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ORIGINAL ARTICLE

# Chemical constituents of *Helichrysum italicum* (Roth) G. Don essential oil and their antimicrobial activity against Gram-positive and Gram-negative bacteria, filamentous fungi and *Candida albicans*

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Antifungal activity

**Abstract** The aerial parts of *Helichrysum italicum* (Roth) G. Don were subjected to hydrodistillation to obtain essential oils which had been analyzed by gas chromatography and gas chromatography coupled with mass spectrometry and tested for antimicrobial activity against 12 bacteria, two yeasts and four fungi by agar diffusion method. The essential oil yielded 0.44% (v/w) and 67 compounds accounting for 99.24% of the oil were identified with a high content of oxygenated sesquiterpenes (61.42%). The most oxygenated sesquiterpene compounds were α-Cedrene (13.61%), α-Curcumene (11.41%), Geranyl acetate (10.05%), Limonene (6.07%), Nerol (5.04%), Neryl acetate (4.91%) and α-Pinene (3.78%). The antimicrobial activity of the essential oil was assayed by using the disk diffusion method on *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* ATCC 4698, *Klebsiella pneumonia* ATCC 4352, *Enterococcus cereus* ATCC 2035, *Bacillus cereus* ATCC 10876, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 9372, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 49452, *Proteus mirabilis* ATCC 35659, *Listeria monocytogenes* ATCC 15313 and yeasts *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763 and fungi, *Fusarium solani* var. *coeruleum*,

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*Aspergillus niger*, *Alternaria alternata*, *Ascochyta rabiei*. *H. italicum* inhibited the growth of all the tested microorganisms except three bacteria, *E. coli* ATCC 25922, *K. pneumonia* ATCC 4352 and *L. monocytogenes* ATCC 15313. The most sensitive bacterium was *E. cereus ATCC 2035* with minimum inhibitory and bactericidal concentrations of 0.79 μg $ml^{-1}$. A minimum fungistatic and fungicide concentration of 6.325 μg $ml^{-1}$ and 12.65 μg $ml^{-1}$ respectively was obtained with *C. albicans* ATCC 10231 and *S. cerevisiae* ATCC 9763. However the four fungi were more resistant with fungistatic minimum concentration ranging from 6.325 μg $ml^{-1}$ to 50.6 μg $ml^{-1}$ and a fungicide minimum concentration of 50.6 μg $ml^{-1}$. This antimicrobial activity could be attributed to the essential oil chemical composition. Thus this study represents a first step in the study of the chemical composition of *H. italicum* (Roth) G. Don collected from north Algeria and its antimicrobial properties.



## 1. Introduction

The genus *Helichrysum* belongs to the family of *Asteraceae* and consists of few hundred species, widespread throughout the world. *Helichrysum* genus naturally occurs in the Mediterranean areas, including Algeria. The essential oil is present in all the green parts of the plant, and its extracts are used in popular medicine in the Mediterranean region (Mastelic et al., 2005).

Dried flowers of *Helichrysum italicum* had a great reputation in traditional medicine as choleretic, diuretic and expectorant (Chinou et al., 1996). The plant has been found to possess anti-inflammatory (Sala et al., 2002), anti-oxidant (Rosa et al., 2007), antimicrobial (Nostro et al., 2001), antiviral (Nostro et al., 2003) and anti-HIV (Appendino et al., 2007) properties. The chemical composition of *H. italicum* essential oil varies in function of the geographic origin and the vegetation cycle (Paolini et al., 2006). Furthermore, there is no study on the essential oil of Algerian *H. italicum*.

The aim of this study was to investigate the chemical composition, and the antimicrobial activity of the aerial parts essential oils of *H. italicum* collected from the North of Algeria. These compounds represent novel leads and future studies may allow the development of a medicine and a pharmacologically acceptable antimicrobial agent.

## 2. Materials and methods

### 2.1. Plant material

Aerial parts of *H. italicum* (Fig. 1) were collected in Jun 2013 during the flowering period from Béjaia (North of Algeria). After dryness, at laboratory temperature and obscurity, the plant material was cut to small pieces with a universal knife.

### 2.2. Essential oil extraction

100 g of the air-dried aerial parts of the plant was subjected to hydrodistillation for 3 h with 500 ml distilled water, using a Clevenger-type apparatus in the laboratory of Natural Resources Valorization belonging to University Ferhat Abbas Setif 1 in Algeria. The obtained oil was collected and stored in screw capped glass vials in a refrigerator at 4–5 °C prior to analysis.

