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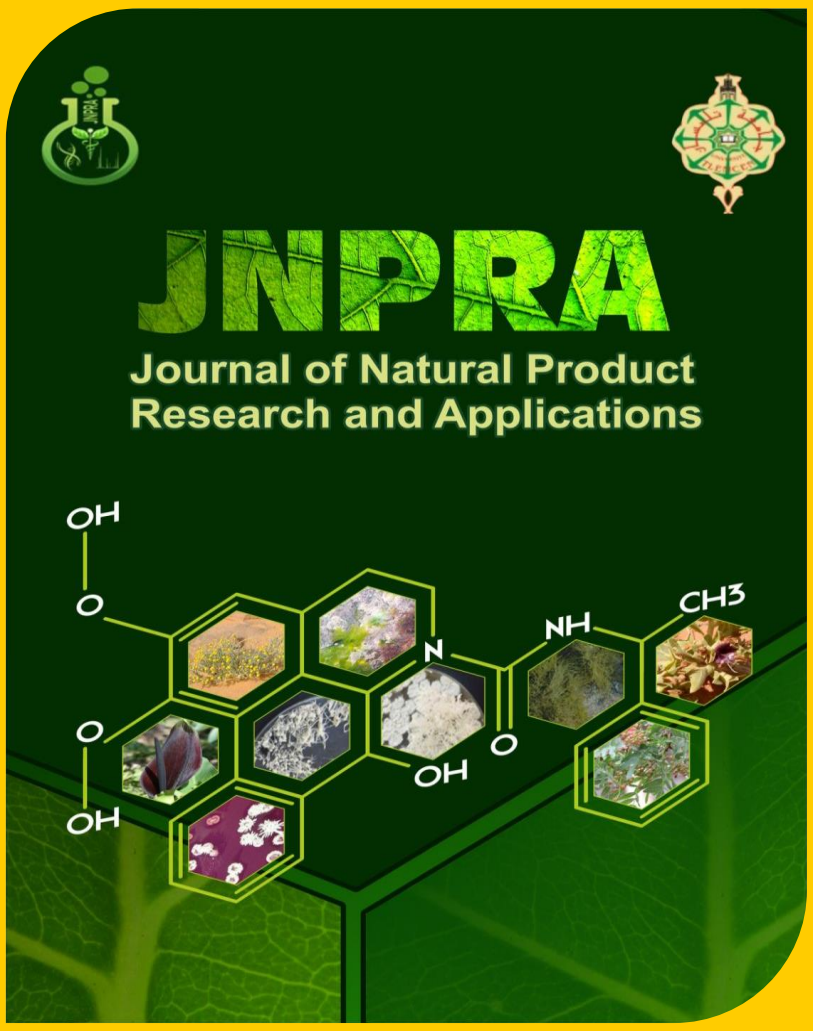

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Research Article

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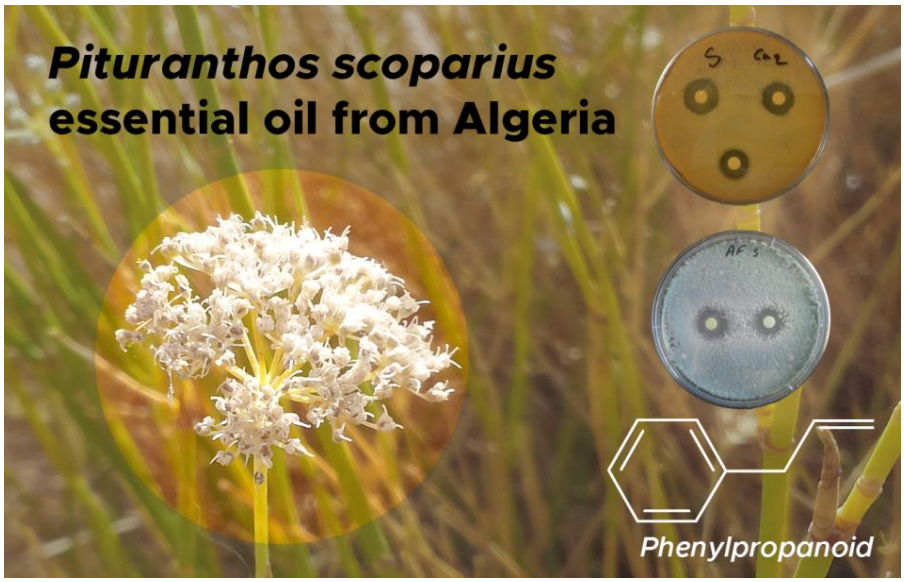
Highlights

