.24, 0.22, 1.21, 0.05 and 0.21%, respectively. However, Kamaliroosta et al. (2016) reported that the essential oil yields were 1.48% for *Citrus sinensis*, 0.90% for *Citrus reticulata* var. tangerine, 0.54% for *Citrus limetta* and 0.56% for *Citrus aurantium* peels from Iran. According to Bourgou et al. (2012), these variations of peel essential oils between species could be due to the influence of extraction procedure as well as environmental genetic factors.

3.2. Essential oil composition

Analysis of *Citrus* peel essential oils showed 32 identified compounds representing 96.1% for pummelo, 97.5% for clementine, 99.7% for sweet orange, 99.9% for tangelo, 99.9% for tangerine, 99.4% sour orange and 96.9% lemon (Table 1). Analysis of the essential oil indicated that it is made essentially from monoterpenes hydrocarbons which constituted the main class in all *Citrus* species (88.4-99.9%). Oxygenated monoterpenes were the second class present with appreciable levels in all *Citrus* species (1.1-7.3%) while it was absent in sweet orange essential oil. Sesquiterpene hydrocarbons were weakly represented in all samples (0.5-2.5%). Analysis of the volatile composition showed the predominance of limonene with 62.1% for tangerine, 69.7% for lemon, 72.2% for tangelo, 76.4% for clementine, 89.8% for pummelo, 92.8% for sour orange and 94.1% for sweet orange. Limonene contributes to the aromatic odor of the oil and hence *Citrus* peel essential oils belong to the limonene chemotype (Chutia et al., 2009). The high concentration of limonene in different *Citrus* peel essential oils was in accordance with previous reports (Lota et al., 2001; Minh Tu et al., 2002; Njoroge et al., 2005; Sawamura et al., 2005; Pawar and thaker, 2006; Sharma and Tripathi, 2008; Chutia et al., 2009; Hosni et al., 2010; Singh et al., 2010; Bourgou et al., 2012; Thi Nguyen et al., 2015; Sajid et al., 2016, Bozkurt et al., 2017; Ajayi-Moses et al., 2019; Sedeek et al., 2021). Limonene was the main component of all *Citrus* species but with different proportions owing to genetic and/or environmental factors. Other bioactive components