### 2.3. Gas chromatography-mass spectrometry (GC/MS) analysis

Gas chromatography (GC) coupled with mass spectrometry was carried out by using a Hewlett-Packard GC-MS system (GC: 6890 series II; MS-HP 6972). The fused-silica HP5 capillary column (30 m long, 0.25 mm internal diameter, film thickness of 0.25 μm) was set at 250 °C and directly coupled to the MS.

The temperature is set from 100 to 325 °C at a rate of 5 °C/min. The carrier gas was helium, with a flow rate of 20 ml/min, split ratio of 1:50, injector port: 280 °C, volume injected: 1 μl of (1/20v/v) solution (diluted in methanol). The device is controlled by a computer system of ûHP ChemStationý type, managing the operation of the appliance and to follow the evolution of the chromatographic analyses and a library of NIST 2008 mass spectrum.

### 2.4. Compound determination

The determination of the components was based on comparison of their mass spectra with those of NIST 08 mass spectral library and on the basis of calculation of retention indices. The percentage composition of the samples was computed from the GC peak areas. In some cases, when identical spectra were not found, only the structural type of the corresponding component was proposed on the basis of its mass spectral

**Figure 1** Photography of aerial parts of *Helichrysum italicum* (Roth) G. Don collected from the North of Algeria.

fragmentation. If available, the reference compounds have been co-chromatography to confirm the GC retention times

### 2.5. Antimicrobial activity

#### 2.5.1. Microbial strains

The essential oil was tested against 12 bacteria, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* ATCC 4698, *Klebsiella pneumonia* ATCC 4352, *Enterococcus cereus* ATCC 2035, *Bacillus cereus* ATCC 10876, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 9372, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 49452, *Proteus mirabilis* ATCC 35659 and *Listeria monocytogenes* ATCC 15313, two yeasts, yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763 from the American Type Culture and four fungi, *Fusarium solani* var. *coeruleum, Aspergillus niger, Alternaria alternata,* and *Ascochyta rabiei* from the laboratory of Applied Microbiology, Faculty of Nature and Life Sciences, University Ferhat Abbas, Sétif 1, were used.

#### 2.5.2. Antimicrobial screening

A preliminary antimicrobial activity of the essential oil was screened following two agar diffusion methods (Bauer et al., 1966; Hayes and Markovic, 2002) by using sterile 6-mm filter paper disks (Whatman N°1). Bacterial, yeast and fungal spores suspensions were standardized at $10^8$ CFU/mL (0.5 of Mac Farland), $10^7$ CFU/mL and $10^6$ spore/mL for fungi, respectively. Suspensions were spread on Muller-Hinton Agar (MHA) and Potato Dextrose Agar (PDA).

Disks were impregnated with 15 µL of the crude essential oil solution or diluted essential oil (1/2, 1/5 and 1/10 v/v) in 10% Dimethyl sulfoxid (DMSO, Sigma-Aldrich), and deposited at equal distances on the surface of the inoculated agar MHA and PDA. 10% DMSO was used as a negative control, Gentamycin (GEN 10 µg/ml) and 5Fluorocytosin (5FC10 µg/ml) were used as positive controls.

Plates were left in at 4 °C for 12hours before incubation to ensure a good diffusion of the oil in the agar. The diameter of inhibition was measured after 24 h at 37 °C for bacteria, 48 h at 37 °C for the yeast and 7 days at 28 °C for the fungi. The essay was carried out in triplicate.

#### 2.5.3. Determination of minimal inhibitory concentration (MIC) and minimum bactericidal activity (MBC)

The essential was dissolved in DMSO 10% at a ratio of 1:20 V/V. One ml (1 ml) of each final concentration (101.2, 50.6, 25.3, 12.65, 6.325, 3.162, 1.581, and 0.79 µg/ml) was aseptically added to nineteen milliliters (19 ml) of MHA containing 0.5% (v/v) of tween 80. The obtained medium (agar solution and DMSO solution) was vortexed for 1 min and immediately poured into sterile Petri dishes and left to set for 30 min.