- The essential oil from aerial parts of *Pituranthos scoparius* was analyzed by GC, GC/MS and ^{13}C NMR.
- The essential oil evaluated for its antimicrobial and antioxidant activities.
- The tested oils possessed inhibitory action against *A. fumigatus*, *S. aureus* and *C. albicans*.
- All samples exhibited weak scavenging activity against DPPH.

Graphical Abstract



Abstract
The essential oil from aerial parts of *Pituranthos scoparius* obtained by hydrodistillation was analyzed by GC, GC/MS and ^{13}C NMR and evaluated for antimicrobial and antioxidant activities. The yields were showed a very high variability ranging from 0.16 to 0.99%. Sixty two components were identified and the results indicated that the essential oil was rich in hydrocarbons monoterpenes and phenylpropanoid compounds. 6-Methoxyelemicine (0.1-47.0%), sabinene (1.4-35.5%), limonene (0.6-24.0%), myristicine (0.2-18.9%), α-pinene (3.1-14.7%) and dill apiole (0.0-10.6%) were the major compounds. The essential oils were tested against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, *Candida albicans*, *Fusarium oxysporum*, *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 2 to 3 µL/mL against *Aspergillus fumigatus*, *Staphylococcus aureus* and *Candida albicans*. The antimicrobial activity of essential oil from *P. Scoparius* well be due probably to the presence of synergy between myristicine and terpinen-4-ol. The antioxidant activity was evaluated through DPPH assay. All samples exhibited weak scavenging activity against DPPH, compared to standard antioxidant tested. Essential oil with IC_{50} of the order of 23.65±0.77 and 25.95±2.13 mg/mL could be attributed probably to the presence of limonene and germacrene D in larger amounts.

Keywords: *Pituranthos scoparius* (Coss. &Durieu) Benth. & Hook; essential oil composition; antimicrobial activity; antioxidant activity.

1. Introduction

Antimicrobial resistance has a global public health problem. Indeed, many microbial infections such as nosocomial diseases have became very resistant to antibiotics. Therefore, ithas an urgent need to find new targets for new antimicrobials. Essential oils have proven their invaluable health value over the past decades. Thus, they are currently considered as very promising alternatives to different antibiotics for the treatment of various infectious diseases. Likewise, the essential oils are the subject of much research and investigation for their antioxidant properties, again to find natural substitutes for synthetic antioxidants, some of which present dangers to our health.

P. scoparius, commonly known as 'Guezzah' (Quézel and Santa, 1963), has been used in folk medicine for the treatment of spasms, pains, diabetes, hepatitis, digestive disorders, urinary infections and for postpartum care (Hammiche et al., 2006; Boudjelal et al., 2013).

P. scoparius (Coss. & Durieu) Benth. & Hook (Syn. *Deverra scoparia* Coss. & Durieu) (Dobignard et al., 2011; Ozenda, 1991), is an endemic plant of North Africa (Mauritania, Morocco, Algeria and Tunisia), growing spontaneously in rocky pastures and widespread in high plateau and throughout the Sahara (Quézel and Santa, 1963; Dobignard et al., 2011; El Oualidi et al., 2012).

The antimicrobial activity of *P. scoparius* essential oil has been the subject of a few studies. Indeed, Boutaghane et al. in 2004 reported that the antimicrobial activity for essential oil samples obtained from the stems and seeds of *P. scoparius* collected in Ghardaïa region varied between 0.156 and 40 mg/mL and between 1 and 256 mg/mL, respectively. More recently, Ksouri et al. (2017), carried out a study on the antimicrobial activity of the essential oil of the aerial parts of *P. scoparius* against six bacterial and four fungal strains. They were concluded that fungi, namely: *Candida albicans*, *Mucor* sp., *Aspergillus flavus* and *Penicillium expansum* were found to be the most sensitive, with inhibition zone diameters of the order of 15.8 to 20 mm and very low MIC varying between 0.02 at 1.25 mg/mL. In

contrast, *Staphylococcus aureus* is the only bacterial strain that has been shown to be susceptible with an MIC of around 1 mg/mL.