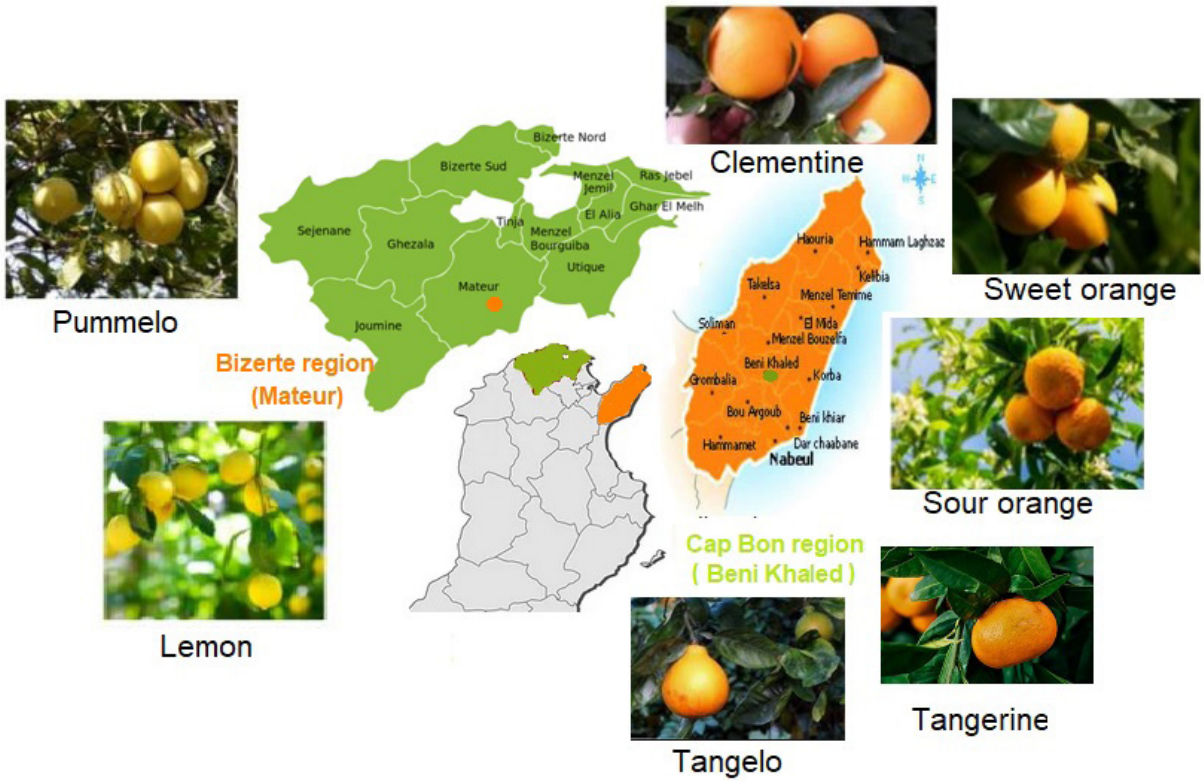

Fig. 1. Geographic distribution of *Citrus* orchards in Beni Kahled and Mateur regions.

were found in *Citrus* peel essential oils with important concentrations such as γ-terpinene in tangelo (15.4%), tangelo (13%) and lemon (10%) as well as sabinene in lemon (5.8%), β-myrcene (5.2%) and α-thujene (3.4%) in tangelo. Bourgou et al. (2012) also found that Tunisian mandarin and lemon essential oils had appreciable levels of γ-terpinene with 14 and 10%, respectively. Lota et al. (2001) found limonene and γ-terpinene as the main compounds of the peel essential oils from nine French mandarin cultivars. In our study, α-pinene had an appreciable presence in tangerine (2.1%), clementine (1.3%) and lemon (1.1%). Hosni et al. (2010) reported that the proportion of limonene can be risen up to 97.3% in *Citrus* essential oils. They also noted the presence of β-pinene with a proportion of 1.8% in Tunisian sweet orange. In our study, tangerine had the higher level of β-pinene (2.1%) than the other *Citrus* species. Bozkurt et al. (2017) evaluated the chemical composition of peel essential oils from some Turkish *Citrus* species as Satsuma mandarin (*Citrus unshiu* Marc.), Clementine mandarin (*Citrus reticulata* Blanco), Myer lemon (*Citrus meyeri*), Interdonato lemon (*Citrus* x *limon* L. Burmf), Washington Navel orange (*Citrus sinensis* (L.) Osbeck), Star Ruby grapefruit (*Citrus paradisi* (Macf.)), Sour orange (*Citrus aurantium*) and Moro blood orange (*Citrus sinensis* (L.) Osbeck). These authors reported that the other main components of oils were determined to be α-pinene, sabinene, β-pinene, β-myrcene, linalool, *m*-cymene and 4-terpineol in addition to limonene.

3.2. In vitro antifungal activity

The antifungal activity of *Citrus* essential oils was tested against *Alternaria alternata* strain. The minimal concentration of these *Citrus* essential oils ranged between 8000 and 12000 µg/mL indicating the total inhibition of *Alternaria alternata* growth. The results presented in Table 2 showed that all *Citrus* essential oils had a weak antifungal activity against *Alternaria alternata* strain as compared to the positive control F (MIC = 2000 µg/mL). In previous study, Sedeek et al. (2021) found that the essential oils of sweet orange (*Citrus sinensis*), lime (*Citrus aurantifolia*), and pummelo (*Citrus maxima*) did not inhibit *Alternaria alternaria* growth while only *Citrus limon* had a weak antifungal activity. Lu et al. (2019) reported that tangerine peel essential oil had promoted the growth of *Alternaria alternata* while *Citrus limon* had inhibited the growth of *Alternaria alternata* with a proportion of 77.2%. It can be seen that the antifungal activity of essential oils was mainly associated with phytochemical components. This is in agreement with Bourgou et al. (2012) who found that limonene had a weak antimicrobial activity. In previous study, Grati Affes et al. (2022) had determined the *in vitro* efficiency of myrtle (3000 µg/mL), laurel (2000 µg/mL) and peppermint (1500 µg/mL) essential oils in the inhibition of *Alternaria alternata* srain owing to their richness in bioactive compounds belonging

