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# Research Article
# Study of Essential Oil Composition and Antifungal Activity of *Lavandula mairei*, *L. dentata* and *Tetraclinis articulata*

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## Abstract

**Back Ground and Objective:** Medicinal plants are getting popular day by day because of their easy accessibility and reasonable costs. This study investigated the chemical composition and antifungal activity of *Lavandula mairei*, *Lavandula dentata* and *Tetraclinis articulata* plants essential oils against *Penicillium digitatum*, *Penicillium italicum* and *Geotrichum citri-aurantii*, the main post-harvest pathogens in citrus. **Materials and Methods:** Essential oils were obtained by hydrodistillation from areal parts of tested plants. Afterwards, they were analyzed by means of GC-MS and their antifungal efficacy was tested *in vitro* by using the agar plate's method. **Results:** The main constituents were carvacrol for *L. mairei*, camphor, linalool and β-pinene for *L. dentata* and bornyl acetate, α-pinene, borneol and limonene for *T. articulata*. In the *in vitro* assay, the effect of essential oils on mycelial growth and spore germination varied significantly between tested plant species. Complete growth inhibition of the three pathogens was obtained by *L. mairei* essential oil. Also, *L. mairei* displayed the highest bioactivity, inhibiting completely the spore germination of the three pathogens. Moreover, this species showed fungistatic and fungicidal activity on the three fungal pathogens. **Conclusion:** In this study, *L. mairei* essential oil showed great antifungal activity which could represent a potential alternative to synthetic fungicides for the control of citrus fruit fungal pathogens.

Key words: *Lavandula mairei*, *Lavandula dentata*, *Tetraclinis articulata*, essential oils, *Penicillium digitatum*, synthetic fungicides, *Geotrichum citri-aurantii*, antifungal activity

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Post-harvest diseases render heavy losses to fruits and vegetables during storage and transit[1]. In the case of citrus fruit, the most common and serious diseases are green and blue moulds caused, respectively, by *Penicillium digitatum* (Pers.:Fr.) Sacc. and *Penicillium italicum* Wehmer followed in importance by sour rot caused by *Geotrichum citri-aurantii* Link ex Persn[2].

Disease control is achieved mainly through the use of synthetic fungicides, especially imazalil (IMZ), thiabendazole (TBZ), fludioxonil and pyrimethanil[3]. However, the post-harvest use of these fungicides is subject to registration and permission for use in various countries. Furthermore, repeated application of synthetic fungicides has resulted in artificial selection of resistant fungi with multiple fungicide resistances, which further complicate the management of the diseases (especially Penicillium rots)[4,5]. In addition, these fungicides are not effective against all important pathogens. Indeed, sour rot is difficult to control with IMZ and TBZ[4-6]. Besides leading to an increase in the cost of post-harvest control of *Penicillium rots* due to development of resistance, the use of synthetic fungicides is increasingly becoming restricted owing to stringent regulation, high and acute residual toxicity, environmental pollution and growing public concern about chemical residues in fruit[3,7]. Therefore, the challenge is to develop safer and eco-friendly alternative strategies of controlling citrus post-harvest diseases, which pose less risk to human health and environment. Recently, natural products including plant extracts and essential oils have been proposed as potential alternatives to synthetic fungicides for the control of post-harvest citrus diseases[8-10]. The EOs are natural, volatile, complex compounds known for their antibacterial, antifungal, antiviral and medicinal properties[11-15].

The genus *Lavandula* is represented in Moroccan flora by 9 species and subspecies of which 5 are endemic[16]. Among these endemic species, *Lavandula mairei* Humbert is considered as rare species[17]. *Lavandula dentata* L. is widely distributed in the Mediterranean region. These *Lavandula* species are widely used in traditional medicine for the treatment of various diseases such as gastrointestinal ailments, microbial infection, cough and asthma[18]. *Tetraclinis articulata* (Vahl) Masters, which belongs to the family of Cupressaceae is a well-known species used extensively throughout the Mediterranean basin in folk medicine for the treatment of a variety of ailments[18,19].