These same authors Ksouri et al. (2017) also studied the antioxidant activity of the essential oil of the aerial parts of *P. scoparius* by the DPPH free radical scavenging test. They show moderate activity with an IC_{50} of 11.21±0.26 mg/mL compared to the standards tested, namely: ascorbic acid (IC_{50} = 4 µg/mL), α-tocopherol (IC_{50} = 9.55 µg/mL) and BHT (IC_{50} = 72.16 µg/mL) which are highly active. They were evaluated the antioxidant power of these essential oils by a second test, that of the bleaching of *β*-carotene, which were showed that the essential oil is not able to effectively inhibit the oxidation of linoleic acid with a value of the order of 38% obtained at a concentration of 2 mg/mL, very low to the positive control BHT (93.56±0.37%) at the same concentration.

It appears from literature data that the essential oilfrom the aerial parts of *P. scoparius* exhibited a large chemical variability. All the investigated oil samples were characterized by the occurrence of monoterpene hydrocarbons (α-pinene, sabinene and limonene) associated with phenylpropanoids (myristicine and dill apiole). The contents of those compounds varied drastically from sample to sample (Vernin et al., 1999; Vérité et al., 2004; Gourine et al., 2011; Smaili et al., 2011; Lograda et al., 2013; Chikhoune et al., 2016; Ksouri et al., 2017). From our investigations on the chemical composition of *P. scoparius*, 93 oil samples were isolated by hydrodistillation from the aerial parts from individual plants collected in five locations in Algeria. Various compounds dominated the essential oil compositions and their contents varied drastically from sample to sample: 6-methoxyelemicine (0.0-59.6%), sabinene (0.8-55.6%), limonene (0.3-44.0%), α-pinene (0.7-31.0%), myristicine (0.0-32.4%), elemicine (0.0-29.1%) and dill apiole (0.0-31.4%). Other components were present at appreciable amounts: α-phellandrene (up to 17.8%), β-pinene (up to 9.6%), δ-3-carene (up to 12.4%), *p*-cymene (up to 9.6%), terpinen-4-ol (up to 10.9%), (Z)-ligustilide (up to 9.7%), germacrene D (up to 9.5%) and (Z)-β-ocimene (up to 7.1%). The 93 samples were submitted to statistical analyses. Combination of hierarchical clustering dendrogramand principal components analysis (PCA), suggested the existence of three principal groups, which were distinguished on the basis of 6-methoxyelemicine, sabinene, limonene, α-pinene, myristicine, elemicine and dill apiole contents (Malti et al., 2018).

In this context, we tested the antimicrobial and antioxidant activities of eight samples of essential oil of the aerial parts of *Pituranthos scoparius*, which were presented different chemical compositions.

2. Materials and Methods

2.1. Plant Material and Oil Distillation

Total aerial parts (stems and flowers or fruits/seeds) from 93 individual plants of *Pituranthos scoparius* were collected in five locations of Algeria: Ghardaïa, Biskra, Batna (Bouilef,Djerma) and Béchar (Malti et al., 2018).

Identification of the plants was performed by Dr. F. Hassani (University of Tlemcen, Algeria). A voucher specimen has been deposited with the Laboratory of Natural Products (Department of Biology, University of Tlemcen) under the accession N° A. 1941. Dry plants (140 - 300 g) were submitted to hydrodistillation for 2 h. Yields have been calculated from dry material (w/w) (Malti et al., 2018).

2.2. Analytical GC

GC analyzes were performed on a Perkin-Elmer Clarus 500 gas chromatograph (FID) equipped two fused silica capillary columns (50 m x 0.22 mm, 0.25 µm film thickness), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min,

injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: hydrogen (0.8 mL/min); split: 1/60. The relative proportions of the oil constituents were expressed as percentages obtained by peak area normalization, without using correcting factors. Retention indices (RI) were determined relative to the retention times of a series of *n*-alkanes with linear interpolation ("Target Compounds" software from Perkin-Elmer).

