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*Research Article*

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# Antimicrobial activity of *Tetraclinis articulata* aerial parts essential oil from Tlemcen, Northwestern of Algeria

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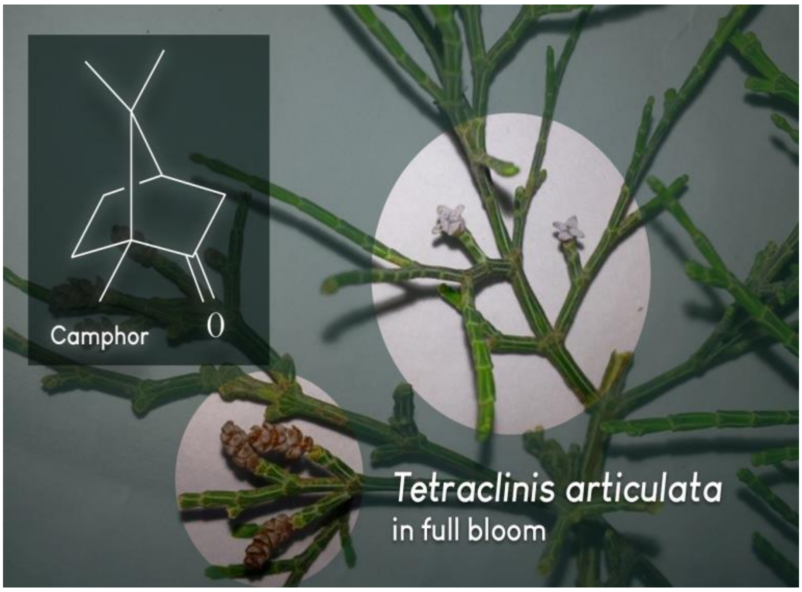
## Highlights

Three different compositions (collective samples) of aerial parts essential oil of *Tetraclinis articulata* were evaluated for antimicrobial activity. The tested oils have been shown to possess inhibitory action in the range from 1 to 6 µL/mL. However, a rich oil of camphor was more active, with MICs varied between 1 to 3 µL/mL against *Aspergillus fumigatus*, *A. niger* and *Staphylococcus aureus*.

## Graphical Abstract



**Abstract**
The essential oil from aerial parts of *Tetraclinis articulata* (Vahl) Masters, obtained by hydrodistillation was analyzed by GC, GC/MS and $^{13}$C-NMR and evaluated for antimicrobial activity. The essential oil was rich in monoterpenes which *α*-pinene, bornyl acetate and camphor being the main constituents.Three essential oils were tested against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, *Aspergillus fumigatus* and *Aspergillus flavus* and the minimum inhibitory concentration was determined.The tested oils have been shown to possess inhibitory action in the range from 1 to 6 µL/mL against *Aspergillus fumigatus*, *A. niger* and *Staphylococcus aureus*. However, essential oil with camphor as major component was the most interesting, with CMIs ranging between 1 and 3 µL/mL. While, essential oil with *α*-pinene as major compound, remains the less effective, with CMIs ranging between 2 and 6 µL/mL.

**Keywords:** *Tetraclinis articulata* (Vahl) Masters (Barbary Thuya); essential oil composition; chemical variability; antimicrobial activity, Algeria.

## 1. Introduction

In the last few years, the search continues for safe and effective antimicrobial agents with which can be treated a wide variety of bacterial infections. This need has been heightened recently by the emergence of many antimicrobial-resistant organisms (Lahlou, 2004; Preuss et al., 2005). A growing body of research in biology and medicine has been devoted to essential oils and their pure components due to their non-toxic nature and a wide spectrum of biological activities (Djouahri et al., 2015).

*Tetraclinis articulata* (Vahl) Masters (Cupressaceae family) is found in Europe on the coast of southeastern Spain and in Malta (Quèzel and Santa, 1962; Quèzel and Médail, 2003). It is widespread in North Africa, in Morocco (from North of Rif to Anti-Atlas), in Tunisia (between Bizerte and Zaghouan) and in Algeria (Northwestern part of the country and particularly in Tlemcen Province) (Quézel and Médail, 2003).