Despite their medicinal properties, these plants have never been examined as potential source of antifungal compounds against the main postharvest fungal pathogens of citrus fruit. Hence, the objective of this study was to evaluate the effectiveness of EOs obtained from *L. mairei*, *L. dentata* and *T. articulata* plants against *P. digitatum*, *P. italicum* and *G. citri-aurantii* for management of post-harvest citrus diseases. As far as we know, this is the first report on the antifungal activity of *L. mairei*, *L. dentata* and *T. articulata* EOs against postharvest fungal pathogens of citrus.

## MATERIALS AND METHODS

**Plant material:** Fresh plant samples of *L. mairei*, *L. dentata* and *T. articulata* were collected from their wild habitat, between March and May 2016, from two different locations in Morocco: *L. mairei* in Tafraout region (Western Anti Atlas), *L. dentata* in Tamri region (Western high Atlas) and *T. articulata* in Immouzzer region (Western high Atlas). Voucher specimens were deposited in the herbarium of the Laboratory of Biotechnology and Valorization of the Natural Resources, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco. Plant samples were cleaned, air dried in the shade and stored in the dark at 4°C until use.

**Extraction of essential oil:** The essential oils (EOs) were obtained from dried (200 g) aerial plant materials by hydrodistillation using a Clevenger type apparatus for 4 h as recommended by European Pharmacopoeia[20]. The EOs obtained were dried over anhydrous sodium sulfate and stored in an amber bottle at 4°C until used.

**Gas chromatography-mass spectrometry analysis:** Plants essential oils compositions were determined by GC/MS analysis according to Boubaker *et al.*[9]. Identification of compounds were based on the comparison of their mass spectra with those of Wiley and NIST libraries as well as by comparison of their retention indices with those of authentic samples. Oil chemical relative composition was determined by peaks area for compounds contributing for more than 0.1% of the total composition.

### Antifungal activities of essential oils

**Fungal cultures:** The fungi used in this study, *P. digitatum*, *P. italicum* and *G. citri-aurantii* were isolated from decayed citrus fruit. Single spore strains of these fungi were maintained on potato dextrose agar (PDA) plates at 5°C. A 1 week old culture of each fungus was used to inoculate the agar plates.

**Determination of antifungal effects of the essential oils on mycelial growth:** The agar dilution method was employed for the determination of the essential oils antifungal activity according to the method of Ameziane *et al.*[21]. All tests were performed in PDA supplemented with 0.05% (v/v) Tween 80 to enhance oil solubility[22]. Control consisted of unamended PDA medium supplemented with 0.05% Tween 80. The antifungal activity was expressed in terms of percentage of mycelial radial growth inhibition and calculated according to the following equation:

$$MGI\ (\%) = \left[\frac{(C-T)}{C}\right] \times 100$$

where, C and T represent mycelial growth diameter in control and EO treated Petri plates, respectively. Three plates were used for each treatment as replications. The experiment was repeated twice and similar results were obtained in each experiment.

**Effect of essential oils on spore germination:** For the germination test, plants essentials oils were used and prepared according to the method described by Boubaker *et al.*[9]. Different concentrations, ranging from 0.25-4.0 μL $mL^{-1}$ of essential oils were prepared by dissolving the requisite amounts in 80 μL of sterilized (0.2 μm filter) orange juice and 0.5% (v/v) Tween 80 and transferred to sterile depression slides[9]. The results were expressed as percent spore germination inhibition and calculated by using the following equation:

$$GI\ (\%) = \left[\frac{(Gc-Gt)}{Gc}\right] \times 100$$

where, Gc and Gt represent the mean number of germinated spores in control and treated slides, respectively. Each treatment included three replicates and the experiment was conducted twice.

**Determination of minimum inhibitory concentration and minimum fungicidal concentration:** The minimum inhibitory concentrations (MICs) of the plant EOs were determined by the agar dilution method[9]. The MICs were recorded by reading the lowest EO concentration that allowed no visible growth of the pathogen[23]. The MFCs were determined by taking agar plugs from well showing no visible mycelial growth and re-inoculating them on unamended PDA medium. The MFC was regarded as the lowest concentration of the EOs that prevented growth of the pathogen after the period of incubation. There were three replicates for each plant EO at each concentration and the experiment was conducted twice.