2.3. GC/MS Analysis

The essential oils were analyzed with a Perkin-Elmer TurboMass detector (quadrupole), directly coupled to a Perkin-Elmer Autosystem XL, equipped with a fused-silica capillary column (50 m x 0.22 mm i.d., film thickness 0.25 µm), BP-1 (dimethylpolysiloxane). Carrier gas, helium at 0.8 mL/min; split, 1/60; injection volume, 0.5 µL; injector temperature, 250 °C; oven temperature programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal (20 min); Ion source temperature, 250 °C; energy ionization, 70 eV; electron ionization mass spectra were acquired over the mass range 40-400 Da.

2.4. ^{13}C-NMR Analysis

^{13}C NMR analyzes were performed on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.623 MHz for ^{13}C, equipped with a 5 mm probe, in deuterated chloroform ($CDCl_3$), with all shifts referred to internal tetramethylsilane (TMS). ^{13}CNMR spectra were recorded with the following parameters: pulse width (PW), 4 µs (flip angle 45°); acquisition time, 2.73 s for 128 K data table with a spectral width (SW) of 220 000 Hz (220 ppm); CPD mode decoupling; digital resolution 0.183 Hz/pt. The number of accumulated scans ranged 2 000-3 000 for each sample (around 40 mg of oil in 0.5 mL of $CDCl_3$). Exponential line broadening multiplication (1.0 Hz) of the free induction decay was applied before Fourier transformation.

2.5. Identification of Components

Identification of the components was based: i) on comparison of their GC retention indices (RIs) on polar and apolar columns, with those of authentic compounds and with reference data (König et al., 2001) on computer matching against commercial mass spectral libraries (König et al., 2001; NIST, 1999; Adams, 2007; NIST, 2014) and ii) on comparison of the signals in the ^{13}C-NMR spectra of essential oils with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi et al., 1995; Tomi and Casanova, 2006; Ouattara et al., 2014). In the investigated samples, individual components were identified by NMR at contents as low as 0.3-0.4%.

2.6. Antimicrobial Activity of the Essential Oil

2.6.1. Microbial Strains

Antimicrobial activity of the essential oil (collective sample) were evaluated against two Gram positive bacteria (*Staphylococcus aureus* ATCC 6538 and *Bacillus cereus* ATCC 25921) and two Gram negative bacteria (*Escherichia coli* ATCC 8739 and *Klebsiella pneumonia* ATCC 700603), two yeasts (*Candida albicans* ATCC 10231, *Candida albicans* ATCC 26790) and three filamentous fungi (*Fusarium oxysporum* MNHN 963917, *Aspergillus fumigatus* MNHN 566 and *Aspergillus flavus* MNHN 994294).

2.6.2. 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 plates, 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 plates. 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), chloramphenicol (CHL 30 µg/disc), vancomycin (VAN 30 µg/disc) and gentamicin (GMN 10 µg/disc) were used as positive controls against bacteria. DMSO was used as negative control. Each test was performed in triplicate.

2.6.2. 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.

2.7. DPPH Radical Scavenging Activity

The antioxidant activity was measured on a sample of essential oil.The antioxidant activity of *P. scoparius* EO was measured on the basis to scavenge of the 2.2-diphenyl-1-picrylhydrazil ($DPPH^{\bullet}$) free radical, according to the experimental protocol described by Kouame et al. (2017). A volume of 2.5 mL with various concentrations (256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 mg/mL) of the EO in absolute ethanol were added to 1 mL of an ethanolic solution of $DPPH^{\bullet}$ at 0.03 mg/mL. For each concentration, a blank was prepared. In parallel, a negative control is prepared by mixing 2.5 mL of absolute ethanol with 1 mL of ethanolic solution of $DPPH^{\bullet}$. After incubation in the dark for 30 min at room temperature, the absorbance was measured against a blank at 517 nm. The activity of the EO was compared to ascorbic acid as a positive control. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$DPPH^{\bullet} \text{ scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where: $A_{control}$ is the absorbance of the negative control; A_{sample} is the absorbance of the tested sample.