*T. articulata* (Vahl) Masters (Barbary Thuya) commonly known as “Ahrar” (Quèzel and Santa, 1962), has been used in folk medicine against respiratory and intestinal infections, gastric pains, hypertension and diarrhea and to treat fever (leaves, aerial parts). It has been used as a diuretic, antipyretic, anti-rheumatic and oral hypoglycemic (Bellakhdar et al., 1982; Ziyyat et al., 1997). In parallel, various biological activities have been reported for extracts and essential oils (seeds, leaves, aerial parts) such as cytotoxic (Buhagiar et al., 1999; Aouinty et al., 2006), vasorelaxant (Zidane et al., 2014), antimicrobial (Bourkhiss et al., 2007a; Abi-Ayad et al., 2011; 2013; Chikhoune et al., 2013; Djouahri et al., 2016; Ben Ghnaya et al., 2016; Ibrahim et al., 2017; Rabib et al., 2019; 2020), antioxidant and anti-inflammatory activities (Bourkhiss et al., 2010; Djouahri et al., 2013).

The antimicrobial activity of *T. articulata* essential oil has been the subject of a many studies. Indeed, the antimicrobial activity of the essential oil isolated from leaves of *T. articulata* was evaluated against *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus flavus*. A high activity was observed, with minimal inhibitory concentrations varied between 1/500 and 1/5000 (Bourkhiss et al., 2007a). Chikhoune et al. in 2013 reported the antimicrobial activity of leaf essential oil samples of *T. articulata* collected from two different locations in Algeria. The essential oil showed a significant activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*, varied between 0.2 and 1.0 µg/mL. Another oil sample isolated from the leaves of *T. articulata* exhibited very good sensitivity against six bacteria: *Salmonella enterica*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *S. aureus*, *E. coli*, *Fusarium culmorum*, *Aspergillus flavus* and *Candida albicans*, with inhibition zones diameters in the range of 11.5 - 25.3 mm and MIC values ranging between 0.25 and 50 µg/mL (Djouahri et al., 2016). Rabib et al. (2019; 2020) also reported very low MICs values (1.56 – 50 µg/ml) for leaves essential oil of *T. Articulata*, against *Pseudomonas aeruginosa*, *S. aureus* and *E. coli*. Ibrahim et al. in 2017 reported on the antibacterial activity of *T. articulata* leaf oil. Essential oil inhibited the growth of seven tested bacteria (*Staphylococcus aureus*, *S. epidermidis*, *E. coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Shigella boydi*) and two tested yeasts: *Candida albicans* and *Candida parapsilosis*. However, the reported MICs values (0.023 – 3.031µL/mL) were very low. In contrast, Abi-Ayad et al. in 2011 and 2013 were determined the antimicrobial activity of the leaves essential oil of *T. articulata*, on several strains, in particular *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus flavus* and *A. niger*. These authors noted a low antimicrobial activity, with a concentration very higher varied between 7.5 and 15 µL/mL. The same, the antifungal activity of *T. articulata* essential oil was evaluated against five filamentous fungi: *Botrytis cinerea*, *Fusarium avenaceum*, *Fusarium oxysporum*, *Fusarium culmorum*, *Fusarium solani*. A low sensitivity was observed (percentage of inhibition: 25.36 -71.17%) (Ben Ghnaya et al., 2016).

Various works reported on the composition of *T. articulata* leaf essential oils. All the investigated oil samples were characterized by the occurrence of monoterpenes as major components. However, several compositions were observed with respect to the contents of the three principal components, mainly *α*-pinene, camphor and bornyl acetate (Barrero et al., 2005; Bourkhiss et al., 2007b; Achak et al., 2009; Ben Jemia et al., 2013).

In a recent study, the chemical composition of fifty *T. articulata* oil samples, isolated by hydrodistillation from individual plants (aerial parts) collected in eight locations in Algeria was reported. Fifty oil samples were submitted to GC(FID) analysis and retention indices (RIs) of individual components were measured on two columns of different polarity. Among them, 7 samples, selected on the basis of their chromatographic profile, were also analyzed by MS and $^{13}$C-NMR. In total, 35 components and accounting for 94.8 - 99.1% of the whole oil composition, were identified in the 50 oil samples and they were considered in the statistical analysis (Boussaïd et al., 2015).