**Statistical analysis:** All data were subjected to statistical analysis of variance (ANOVA) using STATISTICA software, ver. 6 (Stat-Soft, 2001, Créteil, France). Percentage values were subjected to arcsine square root transformation before analysis of variance. Mean separation was performed following the Newman and Keuls test at $p<0.05$.

## RESULTS

**Chemical composition of the essential oils:** The chemical analyses of *L. mairei*, *L. dentata* and *T. articulata* EOs led to the identification and quantification of 25, 28 and 24 compounds, respectively (Table 1). The major compound of *L. mairei* EO is carvacrol (83.2%) followed by caryophyllene oxide (3.06%), p-cymen-8-ol (2.02%), octen-3-ol (1.94%), spathulenol (1.62%) and carvacrol methyl ether (1.07%), which accounted for 92.91% of the total essential oil. While the major components (73.24% of the total oil) identified in *L. dentata* EO were camphor (64.43%), linalool (4.87%) and β-pinene (3.94%). Bornyl acetate (43.78%), α-pinene (20.3%), borneol (12.45%) and limonene (5.2%) were the main compounds identified in *T. articulata* EO.

**Effects of essential oils on mycelial growth:** The results of this study confirm that EOs from *L. mairei*, *L. dentata* and *T. articulata* plants possess antifungal activity against *P. digitatum*, *P. italicum* and *G. citri-aurantii* (Fig. 1). Of the plant species tested EO from *L. mairei* produced highest antifungal activity against the three fungi. Indeed, this plant had completely (100%) inhibited the mycelial growth of the three fungal pathogens at a concentration of 1.0 μL $mL^{-1}$ (Fig. 1). For *L. dentata* EO, the inhibition percentages after 7 days of incubation were 24, 52 and 13% against *P. digitatum*, *P. italicum* and *G. citri-aurantii*, respectively. On the other hand, essential oil obtained from *T. articulata* were found to possess weaker antifungal activity against *P. digitatum*, *P. italicum* and *G. citri-aurantii* with a mycelial growth inhibition of only 17, 41 and 10%, respectively.

**Effect of essential oils on spore germination:** The results shown in Table 2 indicate that *L. mairei* EO inhibited the spore germination of *P. italicum*, *P. digitatum* and *G. citri-aurantii* in a dose-dependent manner. Indeed, tested at 1.0 μL $mL^{-1}$, the EO inhibited the spore germination of *P. digitatum*, *P. italicum*

Table 1: Chemical relative composition of *Lavandula mairei*, *L. dentata* and *Tetraclinis articulata* essential oils