The concentration of the EO required for the 50% reduction in the initial concentration of DPPH (IC_{50}) was calculated from the graph plotted of percentage inhibition against essential oil concentrations. Each test was performed in triplicate.

3. Results and discussion

3.1. Extraction yields

The essential oils of the aerial parts of *Pituranthos scoparius* have been obtained by hydrodistillation. The yields are calculated w/w *vs.* dry material and show a very high variability ranging from 0.16 to 0.99%.The highest yields were obtained for the Sahara regions, ranging from 0.24 to 0.70% for the Ghardaïa region and from 0.25 to 0.99% for the Béchar region. As for the lowest yields, they were observed for plants collected in the highlands ranging from 0.17 to 0.43% for the Biskra region, from 0.16 to 0.37% for the Batna-Bouilef region and from 0.17 to 0.33% for the Batna-Djerma region (Malti et al., 2018).

3.2. Chemical composition

The chemical characterization of the essential oil of *P. scoparius* growing spontaneously in five different stations in Algeria: Ghardaïa, Biskra, Batna (Bouilef and Djerma) and Béchar (Figure 1), by exploiting the complementarity of analytical techniques (GC-FID, GC/MS and ^{13}C-NMR), allowed us to identify 62 compounds. All samples are characterized by a high proportion of monoterpene hydrocarbons (sabinene: 1.4-35.5%; limonene: 0.6-24.0%; α-pinene: 3.1-14.7%; terpinen-4-ol (0.4 – 5.4%) and phenylpropanoids (6-methoxyelemicine: 0.1-47.0%; myristicine: 0.2-18.9%; dill apiole: 0.0-10.6%). We reported in table 1, the major components. It should be noted that the chemical composition of the essential oil of *P. scoparius*, characterized by a high content of 6-methoxyelemicine has never been reported in the literature (Malti et al., 2018). The samples exhibited noticeable different chemical compositions. The samples of Béchar were characterized by the preeminence of 6-methoxyelemicine (23.7-47.0%), followed by limonene (15.0-24.0%). Limonene (19.1%) and myristicine (16.1%) were the major components of the sample of Ghardaïa, followed by α-pinene (12.7%). α and β-Phellandrene (9.7%; 5.3%) and (Z)-ligustilide (3.5%) reached appreciable contents in this sample. The samples of Biskra, Bouilef and Djerma (Dj 1) contained sabinene (18.3-29.6%) as major compound and followed by terpinen-4-ol (7.0-14.7%), myristicine (9.1-12.1%) and dill apiole (6.3-10.6%). The sample of Djerma (Dj 2) exhibited a composition dominated by sabinene (35.5%) accompanied by myristicine (18.9%).

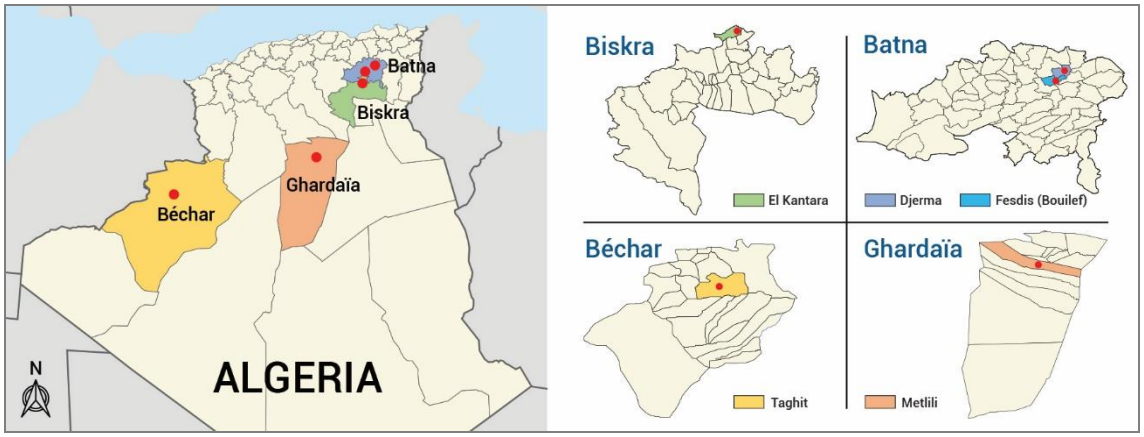


Figure 1. Sampling locations of *Pituranthos scoparius* from Algeria (Malti et al., 2018).