Monoterpenes were the main constituents of all samples (57.1-95.4%). However, the concentration of the three major components varied drastically from sample to sample; *α*-pinene (9.2-56.5%), camphor (0.5-40.3%) and bornyl acetate (1.2-45.1%). Other monoterpenes present in appreciable contents were myrcene, limonene and borneol. The main sesquiterpenes detected were *(E)*-*β*-caryophyllene and germacrene D. A combination of hierarchical clustering dendrogram and principal components analysis (PCA), in which the plan defined by the two first axes described 89.5% of the total variance of the population, suggested the existence of two principal groups, which were distinguished on the basis of *α*-pinene, camphor and bornyl acetate contents (Boussaïd et al., 2015).

In this context, we tested the antimicrobial activity of three collective samples of essential oil of the aerial parts of *T. articulata* presenting different chemical compositions (*α*-pinene, or camphor or bornyl acetate as major components).

## 2. Materials and Methods

### 2.1. Plant Material and Oil Distillation

Fifty samples of aerial parts (leaves and flowers) of individual adult trees of *T. articulata* (Vahl) Masters were collected during the flowering period in March 2014 at different altitudes (42 m to 1210 m) inTlemcen Province: Coastal township (Honaïne, Beni Khellad, Souk Tlata, Ghazaouet); highlands (Sebdou, Ouled Mimoun, Aïn Kebira and Sabra) (Boussaïd et al., 2015). Botanical identification of the species was performed by Pr M. Bouazza (Laboratory of Ecology and Management of Natural Ecosystems, University of Tlemcen, Algeria). A voucher specimen has been deposited at the Laboratory of Natural Products (Department of Biology, University of Tlemcen), under the accession No. C. 46. Crushed aerial parts were submitted to hydrodistillation for 2.5 h using a Clevenger-type apparatus. Yields have been calculated from dry material (Boussaïd et al., 2015).

### 2.2. GC (FID), GC-MS and $^{13}$C-NMR analyses

The samples were submitted to GC in combination with retention indices on two columns of different polarity, GC/MS and $^{13}$C-NMR, following a computerized method developed at the University of Corsica (Tomi et al., 1995; Tomi and Casanova, 2006; Ouattara et al., 2014). Analyses have been carried out as previously reported by Boussaïd et al. (2015).

### 2.3. Identification of components

Identification of the components has been carried out as previously reported by Boussaïd et al. (2015).

### 2.3. Antimicrobial Activity of the Essential Oil

#### *2.3.1. Microbial Strains*

Antimicrobial activity of essential oils (collective samples) was evaluated against three Gram positive bacteria (*Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC25923 and *Enterococcus faecalis* ATCC13047), three Gram negative bacteria (*Escherichia coli* ATCC 8739 and *Klebsiella pneumonia* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853), two yeasts (*Candida albicans* ATCC 10231, *C. albicans* CIP 444) and two filamentous fungi (*Aspergillus fumigatus* MNHN 566 and *A. flavus* MNHN 994294).

#### *2.3.2. Screening of Antimicrobial Activity*

The agar diffusion method was used for the determination of antimicrobial activity of the Eos (NCCLS, 2001). Briefly, a suspension of the tested microorganisms (1 mL of a suspension at $10^6$ cells/mL for bacteria and yeast, $10^7$ cells/mL for *S. aureus* and $10^4$ spores/mL for filamentous fungi) was spread on the solid media Petri dishes, using Mueller-Hinton agar for bacteria, Sabouraud dextrose for yeast and PDA for filamentous fungi. Filter paper discs (6 mm in diameter) were impregnated with 15 µL of the oil and 5 µL of DMSO and placed on the surface of inoculated Petri dishes. The activity was determined by measuring the inhibitory zone diameter in mm after incubation for 24 h at 37 °C for bacteria, 24-48 h at 30 °C for yeast and 5 days at 25 °C for filamentous fungi. Fluconazole (FLU 25 µg/disc) and nystatin (NY 30 µg/disc) were used as reference antifungal against yeast and filamentous fungi; ciprofloxacin (CIP 10 µg/disc), vancomycin (VAN 30 µg/disc), oxacillin (OX 5 µg/disc), penicillin (PEN 6 µg/disc), and amoxicillin (AM10 µg/disc) were used as positive controls against bacteria. DMSO was used as negative control. Each test was performed in triplicate.

#### *2.3.3. Determination of minimum inhibitory concentration (MIC)*

The MIC was defined as the lowest concentration of tested sample that resulted in complete inhibition of visible growth. Dilutions of oil were made in culture medium over the concentration range 2-4 µL/mL. 1 mL of standardized suspension was added. Inoculated plates were incubated at 37 ºC for 24 h for bacteria, 24-48 h at 37 °C for yeast and 5 days at 25 °C for filamentous fungi. MICs were determined as the minimum concentration with no visible growth. Each test was performed in duplicate or in triplicate.