Plates were then inoculated by spotting 1 mL of a suspension of the desired microorganism from the prepared inoculum. They were incubated at 37 °C for 24–48 h. Control consists of $H_2O$–DMSO. Each test was performed in triplicate. The MIC represents the lowest concentration of essential oil inhibiting all visible growth after 24 h of incubation at 37 °C. Furthermore, minimum bacteriostatic concentration (MBC) represents the lowest concentration of essential oil inhibiting all visible growth after 5 days of incubation at 37 °C. The bactericidal and bacteriostatic effects of essential oil were determined by calculating the ratio of MBC/MIC. The oil effect is bacteriostatic with a ratio greater than 4 and bactericidal when the ratio is less than or equal to 4 (Bajpai and Kang, 2010).

#### 2.5.4. Determination of minimum fungistatic concentrations ($MFC_s$) and minimum fungicide concentrations ($MFC_C$)

$MFC_S$ and $MFC_C$ of the essential oil were determined by the broth dilution method reported by Bajpai and Kang (2010) derived from the original method of Murray et al. (1995). The essential oil was first diluted in DMSO 10% to obtain a concentration of 2024 µg/ml. From this solution, 1 ml was introduced into a tube containing 9 ml of potato dextrose broth (PDB), containing 0.5% Tween 80, to have a concentration of 202.4 µg/ml. A twofold dilution series were made from this concentration to obtain 101.2, 50.6, 25.3, 12.65 and 6.325 µg/ml in test tubes. The medium with different concentrations was inoculated by 10 µL of the four fungal strains spores suspension ($10^6$ spores/ml). The mixture was homogenized and incubated at 30 °C for 2–7 days. The positive control was the PDB medium without essential oil and the negative control was the DMSO (10%) with tween 80 (0.5%).

After 2–7 days of incubation, the first tubes with total inhibition, compared to the control are reseeded in the boxes containing 20 ml of the PDA culture medium and incubated from one to four days at 30 °C. The resumption of mycelia growth indicated that the oil is fungistatic ($MFC_S$), and no growth indicated that the effect is fungicide ($MFC_c$). The oil effect is fungicide with a ratio less than 4 and fungistatic when the ratio is higher or equal to 4 (Derwich et al., 2010).

### 2.6. Statistical analysis

All experiments were done in triplicate and results were reported as mean ± SD. Data were analyzed by one-way ANOVA. Statistically significant effects were determined using T test at $p < 0.05$.

## 3. Results and discussion

### 3.1. Chemical composition

The hydro distillation of *H. italicum* aerial parts gave pale yellow oil with a yield of 0.44% (v/w). The analysis revealed 67 constituents representing 99.24% of the total oil (Table 1). This oil was characterized by the dominance of Oxygen–containing Sesquiterpenes (76.7%) and Oxygen-containing monoterpene (61.42%; Fig. 2). The major constituents of the oil were α-Cedrene (13.61%), α-Curcumene (11.41%), Geranyl acetate (10.05%), Limonene (6.07%), Nerol (5.04%), Neryl acetate (4.91%) and α-Pinene (3.78%).

According to our obtained results, the chemical composition of the essential oil of *H. italicum* from the North of Algeria is different from those of other countries. This difference is characterized by the presence of α-Cedrene, α-Curcumene and Geranyl acetate as the majoritarian constituents and by the small percentage of Geraniol (0.02%). *H. italicum* of the Adriatic coast (sub-species not specified) produces essential oil with

**Table 1** Percentage chemical composition and retention indices of the essential oil extracted from *Helichrsyum italicum* growing wild in the North of Algeria.