| [a]RI | [b]Compounds | *L. mairei* | *L. dentata* | *T. articulata* |
|---|---|---|---|---|
| 931 | α-Thujene | | | 1.59 |
| 939 | α-pinene | | 0.91 | 20.3 |
| 954 | Camphene | | 0.81 | 2.13 |
| 972 | Octen-3-ol | 1.94 | 0.3 | |
| 979 | Sabinene | | 0.5 | 0.15 |
| 980 | β-pinene | | 3.94 | 0.58 |
| 984 | Octan-3-one | 0.35 | | |
| 991 | Myrcene | | 0.1 | 2.1 |
| 996 | Octan-3-ol | 0.10 | | |
| 1014 | α-Terpinene | | | 0.45 |
| 1020 | α-Phellandrene | | | 0.2 |
| 1026 | p-Cymene | | 0.5 | 0.78 |
| 1030 | limonene | | 1.09 | 5.20 |
| 1033 | 1,8-Cineole | | 1.79 | |
| 1034 | Eucalyptol | 0.23 | | |
| 1087 | Fenchone | | 0.37 | |
| 1088 | Terpinolene | | 3.25 | 0.45 |
| 1096 | Linalool | 0.34 | 4.87 | |
| 1115 | β-Thujone | | | |
| 1143 | Camphor | | 64.43 | 1.45 |
| 1147 | Terpinolene oxide | 0.55 | | |
| 1165 | Borneol | | 0.3 | 12.45 |
| 1178 | Terpinene-4-ol | | 1.49 | |
| 1181 | Limonen-4-ol | 0.48 | | |
| 1189 | *p*-Cymen-8-ol | 2.02 | 3.86 | 1.48 |
| 1197 | α-terpineol | 0.32 | 1.08 | 1.7 |
| 1194 | Myrtenol | | 1.9 | 0.4 |
| 1222 | Carvomenthenal isomer 1 | 0.12 | | |
| 1224 | Carvomenthenal isomer 2 | 0.15 | | |
| 1228 | Trans-carveol | | 0.43 | 0.3 |
| | Cis-carveol | | | 0.2 |
| 1242 | Carvone | | 0.08 | 0.2 |
| 1248 | Carvacrol methyl ether | 1.07 | | |
| 1294 | Thymol | 0.17 | 0.68 | |
| 1308 | Carvacrol | 83.2 | 0.25 | |
| 1295 | Bornyl acetate | | | 43.78 |
| 1328 | n.i. | 0.20 | | |
| 1360 | 4-Methoxyacetophenone | 0.13 | | |
| 1377 | 8,9-Dehydrocarvacrol | 0.09 | | |
| 1429 | β-Caryophyllene | 0.50 | | 0.6 |
| 1449 | Aromadendrene | 0.08 | | |
| 1480 | α-Humulene | | | 0.71 |
| 1505 | Ledene | 0.09 | | |
| 1524 | γ-Cadinene | 0.08 | 0.14 | Tr |
| RI | Compounds | *L. mairei* | *L. dentata* | *T. articulata* |
| 1526 | δ-Cadinene | | 0.03 | |
| 1587 | Spathulenol | 1.62 | | |
| 1594 | Caryophyllene oxide | 3.06 | 0.28 | 0.9 |
| 1620 | n.i. | 0.11 | | |
| 1650 | t-Cadinol | 0.26 | 0.05 | |
| 1662 | Cadalene | | 3.97 | |
| 1682 | Caryophylla-1(12).7-dien-9-ol | 0.18 | | |
| 1892 | n.i. | 0.15 | | |
| 1993 | Manoyl oxide | 0.38 | | |
| 2037 | n.i. | 0.17 | | |
| 2080 | n.i. | 1.22 | | |
| 2102 | n.i. | 0.18 | | |
| 2190 | α-Bisabolol | | 0.42 | |
| 2199 | n.i. | 0.18 | | |
| 2223 | n.i. | 0.16 | | |
| 2232 | n.i. | 0.15 | | |
| Total (%) | | 99.85% | 97.4% | 98.1% |

[a]RI: Retention indices measured relative to n-alkanes (C-9 to C-24) on the non-polar DB-5 column. [b]Compounds listed in order of elution

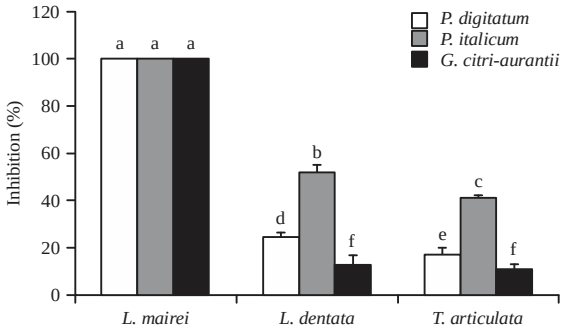


Fig. 1: *In vitro* effects of *L. mairei*, *L. dentata* and *T. articulata* essential oils on mycelial growth of *P. digitatum*, *P. italicum* and *G. citri-aurantii*

Values followed by the same letters were not significantly different ($p<0.05$) according to Newman and Keuls test

Table 2: *In vitro* effect of *L. mairei*, *L. dentata* and *T. articulata* essential oils on spore germination of *P. digitatum*, *P. italicum* and *G. citri-aurantii*