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## 3. Results and discussion

### 3.1. Extraction yields

The essential oil of the aerial parts of *T. articulata* harvested in eight locations from Tlemcen Province (Figure 1) have been obtained by hydrodistillation. The yields (w/w) calculated from dry material, varied drastically from sample to sample, ranging between 0.03 to 0.86%, even within a location (0.04%-0.75% in Ghazaouet, for instance). According to these results, there is no correlation between the yield of essential oil and altitude (Boussaïd et al., 2015).

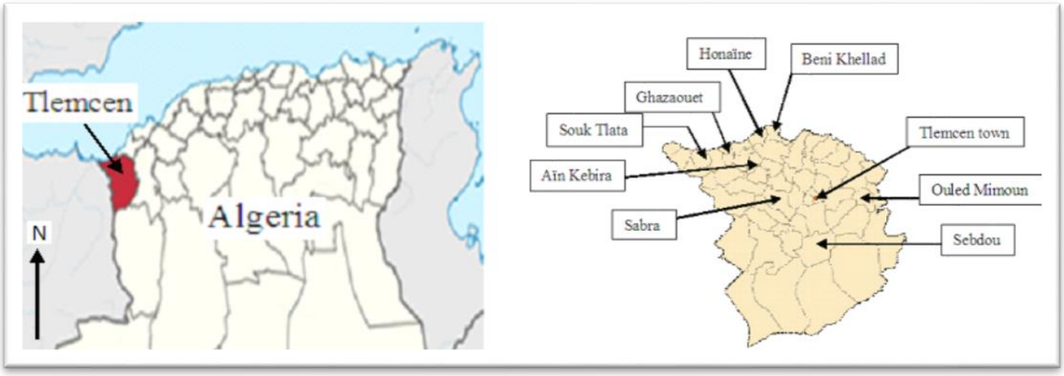


**Figure 1.** Sampling locations of *Tetraclinis articulata* from Tlemcen Province (Boussaïd et al., 2015)

### 3.2. Chemical composition

The chemical characterization of 50 oil samples of *T. articulata* (Vahl) Masters, by exploiting the complementarity of analytical techniques (GC-FID, GC/MS and $^{13}$C-NMR), allowed us to identify 35 compounds. Monoterpenes were the main constituents of all samples (57.1-95.4%). However, the concentration of the three major components varied drastically from sample to sample; *α*-pinene (9.2-56.5%), camphor (0.5-40.3%) and bornyl acetate (1.2-45.1%). Other monoterpenes present in appreciable, contents were myrcene (up to 9.7%), limonene (up to 12.5%) and borneol (up to12.9%). The main sesquiterpenes detected were germacrene D (up to 14.2%) and *(E)-β*-caryophyllene (up to 13.3%) (Boussaïd et al., 2015).
The analysis of 50 samples of aerial parts essential oil, associated with statistical treatment of the results (PCA and Dendrogram), suggested the occurrence of a chemical variability. Samples are divided into two groups (I and II which is subdivided in two sub-groups) based on their contents in i) camphor (group I), ii) *α*-pinene (sub-groups IIA) and iii) association *α*-pinene/bornyl acetate (sub-groups IIB) (Boussaïd et al., 2015).
To obtain enough quantity of essential oil to carry out antimicrobial experiments, various oil samples with similar compositions have been pooled leading three collective samples (S1-S3), whose main components are reported in Table 1. As observed in oil samples previously investigated, the sample S1 was characterized by a high content of camphor, followed by *α*-pinene and bornyl acetate. Limonene and borneol were the other components present in appreciable contents. In this sample, several sesquiterpene hydrocarbons, such as *(E)-β*-caryophyllene and germacrene D were present in low amounts. Sample S2 was characterized by a very high content of *α*-pinene, followed by bornyl acetate and camphor. We also note the occurrence of *(E)-β*-caryophyllene in appreciable contents. This sample exhibited comparable proportions of limonene and borneol, all present in appreciable amounts. Sample S3 was dominated by high contents of bornyl acetate, followed by *α*-pinene accompanied to a lesser extent by camphor, borneol and *(E)-β*-caryophyllene.