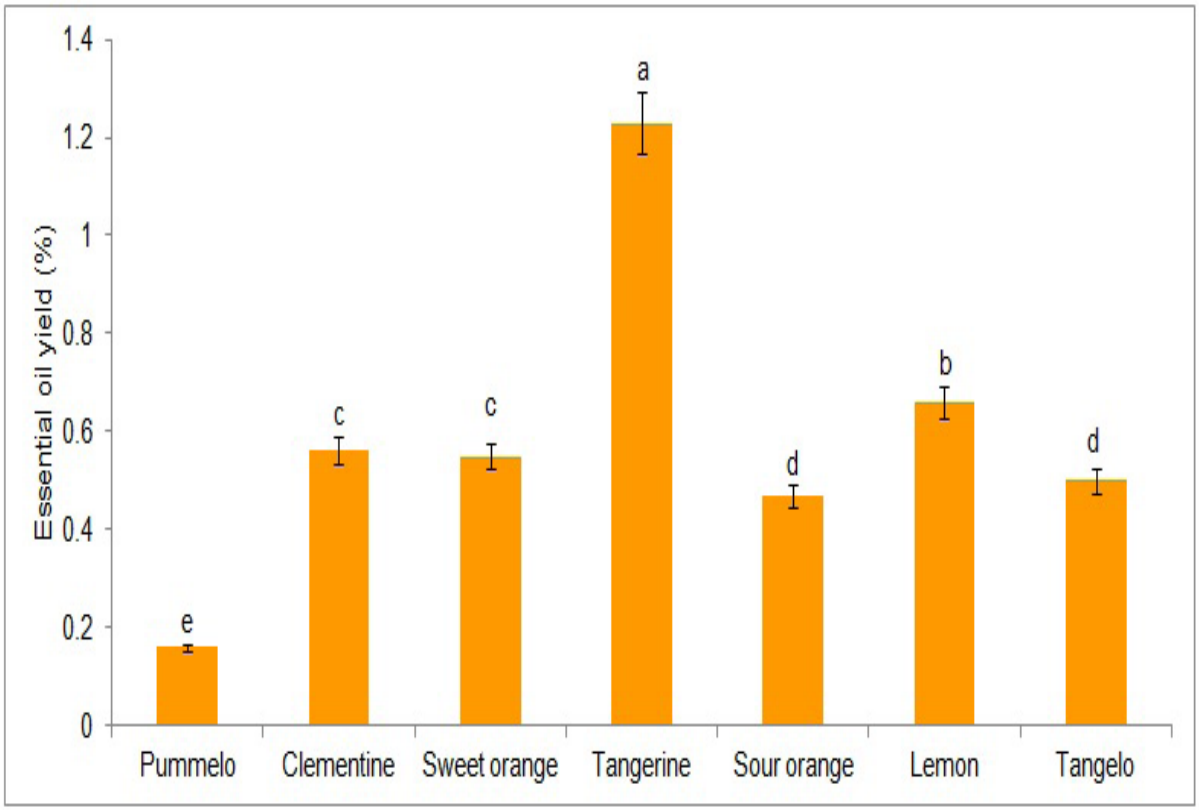

Fig. 2. Essential oil yields of different *Citrus* peels (Values with the different letters (a-e) showed significant differences at $p < 0.05$).