| | | Inhibition of spore germination (%) | | | |
|---|---|---|---|---|---|
| | | Essential oils concentration (μL mL$^{-1}$) | | | |
| Plant species | Pathogens | 0.5 | 1.0 | 2.0 | 4.0 |
| *L. mairei* | *P. digitatum* | 0±0$^{g}$ | 38.0±2.64$^{e}$ | 94.33±4.04$^{b}$ | 100.0±0$^{a}$ |
| | *P. italicum* | 0±0$^{g}$ | 50.0±6.24$^{d}$ | 97.67±5.51$^{ab}$ | 100.0±0$^{a}$ |
| | *G. citri-aurantii* | 0±0$^{g}$ | 85.33±4.5$^{c}$ | 97.66±2.51$^{ab}$ | 100.0±0$^{a}$ |
| *L. dentata* | P. digitatum | 0±0$^{g}$ | 0.0±0$^{g}$ | 0.0±0$^{g}$ | 95.33±2.51$^{a}$ |
| | *P. italicum* | 0±0$^{g}$ | 0.0±0$^{g}$ | 35.0±5$^{e}$ | 100.0±0$^{a}$ |
| | *G. citri-aurantii* | 0±0$^{g}$ | 0.0±0$^{g}$ | 0.0±0$^{g}$ | 13.33±4.04$^{f}$ |
| *T. articulata* | *P. digitatum* | 0±0$^{g}$ | 0.0±0$^{g}$ | 18.7±6.51$^{f}$ | 97.0±10$^{a}$ |
| | *P. italicum* | 0±0$^{g}$ | 0.0±0$^{g}$ | 0.0±0$^{g}$ | 33.34±6.11$^{e}$ |
| | *G. citri-aurantii* | 0±0$^{g}$ | 0.0±0$^{g}$ | 0.0±0$^{g}$ | 14.67±4.51$^{f}$ |

Each value represents the mean of three replicates. Means followed by different letter(s) are significantly different at $p<0.05$. For all control treatments, which contained only Tween 80 in orange juice, spore germination was >90%

Table 3: Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of *Lavandula mairei* and *L. dentata* essential oils

| Species | *P. digitatum* | *P. italicum* | *G. citri-aurantii* |
|---|---|---|---|
| ***L. mairei*** | | | |
| MIC (μL mL$^{-1}$) | 0.5 | 0.25 | 0.5 |
| MFC (μl mL) | 2.0 | 1.0 | 1.0 |
| ***L. dentata*** | | | |
| MIC | 2.0 | 4.0 | 4.0 |
| MFC | 4.0 | 4.0 | 4.0 |
| ***T. articulata*** | | | |
| MIC | 4.0 | 4.0 | 4.0 |
| MFC | 4.0 | 4.0 | 4.0 |

MIC: Concentration that was fungistatic, MFC: Concentration that was fungicidal

and *G. citri-aurantii* by 38.0, 50.0 and 85.33%, respectively. At concentrations of 2.0 and 4.0 μL mL$^{-1}$, *L. mairei* EO inhibited by more than >94% spores germination of *P. digitatum*, *P. italicum* and *G. citri-aurantii*. When tested at 4.0 μL mL$^{-1}$, the EO of *L. dentata* inhibited the germination of spores of *P. digitatum*, *P. italicum* and *G. citri-aurantii* by 95.33, 100 and

13.33%, respectively. *Tetraclinis articulata* EO reduced the germination of spores of *P. digitatum*, *P. italicum* and *G. citri-aurantii* by 97, 33.33 and 14.67%, respectively. For all control treatments, which contained only Tween 80 in orange juice, spore germination was >90%.

**MIC and MFC:** The inhibitory property of the oil obtained from *L. mairei* was observed within a range of 0.25 μL $mL^{-1}$ for *P. italicum* to 0.5 μL $mL^{-1}$ for *P. digitatum* and *G. citri-aurantii* (Table 3). *Lavandula mairei* EO was found to be fungicidal against *P. digitatum*, *P. italicum* and *G. citri-aurantii* at MFCs of 2.0, 1.0 and 1.0 μL $mL^{-1}$, respectively. *Lavandula dentata* EO completely inhibited the growth of *P. digitatum*, *P. italicum* and *G. citri-aurantii* at MICs of 2.0, 4.0 and 4.0 μL $mL^{-1}$, respectively. While the fungicidal effect of *T. articulata* and *L. dentata* EOs against the three pathogens appeared at a higher concentration >4.0 μL $mL^{-1}$.