| Pic | RT | Compound | RI | % |
|---|---|---|---|---|
| **1** | 1.908 | Ethylether | 529 | 0.04 |
| **2** | 5.358 | Methyl butanoate | 721 | 0.22 |
| **4** | 7.203 | 2-Methyl-2-heptene | 981 | 0.29 |
| **5** | 8,672 | 2-methyl-Hexanoic acid | 1027 | 0.53 |
| **6** | 11.083 | δ-3-Carene | 1008 | **1.08** |
| **7** | 12.219 | α-Pinene | 932 | **3.78** |
| **8** | 12.662 | α-Fenchene | 945 | **1.43** |
| **9** | 13.751 | β-Pinene | 974 | **1.52** |
| **10** | 14.38 | Cyclopentanol | 780 | 0.28 |
| **11** | 14.794 | Isopropyl-2-methyl butyrate | 880 | 0.37 |
| **12** | 15.204 | Terpinolene | 1086 | 0.40 |
| **13** | 15.364 | *p*-Methylanisol | 1015 | 0.15 |
| **14** | 15.504 | *p*-Cymene | 1020 | **1.02** |
| **15** | 15.506 | Limonene | 1024 | **6.07** |
| **16** | 16.331 | (E)-β-ocimène | 1044 | 0.37 |
| **17** | 16.466 | 2-Butenoic acid, 3-methyl | 881 | **2.24** |
| **18** | 16.665 | γ-Terpinen | 1054 | **1.26** |
| **19** | 17.767 | 2-Nonanone | 1087 | 0.46 |
| **20** | 17.952 | 3-Hexanone | 945 | 0.34 |
| **21** | 18.096 | Linalool | 1095 | **2.98** |
| **22** | 18.172 | Isovaleric acid | 827 | 0.23 |
| **23** | 18.483 | (+)−Fenchol | 1114 | 0.12 |
| **24** | 19.067 | Adamantane | 1118 | 0.11 |
| **25** | 19.684 | Borneol | 1165 | 0.25 |
| **26** | 19.912 | α-Terpineol | 1014 | **2.47** |
| **27** | 20.11 | 3-Pentanone | 788 | **1.26** |
| **28** | 20.279 | Myrtenol | 1194 | 0.18 |
| **29** | 20.667 | *cis*-Pinocarveol | 1182 | 0,15 |
| **30** | 20.841 | Nerol | 1227 | **5.04** |
| **31** | 20.993 | Neral | 1235 | 0.12 |
| **32** | 21.064 | Camphene | 946 | 0.09 |
| **33** | 21.419 | Geraniol | 1249 | 0.06 |
| **34** | 21.541 | Linalool | 1095 | 0.15 |
| **35** | 21.651 | Bornyl acetate | 1284 | 0.05 |
| **36** | 21.706 | 2-Undecanone | 1293 | 0.3 |
| **37** | 21.761 | *n*-Tridecane | 1300 | 0.07 |
| **38** | 22.436 | α-Terpinyl acetate | 1346 | 0.09 |
| **39** | 22.589 | Geranyl acetate | 1379 | **10.05** |
| **40** | 22.812 | α-Cedrene | 1410 | **13.61** |
| **41** | 22.863 | 1.3.8-ρ-Menthatriene | 1108 | 0.58 |
| **42** | 22.943 | *n*-Tetradecane | 1400 | 0.18 |
| **43** | 23.201 | α-Bergamotene | 1411 | **1.06** |
| **44** | 23.311 | Caryophyllene | 1417 | 0.48 |
| **45** | 23.458 | α-curcumene | 1479 | **11.41** |
| **46** | 23.547 | Neryl acetate | 1359 | **4.91** |
| **47** | 23.678 | β-Himachalene | 1500 | **1.87** |
| **48** | 24,239 | Acetic acid | 1393 | **1.38** |
| **49** | 24,539 | (Z)-Nerolidol | 1531 | **1.65** |
| **50** | 24,725 | β-curcumene | 1514 | **2.25** |
| **51** | 24,865 | *cis*-4-Caranone | 1200 | 0.23 |
| **52** | 24,945 | Guaiol | 1600 | **2.36** |
| **53** | 25,084 | Anisole | 913 | **2.99** |
| **54** | 25.173 | *δ*-Selinene | 1492 | 0.33 |
| **55** | 25.265 | *ρ*-Cresol | 1071 | **2.42** |
| **56** | 25.43 | α-Eudesmol | 1652 | **2.88** |
| **57** | 25.51 | Bulnesol | 1670 | 0.59 |
| **58** | 25.624 | (Z)-α-*trans*-Bergamotol | 1690 | 0.35 |
| **59** | 25.764 | *δ*-Cadinene | 1522 | 0.14 |
| **60** | 25.869 | *cis*-Carveol | 1226 | 0.11 |
| **61** | 25.916 | Dehydro-1.8-Cineole | 988 | 0.39 |
| **62** | 26.074 | o-Cresol | 1050 | 0.12 |
| **63** | 26.456 | Eugenol | 1356 | 0.62 |

(*continued on next page*)

**Table 1** (*continued*)

| Pic | RT | Compound | RI | % |
|---|---|---|---|---|
| **64** | 26.566 | Geranyl butanoate | 1562 | 0.2 |
| **65** | 26.663 | 2-Pentadecanone | 1697 | 0.2 |
| **66** | 30.573 | 2,3-dimethyl-Benzofuran | 1219 | 0.21 |
| | | **Total identified (%)** | | **99.24** |
| | | Monoterpene hydrocarbons | | 10.83 |
| | | Oxygen-containing monoterpene | | 18.95 |
| | | Oxygen–containing Sesquiterpenes | | 61.42 |
| | | Acids | | 4.48 |
| | | Others | | 2.17 |

Bold means the highest percentage of the compounds of the essential oil.