to the class of phenylpropanoids and oxygenated monoterpenes. According to the literature data, phenolic monoterpenes (thymol and carvacrol) and phenylpropanoids (eugenol) in combination with other components were found to increase the bioactivities of essential oils. Most of the studies have focused on the interaction of phenolic monoterpenes and phenylpropanoids with other groups of components, particularly with other phenols, phenylpropanoids and monoterpene alcohols, while monoterpene and sesquiterpene hydrocarbons were used to a lesser extent (Bassolé and Juliani, 2010). However, limonene found to have higher inhibitory activity than the other terpenoids against a wide range of fungi, including *Aspergillus niger, Penicillium digitatum, Rhizoctonia solani, Fusarium oxysporum, Fusarium verticillioides* (Dambolena et al., 2008; Marei et al., 2012).

3.3. Principal component analysis

The principal component analysis (PCA) was applied to assess the chemical composition and the antifungal activity of peel essential oils from various *Citrus* species. So, PCA greatly helps in the interpretation of results from the experiments, the two-dimensional axial systems generated from PCA of *Citrus* essential oils showed that there are three main groups (Fig. 3). In fact, pummelo, sour orange and sweet orange were closer due to the similarities of the highest limonene levels (89.8-94.1%) and of MIC values (8000 µg/mL). *Tangelo* possessed the lowest antifungal activity against *Alternaria alternata* strain with 72.2% of limonene constituting the second cluster. Finally, tangerine, lemon and clementine formed the third cluster characterized by the lowest levels of limonene (76.3-62.1%) having an antifungal activity of 8500 µg/mL. The analysis of Pearson's correlation coefficients between the essential oil compounds and antifungal activity showed a non-significant correlation between limonene and *Alternaria alternata* (r = -0.215). However, there were a strong correlation between *Alternaria alternata* and the minor components α-thujene (r = 0.908), α-pinene (r = -0.600), β-myrcene (r = 0.879) and Δ-2-carene (r = 0.908). In Tunisia, *Alternaria alternata* affects *Citrus* species and in this study, this strain was isolated from tangerine (*Citrus reticulata* var. Fortune). It could be deduced that the essential oils of these *Citrus* species did not promote the increased resistance of plants against *Alternaria alternata*. However, Dayan et al. (2009) had highlighted the contribution of essential oils in increasing plant resistance against external aggression and they provide an important defence strategy for plants, in particular against parasitic herbivore insects and phytopathogenic fungi.

Table 1
Essential oil compositions of different *Citrus* peels.