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**Table 1.** Main compounds identified in each collective essential oil sample of the aerial parts of *T. articulata.*

| Compounds[a] | RIa[b] | RIp[c] | S1 (%) | S2 (%) | S3 (%) |
|---|---|---|---|---|---|
| **$\alpha$-Pinene** | 932 | 1018 | 18.0 | **50.2** | 19.0 |
| **Myrcene** | 982 | 1161 | 2.6 | 2.3 | 2.8 |
| **Limonene** | 1022 | 1202 | 6.4 | 4.7 | 7.9 |
| **Camphor** | 1122 | 1514 | **33.7** | 11.2 | 8.4 |
| **Borneol** | 1150 | 1694 | 4.0 | 3.4 | 2.9 |
| **Bornyl acetate** | 1271 | 1579 | 13.5 | 13.5 | **42.5** |
| **(*E*)-$\beta$-Caryophyllene** | 1419 | 1595 | 1.3 | 4.4 | 0.8 |
| **Germacrene D** | 1477 | 1705 | 0.4 | 0.9 | 0.9 |

[a]: Order of elution and percentages are given on apolar column (BP-1). [b]RIa: Retention indices measured on apolar column (BP-1). [c]RIp: Retention indices measured on polar column (BP-20). S: Sample. %: Percentage.

### 3.3. Antimicrobial activity

The antimicrobial activity of three essential oils was tested against six bacteria, two yeasts and two filamentous fungi by applying the agar diffusion method, and in the affirmation, we have determined the minimum inhibitory concentration (MIC) using the direct contact method in agar medium.

#### *3.3.1. Screening of Antimicrobial Activity*

The results of the antimicrobial activity of the essential oil of the aerial parts of *T. articulata* and the antibiotic resistance of each strain against the positive controls are given in Table 2.

The essential oil of the aerial parts of *T. articulata* presenting three different chemical compositions ($\alpha$-pinene, or camphor or bornyl acetate as major compounds), displayed the highest activity against both *S. aureus*, *A. fumigatus* and *C. albicans*, with inhibition zones diameters ranging between 19.0 and 38.5 mm. We observed that, essential oil with camphor as major component, was the most interesting. Furthermore, all the essential oils tested, were inactive with respect to the bacterial strains: *P. aeruginosa* and *K. pneumoniae*, which appear to be very recognized. The other microbial strains have been shown to be more or less sensitive to all these essential oils, with inhibition zones diameters varying between 9.3 and 15.3 mm.

These results were consistent with those reported by Chikhoune et al. (2013) *i.e.* a low activity of the leaf essential oil of Thuja against *P. aeruginosa* and *E. coli*, with inhibition zones diameters ranging between 7 and 11 mm. They also suggest a significant sensitivity of *S. aureus* with inhibition zones diameters varying between 18 and 22 mm. This essential oil contained a high content of bornyl acetate, with percentages of 40.2 and 59.2% in two samples collected at two different locations. Djouhri et al. (2016) also reported a good activity against *S. aureus* with inhibition zone diameter in order of 25.3 mm. The main components in this sample were α-pinene (29.0%), bornyl acetate (25.5%) and camphor (16.6%).

**Table 2.** Antimicrobial activity of the three collective samples essential oil from the aerial parts of *T. articulata* determined by the disc diffusion assay (mm).