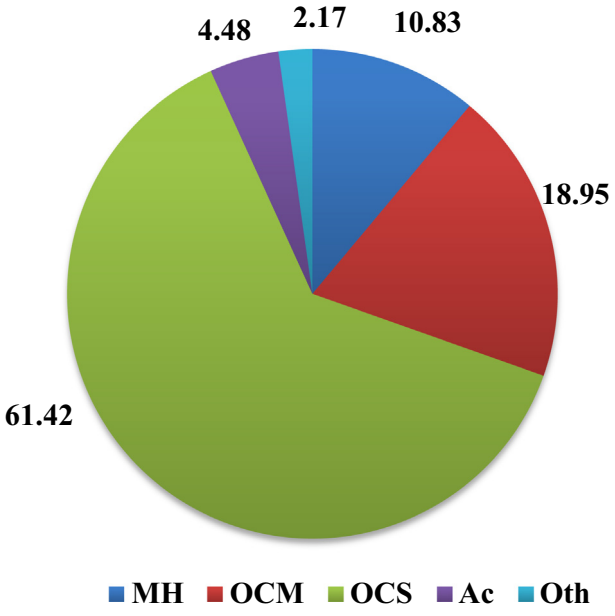


**Figure 2** Percentage of the different chemical groups of components present in *H. italicum* essential oil. **MH:** Monoterpene hydrocarbons; **OCM:** Oxygen-containing monoterpene; **OCS:** Oxygene–containing Sesquiterpenes; **Ac:** Acids; **Other:** other compound.

α-pinene, α- and γ-curcumene as major components (Conti et al., 2010). Essential oil (subsp *italicum*) from Tuscany contained mainly α-pinene and neryl acetate (Bianchini et al., 2001) while an oil sample from Southern Italy was dominated by *iso*-italicene epoxide (Mancini et al., 2011). Another Italian oil contained mainly γ-curcumene, β-selinene and α-selinene (Morone-Fortunato et al., 2010). Neryl acetate was also reported as one of the main components of the *H. italicum* ssp. *italicum* oil from North America (Mastelic et al., 2005) and in the oil of *H. italicum* from South Croatia (Tucker et al., 1997).

Nerol and its esters have also been found as the main components of the essential oil from flowers of some genotypes of *H. italicum* ssp. *Microphyllum* (Satta et al., 1999). This difference can be explained by various factors, e.g. the harvest time and local climatic geographic seasonal factors (Morone-Fortunato et al., 2010). Correlations between the essential oil composition and various parameters were shown: soil texture and acidity, inorganic composition of plant and soil, vegetative stage of development (Ens et al., 2008).

### 3.2. Antimicrobial activity

The results indicate that the essential oil of *H. italicum* had no effect on all the tested microorganisms, bacteria and fungi at 1/10 dilution (Tables 2 and 4).

The antibacterial assay indicated that the most resistant strains to all dilution were *E. coli* ATCC 25922, *K. pneumonia* ATCC 4352 and *L. monocytogenes* ATCC 15313. In contrast the most sensitive one were *M. luteus* ATCC 1533 with 30 ± 0.2 mm inhibition and *S. aureus* ATCC 6538 with 27 ± 0.1 inhibition zone (Table 2).

The MIC and MBC obtained by the agar dilution method were from 50.6 μg $ml^{-1}$ to 0.79 μg $ml^{-1}$. These values indicated that essential oil had a bactericidal effect (Table 3).

The antifungal activity indicted that essential oil inhibited the growth of all tested fungi at all dilutions, except *A. alternata* which was resistant to the dilution of 1/5 (Table 4).

The results showed that the most sensitive fungus was the *C. albicans* ATCC 10231 followed by *S. cerevisiae* ATCC 9763 and the most resistant one was *A. alternata* at all dilutions. An exception was recorded for *F. solani* var. *coeruleum*, and for this fungus the same zone of inhibition 12 ± 0.1 mm was recorded at dilutions 1/2 and 1/5 (Table 4).