Sr. Num.	Volatile compounds	KRI[a]	KRI[b]	Pummelo	Clementine	Sweet orange	Tangerine	Sour orange	Lemon	Tangelo
1	α-Thujene	923	836	0.1 ± 0.04^{d}	-	-	1.1 ± 0.12^{b}	-	0.3 ± 0.09^{c}	3.4 ± 0.74^{a}
2	α-Pinene	934	982	0.3 ± 0.02^{e}	1.3 ± 0.13^{b}	0.5 ± 0.01^{d}	2.2 ± 0.14^{a}	0.5 ± 0.01^{d}	1.1 ± 0.62^{c}	-
3	β-Pinene	937	1113	0.2 ± 0.03^{e}	0.8 ± 0.19^{c}	0.1 ± 0.01^{ef}	2.1 ± 0.14^{a}	0.2 ± 0.01^{f}	0.6 ± 0.11^{d}	1.3 ± 0.14^{b}
4	Camphene	952	1077	-	0.7 ± 0.18^{a}	-	0.6 ± 0.15^{b}	-	0.5 ± 0.01^{c}	-
5	Sabinene	983	1111	0.2 ± 0.02^{c}	0.9 ± 0.18^{b}	0.1 ± 0.01^{d}	0.8 ± 0.15^{b}	0.1 ± 0.00^{e}	5.8 ± 0.12^{a}	-
6	β-Myrcene	991	1168	0.5 ± 0.09^{f}	2.1 ± 0.12^{b}	0.8 ± 0.05^{e}	2.2 ± 0.16^{b}	1.5 ± 0.03^{c}	1 ± 0.06^{d}	5.2 ± 0.95^{a}
7	δ-2-Carene	1002	1150	0.6 ± 0.07^{d}	1.2 ± 0.27^{b}	0.6 ± 0.00c	-	-	-	2.3 ± 0.23^{a}
8	α-Phellandrene	1005	1025	-	0.9 ± 0.21^{a}	0.1 ± 0.01^{c}	0.9 ± 0.17^{a}	0.2 ± 0.00^{c}	-	0.6 ± 0.15^{b}
9	δ-3-Carene	1011	1159	-	0.9 ± 0.22^{a}	0.2 ± 0.01^{c}	-	-	-	0.4 ± 0.04^{b}
10	α-Terpinene	1018	1255	0.1 ± 0.02^{f}	0.9 ± 0.22^{c}	0.2 ± 0.01^{e}	1.2 ± 0.17^{a}	0.1 ± 0.00^{f}	1.0 ± 0.04^{b}	0.7 ± 0.08^{d}
11	*p*-Cymene	1026	1277	0.2 ± 0.04^{d}	-	0.3 ± 0.15^{c}	1.6 ± 0.17^{a}	0.1 ± 0.00^{e}	0.2 ± 0.02^{d}	0.9 ± 0.09^{b}
12	Limonene	1030	1031	89.8 ± 1.34^{c}	76.4 ± 4.85^{d}	94.1 ± 0.75^{a}	62.1 ± 3.20^{g}	92.8 ± 0.68^{b}	69.7 ± 1.43^{f}	72.2 ± 1.04^{e}
13	(*Z*)-β Ocimene	1045	1044	-	-	0.6 ± 0.09^{b}	0.9 ± 0.20^{a}	0.3 ± 0.01^{c}	-	-
14	(*E*)-β-Ocimene	1052	1022	0.3 ± 0.00^{d}	1.0 ± 0.25^{a}	0.8 ± 0.05^{b}	-	-	-	-
15	γ-Terpinene	1059	1262	0.4 ± 0.00^{g}	1.3 ± 0.24^{d}	0.8 ± 0.02^{e}	15.4 ± 0.72^{a}	0.5 ± 0.01^{f}	10 ± 0.05^{c}	13 ± 0.07^{b}
16	(Z)-linalool oxide	1074	1450	-	-	-	-	0.7 ± 0.11^{a}	-	-
17	(E)-linalool oxide	1081	1437	-	-	-	1.0 ± 0.21^{a}	0.4 ± 0.01^{b}	-	-
18	Linalool	1098	1551	0.4 ± 0.02^{d}	1.8 ± 0.24^{a}	-	1.1 ± 0.23^{b}	0.6 ± 0.01^{c}	0.6 ± 0.03^{c}	-
19	(*E*)-*p*-Menthen-1-ol	1141	1491	-	1.3 ± 0.30^{a}	-	1.0 ± 0.20^{b}	-	-	-
20	Borneol	1165	1642	-	-	-	-	-	2.5 ± 0.16^{a}	-
21	Terpinen-4-ol	1178	1593	0.3 ± 0.06^{c}	1.4 ± 0.24^{b}	-	1.5 ± 0.27^{a}	0.1 ± 0.00^{d}	-	-
22	α-Terpineol	1185	1711	0.4 ± 0.03^{c}	1.4 ± 0.35^{b}	-	1.7 ± 0.25^{a}	0.5 ± 0.14^{c}	1.2 ± 0.12^{b}	-
23	Camphor	1192	1498	-	-	-	1 ± 0.22^{a}	0.1 ± 0.00^{c}	0.6 ± 0.02^{b}	-
24	Linalyl acetate	1257	1556	-	1.3 ± 0.33^{a}	-		-	-	-
25	Geranyl acetate	1383	1599	-	-	-	-	-	0.5 ± 0.01^{a}	-
26	α-Copaene	1395	1391	0.4 ± 0.10^{a}	-	-	-	-	-	-
27	α-Cedrene	1410	1577	-	1.9 ± 0.45^{a}	-	-	-	-	-
28	γ-Elemene	1436	1651	0.5 ± 0.12^{a}	-	-	-	0.3 ± 0.17^{b}	-	-