| Samples | Bacteria | | | | | | Yeasts | | Fungi | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *E. c.* | *E. f.* | *P. a.* | *K. p.* | *S. a.* 25923 | *S. a* 6538 | *C. a* 10 231 | *C. a* CIP | *A. flavus* | *A. fumigatus* |
| **S1** | 10.0±0.0 | 11.7±0.6 | 6.0±0.0 | 6.0±0.0 | 38.5±8.2 | 24.0±4.2 | 12.7±1.2 | 19.0±1.0 | 13.0±1.7 | ---- |
| **S2** | 11.3±0.6 | 15.3±1.2 | 6.0±0.0 | 6.0±0.0 | 24.0±1.4 | 20.0±1.7 | 15.0±0.0 | 11.3±0.6 | 8.3±0.6 | 21.3±4.2 |
| **S3** | 6.0±0,0 | 9.3±0.6 | 6.0±0.0 | 6.0±0.0 | 24.6±0.9 | 20.0±0.0 | 12.0±0.0 | 11.7±1.2 | 12.7±2.1 | 20.0±5.3 |
| **DMSO** | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 |
| **CIP** | 28.5±0.7 | 29.0±1.4 | 29.0±1.0 | 23.0±1.4 | 39.0±4.0 | 21.0±0.0 | ---- | ---- | ---- | ---- |
| **VAN** | 6.0±0.0 | 22.0±2.8 | 6.0± 0.0 | 6.0± 0.0 | 17.5±2.1 | 17.0±0.0 | ---- | ---- | ---- | ---- |
| **OX** | 6.0±0.0 | ---- | 6.0±0.0 | 6.0±0.0 | 16.0±0.0 | 20.0±0.0 | ---- | ---- | ---- | ---- |
| **PEN** | 6.0±0.0 | ---- | 6.0±0.0 | 6.0±0.0 | 34.0±0.0 | ---- | ---- | ---- | ---- | ---- |
| **AM** | 23.0±0.0 | 6.0±0.0 | 6.0±0.0 | ---- | 32.5±2.1 | 37.0±0.0 | ---- | ---- | ---- | ---- |
| **NY** | ---- | ---- | ---- | ---- | ---- | ---- | 16.0±0.0 | 20.0±0.0 | 22.3±0.6 | 33.7±1.2 |
| **FLU** | ---- | ---- | ---- | ---- | ---- | ---- | 6.0±0.0 | 8.0±0.0 | ---- | 6.0±0.0 |

CIP: Ciprofloxacin, VAN: Vancomycin, OX: Oxacillin, PEN: Penicillin, AM: Amoxicillin, FLU: Fluconazole, NY: Nystatin, were used as positive controls. ----: Not tested. Mean values of zones of growth inhibition in mm including disc diameter of 6 mm±Standard deviation. *E. c.*: *Escherichia coli*. *E. f.*: *Enterococcus faecalis*. *P. a.*: *Pseudomonas aeruginosa*. *K. p.*: *Klebsiella pneumonia*. *S. a.*: *Staphylococcus aureus*. *C.a.*: *Candida albicans*. *A.*: *Aspergillus*.

### *3.3.2. Determination of minimum inhibitory concentration (MIC)*

According to the results obtained previously concerning the sensitivity of microbial strains to essential oil, determined by the disc diffusion method, we selected three microbial strains, namely: *S. aureus, A. flavus* and *A. fumigatus,* which possessing a high sensitivity towards the three oils tested for which we determined the minimum inhibitory concentration by the direct contact method in agar medium. The results are reported in Table 3.

**Table 3.** Minimum inhibitory concentrations (MICs) of aerial parts of *T. articulata* essential oil against sensitive strains.

| **Strains tested** | **Essential oil of *T. articulata* (µL/mL)** | | |
|---|---|---|---|
| | **S1** | **S2** | **S3** |
| *Staphylococcus aureus* ATCC 25923 | 3.0 | 6.0 | 4.0 |
| *Staphylococcus aureus* ATCC 6538 | 3.0 | 6.0 | 4.0 |
| *Aspergillus flavus* | > 2.0 | > 2.0 | > 2.0 |
| *Aspergillus fumigatus* | ≤ 1.0 | ≤ 2.0 | 2.0 |

A global interpretation of the results shows that the values of the CMIs generally agree with those of the diameters of inhibition, the essential oils having induced a large zone of inhibition exhibit the smallest MICs on the corresponding strains.