The $MFC_c$ were from 12.65 μg $ml^{-1}$ to 50.6 μg $ml^{-1}$ and the $MFC_s$ were from 6.325 μg $ml^{-1}$ to 50.6 μg $ml^{-1}$. These values indicate that essential oil had a good antifungal activity (Table 5).

On the basis of the obtained results, the *H. italicum* essential oil can be considered to have bactericide and fungicide activity. The obtained MBC/MIC which were between 1 and 4 ($\leqslant 4$), are similar to those obtained by Canillac and Mourey (2001). Similarly the obtained $MFC_c$ and $MFC_s$ which were between 1 and 2 (less than 4) are similar to those of Derwich et al. (2010).

The antimicrobial activity of *H. italicum* can be attributed to its chemical composition. In fact oxygen-containing compounds are probably responsible for the antimicrobial activity of the essential oil of *H. italicum* such as neryl acetate, geranyl acetate, geraniol, and nerol (Bajpai et al., 2013) which are the main components of the Algerian *H. italicum* oil. For example the susceptibility of *C. albicans* was probably due to the pres-

**Table 2** Antibacterial activity of *H. italicum* essential oil measured as diameter of inhibition.

| Bacterial strains | *H. italicum* essential oil diluted in DMSO | | | | Control | |
|---|---|---|---|---|---|---|
| | 1/1 | 1/2 | 1/5 | 1/10 | GEN | DMSO |
| *Escherichia coli* ATCC 25922 | – | – | – | – | 33 | – |
| *Staphylococcus aureus* ATCC 6538 | 27 ± 0.1 | 23 ± 0.2 | 16 ± 0.2 | – | 25 | – |
| *Micrococcus luteus* ATCC 4698 | 30 ± 0.2 | 21 ± 0.6 | 17 ± 0.4 | – | 24 | – |
| *Klebsiella pneumonia* ATCC 4352 | – | – | – | – | 21 | – |
| *Enterococcus cereus* ATCC 2035 | 17 ± 0.4 | 15 ± 0.2 | – | – | 22 | – |
| *Bacillus cereus* ATCC 10876 | 21 ± 0.2 | 19 ± 0.6 | 13 ± 0.1 | – | 30 | – |
| *Staphylococcus epidermidis* ATCC 12228 | 23 ± 0.5 | 18 ± 0.3 | 14 ± 0.2 | – | 33 | – |
| *Bacillus subtilis* ATCC 9372 | 19 ± 0.2 | 16 ± 0.1 | – | – | 33 | – |
| *Pseudomonas aeruginosa* ATCC 27853 | 18 ± 0.4 | 12 ± 0.2 | 09 ± 0.1 | – | 26 | – |
| *Enterococcus faecalis* ATCC 49452 | 19 ± 0.5 | 17 ± 0.1 | 14 ± 0.3 | – | 30 | – |
| *Proteus mirabilis* ATCC 35659 | 18 ± 0.2 | 12 ± 0.4 | 07 ± 0.1 | – | 25 | – |
| *Listeria monocytogenes* ATCC 15313 | – | – | – | – | 23 | – |

– Indicates no growth inhibition. Gent. (Gentamicin) positive control; DMSO (Dimethyl sulfoxid) negative control.

**Table 3** Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of *H. italicum* essential oil.

| Bacterial strain | MIC ($\mu g\ ml^{-1}$) | MBC ($\mu g\ ml^{-1}$) | MBC/MIC |
|---|---|---|---|
| *Staphylococcus aureus* ATCC 6538 | 50.6 | 50.6 | 1 |
| *Micrococcus luteus ATCC 4698* | 6.325 | 12.65 | 2 |
| *Enterococcus cereus ATCC 2035* | 0.79 | 0.79 | 1 |
| *Bacillus cereus ATCC 10876* | 3.162 | 12.65 | 4 |
| *Staphylococcus epidermidis ATCC 12228* | 25.3 | 25.3 | 1 |
| *Bacillus subtilis ATCC 9372* | 12.65 | 50.6 | 4 |
| *Pseudomonas aeruginosa ATCC 27853* | 12.65 | 12.65 | 1 |
| *Enterococcus faecalis ATCC 49452* | 50.6 | 101.2 | 2 |
| *Proteus mirabilis ATCC 35659* | 1.581 | 3.162 | 2 |

ence of geranyl acetate (10.05%), in our essential oil. In the same way, monoterpenes or sesquiterpene hydrocarbons represented 10.83% of the identified compounds, and their oxygenated derivatives exhibited a potential antimicrobial activity (Siti et al., 2010).