Table 1. Main compounds identified in the essential oils of eight samples of *P. scoparius*.

Main compounds[a]	**RI_a**[b]	**RI_p**[c]	**B 1**	**B 2**	**B 3**	**G**	**Bik**	**Bf**	**Dj 1**	**Dj2**
α-Thujene	922	1023	0.5	0.7	0.4	1.1	1.0	0.9	1.3	0.8
***α*-Pinene**	930	1022	**3.4**	**3.4**	**3.1**	**12.7**	**11.4**	**7.0**	**14.7**	**7.8**
Sabinene	964	1126	**33.2**	**15.0**	**19.0**	**1.4**	**24.8**	**29.6**	**18.3**	**35.5**
β-Pinene	969	1115	2.0	1.6	1.4	4.7	3.4	3.5	5.6	3.6
Myrcene	979	1165	1.2	0.6	0.7	1.0	1.0	0.7	0.7	0.7
α-Phellandrene	996	1170	0.3	0.1	0.4	**9.7**	0.7	0.7	0.7	0.5
δ-3-Carene	1003	1151	0.2	0.2	0.1	0.4	5.1	1.4	0.2	0.1
p-Cymene	1011	1275	2.4	2.5	1.7	4.9	4.3	4.7	5.5	3.4
Limonene*	1021	1206	**19.5**	**15.0**	**24.0**	**19.4**	**1.4**	**0.7**	**1.8**	**0.6**
β-Phellandrene*	1021	1214	0.3	0.2	0.2	**5.3**	0.4	0.4	0.5	0.3
(*Z*)-*β*-Ocimene	1024	1235	Tr	0.3	0.1	0.1	0.5	1.3	2.0	1.1
γ-Terpinene	1047	1247	0.7	0.2	1.1	0.6	1.2	1.1	0.8	1.3
trans-Verbenol	1127	1680	0.2	0.2	0.2	0.2	1.0	0.8	1.2	0.9
Terpinen-4-ol	1160	1604	**4.9**	**2.9**	**3.3**	**0.4**	**4.1**	**5.4**	**2.8**	**5.2**
Methyleugenol	1369	2019	0.7	0.9	0.7	1.3	0.9	0.9	1.4	0.9
Germacrene D	1473	1710	1.6	1.6	**3.1**	**3.0**	0.5	0.6	0.6	0.3
Myristicine	1488	2272	**0.2**	**0.2**	**0.4**	**16.1**	**9.1**	**11.4**	**12.1**	**18.9**
δ-Cadinene	1512	1758	0.2	0.2	0.3	0.8	0.4	0.4	0.4	0.3
Elemicine	1518	2226	Tr	0.2	0.1	0.1	4.1	0.7	0.2	0.1
Spathulenol	1561	2125	--	--	--	1.3	1.8	1.7	1.6	0.9
6-Methoxyelemicine	1567	2215	**23.7**	**47.0**	**33.8**	**0.1**	**0.2**	**0.2**	**0.2**	**0.1**
Dill apiole	1590	2363	**--**	**Tr**	**Tr**	**3.0**	**6.3**	**10.5**	**10.6**	**4.8**
t-Muurolol	1624	2187	0.5	0.3	0.2	0.8	1.3	1.1	1.1	0.7
β-Eudesmol	1632	2230	0.5	1.0	0.4	1.4	0.4	0.5	0.7	0.5
(*Z*)-Ligustilide	1691	2579	--	--	--	**3.5**	Tr	--	0.3	0.1

[a]Order of elution and percentages are given on apolar column (BP-1), except for compounds with * (BP-20). [b]RI_a: Retention indices measured on apolar column. [c]RI_p: Retention indices measured on polar column. Tr: Traces. B: Béchar; G: Ghardaïa; Bik: Biskra; Bf: Bouilef; Dj: Djerma.