Table 1 Continued

Sr. Num.	Volatile compounds	KRI[a]	KRI[b]	Pummelo	Clementine	Sweet orange	Tangerine	Sour orange	Lemon	Tangelo
29	(*E*)-Caryophyllene	1446	1608	0.6 ± 0.07[b]	-	-	1.5 ± 0.30[a]	0.4 ± 0.16[c]	-	-
30	Germacrene D	1480	1685	-	-	-	-	-	1.3 ± 0.01[a]	-
31	α-Humulene	1485	1691	0.5 ± 0.11[a]	-	-	-	-	-	-
32	Valencene	1495	1520	0.5 ± 0.12[a]	-	0.5 ± 0.24[a]	-	-	-	-
Monoterpene hydrocarbons				92.5 ± 0.90[c]	88.4 ± 0.85[e]	99.2 ± 1.55[a]	91.9 ± 1.55[b]	96.3 ± 1.53[b]	90.2 ± 1.30[d]	99.9 ± 3.30[a]
Oxygenated monoterpenes				1.1 ± 0.90[e]	7.2 ± 0.75[c]	-	7.3 ± 1.01[a]	2.4 ± 0.55[d]	5.4 ± 0.75[b]	-
Sesquiterpene hydrocarbons				2.5 ± 0.11[a]	1.9 ± 0.05[c]	0.5 ± 0.09[f]	1.5 ± 0.99[b]	0.7 ± 0.05[e]	1.3 ± 0.09[d]	-
Total				96.1 ± 1.37[c]	97.5 ± 2.75[c]	99.7 ± 0.09[a]	99.9 ± 1.77[d]	99.4 ± 1.55[b]	96.9 ± 1.30[c]	99.9 ± 3.30[a]

*Compounds in order of elution on HP-5 MS. [a]KRI: Kovats retention indexes calculated on HP-5 MS column; [b]KRI: Kovats retention indexes calculated on HP Innowax column; Means of three replicates. Values with different superscripts are significantly different at $p < 0.05$.

Table 2

Antifungal activity of peel essential oils from different *Citrus* species.

Species	Minimal concentration of inhibition (µg/mL)							
Citrus species	**Pummelo**	**Clementine**	**Sweet orange**	**Tangerine**	**Sour orange**	**Lemon**	**Tangelo**	**F**
Alternaria alternata	8500 ± 0.00[c]	8500 ± 0.00[c]	8500 ± 0.00[c]	8000 ± 0.0[b]	8000 ± 0.00[b]	8000 ± 0.00[b]	12000 ± 0.00[a]	2000 ± 0.00d

Values with different superscripts are significantly different at $p < 0.05$; F: Fungicide used as positive control: Melody duo with an active ingredient (5.5% Iprovalicarb and 61.3% Propineb)