## DISCUSSION

In the present study, α-pinene, camphor, borneol and bornyl acetate were found to be the principal constituents of *T. articulata* EO. These results are in agreement with those reported by Bourkhiss *et al.*[24], in where it has been established that α-pinene, camphor, borneol and bornyl acetate were the main components of Moroccan *T. articulata* EO. *Lavandula mairei* EO is characterized by a high amount of carvacrol compared to that reported for others *Lavandula* species, Only *L. multifida* has been reported as carvacrol-rich specie[25]. The presence of carvacrol within *L. mairei* EO at very substantial proportions presents a special interest. Indeed, carvacrol possesses a very high antifungal activity against many post-harvest pathogens[26,27] and essential oils containing a high amount of carvacrol could have many applications and effects on both human and plants pathogens. Furthermore, the safe use of medicinal herbs and their individual components has led to their current status of Generally Recognized as Safe (GRAS) food ingredients to control bacterial and fungal diseases[15].

In several studies, it was pointed out that antimicrobial activity of EO obtained from *T. articulata* may be due, in part, to the presence of α-pinene and borneol among the major compounds of this species[24-28]. Prior reports described antifungal activities of plants EOs and several of their individual components against some of the pathogens examined in the present work and demonstrated that the mechanisms involved in control of these pathogens by plant EOs include restriction of their conidial germination and hyphal growth[26,27,29,30]. In the present study, the high antifungal properties of *L. mairei* EO, against the three fungal pathogens, can be attributed to the presence of high concentration of carvacrol (83.2%). According to previous works, thymol, carvacrol, geraniol, eugenol, octanal and citral were recognized as the most active components against citrus fungal pathogens[26,27,30-32].

In the current experiment, *L. mairei*, *L. dentata* and *T. articulata* essential oils have been shown to reduce or completely inhibit the spore germination of *P. digitatum*, *P. italicum* and *G. citri-aurantii.* These results are consistent with reports in the literature that describe carvacrol and/or thymol as an inhibitor of conidial germination and mycelial growth of several postharvest citrus pathogens[26,27,32-34]. In addition, it is also possible that the minor components such as linalool, borneol and α-terpineol might be involved in the antifungal activity with other active components of tested EOs, as also evident by the work of others Tao *et al.*[30], Wolken *et al.*[35] and Wuryatmo *et al.*[36].

Among the three plants EOs tested, essential oils obtained from *L. mairei* showed the most potent antifungal effect against the three fungal pathogens. The MIC values obtained in this study, against *P. digitatum*, *P. italicum* and *G. citri-aurantii* were 0.50, 0.25 and 0.50 μL $mL^{-1}$, respectively. A study by Tao *et al.*[37] demonstrated that octanal, a pure plants EOs constituent, reduced strongly the mycelial growth of *P. italicum* and *P. digitatum*, with an MIC and MFC of 0.5 and 1.0 μL $mL^{-1}$, respectively. In a recent study, essential oils obtained from four Moroccan *Thymus* species showed a MIC values against *P. digitatum*, *P. italicum* and *G. citri-aurantii* ranging from 0.5-4.0 μL $mL^{-1}$ and MFC values[9] from 1.0 to more than 4.0 μL $mL^{-1}$. In a similar study, Regnier *et al.*[27] reported the efficacy of different plants EOs and their major components (citral, eugenol, geraniol, carvacrol and thymol). Their results confirmed the antifungal activity of those substances for *in vitro* and *in vivo* control of post-harvest citrus pathogens[27].

## CONCLUSION

*Lavandula mairei* essential oil exhibited great antifungal which suggests that they may be considered as a potential alternative to the synthetic fungicides for the control of post-harvest citrus fungal pathogens. Further experimental research is needed to assess *in vivo* efficacy and commercial implementation of EOs as post-harvest botanical fungicides in citrus industry with respects to problems related to potential phytotoxicity, organoleptic aspects and compatibility with common post-harvest practices.

## SIGNIFICANCE STATEMENT

This study demonstrates that plant essential oils have a high potential to control post-harvest fungal diseases of citrus fruits. Among the three plants tested, *L. mairei* essential oil showed high antifungal activities against the tested pathogens and will provide a starting point for discovering new compounds with better activity than chemical fungicides currently available. Such natural products therefore represent a sustainable alternative to the use of chemical fungicides.

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