However, the possible synergism of the minor components on the antimicrobial activity should be considered. α-pinene, β-pinene and Limonene have a strong antibacterial activity (Filipiwicz et al., 2003). It has been demonstrated that α-pinene and β-pinene are able to destroy cellular integrity and they inhibit respiration and ion transport processes (Tekaya-Karoui et al., 2011; Tzakou et al., 1998). Glisic et al. (2007) have established that the fractions containing the high concentration of α-pinene and sabinene effectively inhibited the growth of microorganisms, especially against fungi. In addition, the antimicrobial activity was dependent on β-pinene content too.

The obtained are in good agreement with the findings of Cantore et al. (2004) who reported that Gram positive bacteria are more sensitive to plant essential oils than Gram negative bacteria and of two main studies report the antibacterial properties of *H. italicum* essential oil and its related constituents. Rossi et al. (2007) demonstrated that the essential oil, obtained from endemic plants of Corsica, was more effective against the Gram positive bacterium *S. aureus* than against the Gram negative strains *E. coli*, *Enterobacter aerogenes*, and *P. aeruginosa*. It is commonly known that Gram negative bacteria are less susceptible to essential oil than Gram positive bacteria, and this is directly connected to the bacterial cell wall structure. In Gram negative bacteria, the cell wall is a complex envelope constituted by the cytoplasmic membrane, the periplasm and the outer membrane.

**Table 4** Antifungal activity of *H. italicum* essential oil measured as diameter of inhibition (mm).

| Fungi strains | *H. italicum* essential oil diluted control in DMSO (v/v) | | | | | |
|---|---|---|---|---|---|---|
| | 1/1 | 1/2 | 1/5 | 1/10 | 5-FC | DMSO |
| *Candida albicans* ATCC 10231 | 29 ± 0.2 | 22 ± 0.4 | 13 ± 0.1 | – | 24 | – |
| *Saccharomyces cerevisiae* ATCC 9763 | 27 ± 0.7 | 21 ± 0.2 | 11 ± 0.3 | – | 30 | – |
| *Fusarium solani* var. *coeruleum* | 13 ± 0.4 | 12 ± 0.1 | 12 ± 0.1 | – | 27 | – |
| *Aspergillus niger* | 14 ± 0.2 | 11 ± 0.5 | 08 ± 0.2 | – | 65 | – |
| *Alternaria alternata* | 11 ± 0.4 | 07 ± 0.1 | – | – | 34 | – |
| *Ascochyta rabiei* | 14 ± 0.8 | 12 ± 0.4 | 10 ± 0.1 | – | 28 | – |

– Indicates no growth inhibition. 5-FC (5-Fluorocytosin) positive control; DMSO (Dimethyl sulfoxid) negative control.

**Table 5** Fungistatic minimum concentrations $MFC_S$ and fungicide $MFC_C$ of *H. italicum* essential oil.

| Fungal strain | $MFC_S$ ($\mu g\ ml^{-1}$) | $MFC_C$ ($\mu g\ ml^{-1}$) | $MFC_C/MFC_S$ |
|---|---|---|---|
| *Candida albicans* ATCC 10231 | 6.325 | 12.65 | 2 |
| *Saccharomyces cerevisiae* ATCC 9763 | 6.325 | 12.65 | 2 |
| *Fusarium solani* var. *coeruleum* | 50.6 | 50.6 | 1 |
| *Aspergillus niger* | 25.3 | 50.6 | 2 |
| *Alternaria alternata* | 25.3 | 50.6 | 2 |
| *Ascochyta rabiei* | 50.6 | 50.6 | 1 |

Microscopic studies have shown that there may be distortion hyphae with the frequent occurrence of fragmentations and disorganization of reproductive organs, the separation of the cell membrane of the cell wall and the destruction of cellular organelles (Kocić-Tanackov et al., 2012).

## 4. Conclusion

The results presented in this study confirm that *H. italicum* (Roth) G. Don essential oil from Algeria exhibits interesting antimicrobial activity that seems to be due to the large diversity of its chemical contents. Therefore, essential oil of *H. italicum* (Roth) G. Don might be used as a therapeutic agent and these compounds can be applied to medicinal and pharmaceutical purposes. However, future researches are necessary to understand the involved mechanisms.

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