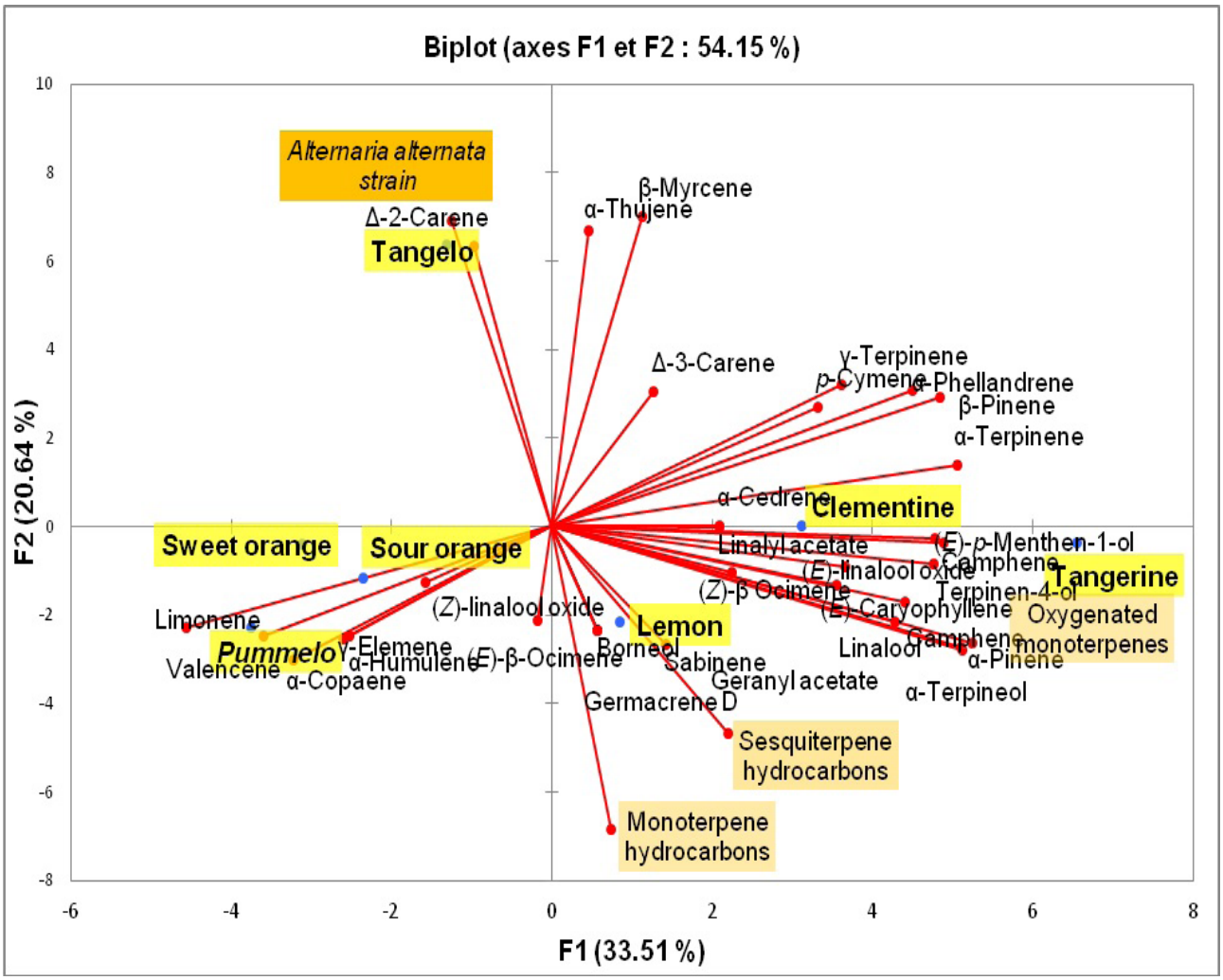

Fig. 3. Biplot obtained from principal component analysis of variables comprising essential oil components, chemical classes and antifungal activities.

4. Concluding remarks

In summary, *Citrus* peel essential oils were characterized by the predominance of limonene. All these *Citrus* essential oils had a weak antifungal activity against *Alternaria alternata*. Additionally, the analysis of Pearson's correlation coefficients between the essential oil compounds and antifungal activity showed a non-significant correlation between limonene and *Alternaria alternata*. So, a possible screening of other plants rich in essential oils could be conducted in further investigations to select their bioactive compounds having a potent antifungal effect against *Alternaria alternata*. These investigations could lead to the development of a new treatment based on natural bioactive substances against *Alternaria alternata*.

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