This is the case with camphor essential oil, which has been shown to be particularly very effective against *A. fumigatus*, with a very low MIC of less than or equal to 1.0 µL/mL. It should be noted that this essential oil also contains an appreciable amount of bornyl acetate, with a percentage of 13.5%. Indeed, the essential oil, which is rich in this compound, also showed good activity, in particular against *A. fumigatus*, with a MIC around 2 µL/mL. By comparison of our results with those of Abi-Ayad et al. in 2013 which determined the antifungal activity of the leaf essential oil of *T. articulata* (containing 52.1% of bornyl acetate), against several filamentous strains, in particular *A. flavus* and *A. niger*, we observed that our essential oils are more active. Indeed, these authors noted a low antifungal activity, with percentages of inhibition of the order of 64.44 and 61.63%, respectively at a concentration of 15 µL/mL. This is probably due to the presence in appreciable quantities of limonene (4.7-7.9% *vs.* 0.6%) in our samples. Indeed, the antimicrobial power of limonene has been demonstrated on several bacterial strains, in particular: *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *E. coli* and *E. faecalis*, with MICs varying between 4 and 125 µg/mL (Erdoğan Orhan et al., 2012; Dai et al., 2013). In addition, we noted a moderate activity of essential oils with camphor and bornyl acetate against the two strains of *S. aureus*, with MICs varying between 3 and 4 µL/mL. Our results agree with those of Ibrahim et al. (2017) who also showed a moderate activity against *S. aureus*, with MIC in order of 3.031 µl/mL. The essential oil was contained camphor and bornyl acetate as major components. Lemos et al. (2015) reported that the essential oil of *Rosmarinus officinalis* which contain between 24.4 and 35.9% camphor, exhibit a significant activity against *S. aureus* with MICs varying between 0.5 and 2.0 µL/mL. They also point out that the essential oil which contain a higher amount of camphor was the most effective. This has already been confirmed by Magiatis et al. in 2002 who worked on several *Achillea* species with camphor as the major compound. Conversely, we found that *α*-pinene essential oil showed low activity against all microbial strains tested. Indeed, *α*- and *β*-pinene have been reported to exhibit very low activity against a panel of microorganisms. MICs values were varied between 7.5 and 20.0 mg/mL against *S. aureus*, *E. coli* and *E. faecalis* (Dorman and Deans, 2000; Sonboli et al., 2006; Jung, 2009; Leite et al., 2007).

Our results agree with those of Bourkhiss et al. in (2007b). Indeed, these authors show a significant inhibitory activity *in vitro*, against four bacteria and two fungi tested. *S. aureus* showed the greatest sensitivity, inhibited at a concentration of 1/5000 (v/v). On the other hand, *Aspergillus niger* is inhibited at a concentration of the order of (1/500 (v/v). The major compounds are bornyl acetate (30.7%), *α*-pinene (23.5%), followed by limonene (23.3%) and camphor (17.3%). Conversely, Abi-Ayad et al. (2011, 2013) suggest higher MICs with respect to *S. aureus* and *Escherichia coli* which varied between 7.5 and 10 µL/mL. Concerning fungal strains, the greatest activity was reported against *Fusarium sp.* species, with percentages of inhibition of the order of 94 and 100% after 6 days, at a concentration of 15 and 20 µL/mL, respectively, followed by *A. flavus* and *A. niger* inhibited at 64.44% and 61.63% respectively at a concentration of 15 µL/mL. The chemical composition of this essential oil was dominated by bornyl acetate (52.1%). Bornyl acetate has been shown to exhibit moderate antimicrobial activity (Duke et al., 2001).

Finally, although the antimicrobial activity of an essential oil is often attributed to its major compound, the synergistic and/or antagonistic effects of the components of the essential oil could also be considered (Chang et al., 2001; Daferera et al., 2003; Burt, 2004) to explain the variation in the degree of sensitivity of microorganisms to the samples tested. However, the essential oils tested, also contain significant amounts of borneol and (*E*)-*β*-caryophyllene. The antimicrobial activity of this last compound has been demonstrated by several studies (Skaltsa et al., 2003; Rather et al., 2012). In addition, the work carried out on essential oil of *Micromeria cristana* subsp. *Phrygia*, rich in borneol, have shown that this compound has great antimicrobial power (Tabanca et al., 2001, Runyoro et al., 2010). Indeed, terpene alcohols such as borneol, are known for their strong antimicrobial power due to their high solubility in water and therefore what gives them a high ability to penetrate through the walls of bacterial and fungal cells (Knobloch et al., 1989).

## 4. Conclusion

Essential oil from the aerial parts of *T. articulata* growing spontaneously in Tlemcen Province exhibits an important chemical variability characterized by the presence of three different compositions. The present study showed that the essential oil of *T. articulata* has significant antimicrobial activity. A camphor-rich essential oil was the most effective against *S. aureus*, *Aspergillus niger* and *A. fumigatus*. The antimicrobial activity of those essential oils is mainly related to its major compound. The synergistic or antagonistic effect of each of its constituents present in low content is also considered.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**Author Contribution Statement**

C. B. conceived the experiments. M. B. performed sampling, extractions and biological activity. C. B. and F. T. performed GC, GC/MS and NMR experiments. M. B., C. B., P. T. and F. T. contributed to the preparation of the manuscript.

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