3.3. Antimicrobial activity

The antimicrobial activity of essential oils were tested against four bacteria, two yeasts and three 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 essential oils of *Pituranthos scoparius* from the eight samples used, acted very differently on the strains tested (Table 2).

Concerning the bacterial strains, the samples of Ghardaïa, Biskra and Djerma (Dj1) were found to be active against *Staphylococcus aureus* with inhibition zone diameters varied between 16 and 18 mm. We also noted moderate activity for the other samples against this

same bacterial strain with inhibition zone diameters varied between 12 and 14 mm. All the samples exhibited very poor activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus cereus*, with inhibition zone diameters ranging between 6 to 8.7 mm.
These results confirm those of Ksouri et al.(2017) who report a low activity of the essential oils of the aerial parts of *Pituranthos scoparius* against *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with inhibition zones ranging from 9.0±1.0 to 11.7±1.5 mm. They also suggest that *Staphylococcus aureus* was the only bacterial strain that was found to be sensitive with an inhibition zone of the order of 20.0±3.0 mm. Conversely, Boutaghane et al. (2004), reported a good activity of *Pituranthos scoparius* essential oils against *Escherichia coli* (14 to 26 mm), *Klebsiella pneumoniae* (16 to 28 mm) and *Staphylococcus aureus* (14 to 28 mm).
Concerning the yeasts, the samples of Ghardaïa, Biskra, Bouilef and Djerma (Dj 2) were found to be active against both *Candida albicans* with inhibition zone diameters varied between 15 and 20 mm. Finally, for the filamentous fungi, the *Aspergillus fumigatus* strain was found to be the most sensitive to essential oils of the samples from Biskra, Bouilef and Djerma (1 and 2) with inhibition zone diameters varied between 21 and 27 mm. Little activity was observed for the rest of the strains against all the samples tested, with diameters varied between 7 to 11 mm.
Ksouri et al. (2017), also report a sensitivity of *Candida albicans* (15.8±2.4 mm) to essential oils from the aerial parts of *Pituranthos scoparius*. Furthermore, they also suggest that filamentous fungi, namely: *Mucor* sp., *Aspergillus flavus* and *Penicillium expansum* were the most sensitive, with inhibition zone diameters in the range of 19.5 to 20 mm.

3.3.2. Determination of minimum inhibitory concentration (MIC)

According to the results obtained previously concerning the sensitivity of microbial strains to essential oils, determined by the disc diffusion method, we selected six microbial strains, namely: *Staphylococcus aureus*, *Candida albicans* ATCC 10231, *Candida albicans* ATCC 26790, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Fusarium oxysporum*, possessing a high sensitivity towards the tested oil samples for which we determined the minimum inhibitory concentration by the direct contact method in agar medium. The results are reported in Table 3.
Indeed, for the essential oils of the samples of Biskra, Bouilef, Djerma (Dj 1) and Djerma (Dj2), which were the most efficient in the disc method against the tested strains, we obtained the lowest MIC. However, the essential oil of the Djerma (Dj 2) sample was shown to be the most active against most of the strains tested, with MIC varied between 2 and 4 µL/mL. The microbial strains *Aspergillus fumigatus* and *Candida albicans* ATCC 26790 were found to be the most sensitive to this essential oil with a low MIC of around 2 µL/mL. Also, *Candida albicans* ATCC 26790 was also shown to be sensitive to the essential oil of the Bouilef sample, with a MIC of 3 µL/mL. In addition, we noted a moderate activity of the essential oil of the Djerma (Dj 2) sample against *Staphylococcus aureus* and *Candida albicans* ATCC 10231, with a MIC of around 3 µL/mL.Furthermore, the essential oils of the samples from Biskra, Bouilef and Djerma (Dj 1) were also shown to be active against *Aspergillus fumigatus* and *Candida albicans* ATCC 26790, but with a MIC of around 3 µL/mL.
The essential oil from the Ghardaïa sample was only active against *Aspergillus fumigatus*, but with a MIC of about 4 µL/mL. On the other hand, all the microbial strains tested were resistant to the essential oils of the three samples from Béchar, even at an MIC of around 4 µL/mL. It should be noted that these samples are rich in 6-methoxyelemicine, up to 47%. Thus, this compound probably does not have antimicrobial activity.

Our results agree with those of Boutaghane et al. (2004) who report a lower activity of essential oils from the stems (rich in α-pinene 34.0%) and seeds (rich in apiole 52.8%), with MIC in the range of 256 mg/mL and between 20 and 40 mg/mL, against *Escherichia coli* and *Staphylococcus aureus*, respectively. In contrast, Ksouri et al. (2017) report a very good activity of the essential oils of the aerial parts of *Pituranthos scoparius*, with MIC varied between 0.02 and 1.25 mg/mL against *Staphylococcus aureus*, *Candida albicans* and *Aspergillus flavus*. It should be noted that this essential oil is characterized by a high content of limonene (46.9%).

In conclusion, 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 taken into account (Deferera et al., 2003; Burt, 2004) to explain the variation in the degree of sensitivity of microorganisms to the samples tested.

Thus, the best activity of the Djerma (Dj 2) sample is probably due to its chemical composition. The major compounds were: sabinene (35.5%), myristicine (18.9%), α-pinene (7.8%), terpinen-4-ol (5.2%) and dill apiole (4.8%). Essential oil contain more hydrocarbon monoterpenes such as sabinene and α-pinene which are reported to exhibit little activity (Dorman and Deans, 2000; Pintore et al., 2002; Angioni et al., 2003; Leite et al., 2007; Jung, 2009; Qadir and Shah, 2014; Šarac et al., 2014).

Conversely, the essential oil sample of Ghardaïa which contains a comparable amount of myristicine (16.1%) but a low content of terpinen-4-ol (0.4%) was less active than the Djerma (Dj 2) sample. So, the significant activity of Djerma sample could be attributed to terpinen-4-ol or synergy between myristicine and terpinen-4-ol. Indeed, Filipowicz et al. (2003) reported that terpinen-4-ol possessed high level of antimicrobial power, but Torbati et al. (2014) suggest a very low antimicrobial power of myristicine. For example, in their study on *Heracleum anisactis* roots, rich oil of myristicine (95.2%), Torbati et al. found very high MIC values, varied between 1.1 to 1.5 (V/V) (so, between 11 to 15 µL/mL) for *E. coli* and *S. aureus*.

Furthermore, this Ghardaïa sample contains an appreciable amount of ligustilide (3.5%) and methyleugenol (1.3%) which probably also participated in this moderate antimicrobial activity against *Aspergillus fumigatus*. Indeed, Rossi et al. in 2007 reported that methyleugenol was shown to be very active against *Campylobacter jejuni* with an MIC value 250 µg/mL. Moreover, the essential oil of the Biskra sample which contains less myristicine (9.1%) shows the same activity as the essential oils of the Bouilef and Djerma (Dj1) samples (11.4% and 12.1%, respectively). This is probably due to its appreciable elemicine content (4.1% *vs.* 0.7% and 0.2%, respectively). Indeed, Rossi et al. in 2007 reported that a fraction of the essential oil of *Daucus carota* very rich in elemicine of the order of 98% was found to be very active against *Campylobacter jejuni* with an MIC of around 250 µg/mL.

Dill apiole probably does not have antimicrobial activity because the Bouilef and Djerma (Dj 2) samples, which contain higher quantities of 10.5 and 10.6% respectively, were less active than the Djerma (Dj 2) sample which contains a lower content of 4.8%. In contrast, Jabrane et al. (2009), advance very low MIC against *S. aureus* and *E. coli* of the order of 1.25 and 2.5 mg/ml, respectively, when studying the antimicrobial activity of essential oil of roots of *Daucus carota*, whose composition is dominated by the dill apiole (46.6%), followed by myristicine (29.7%).

Table 2. Antimicrobial activity of the eight essential oil samples from the aerial parts of *P. scoparius* determined by the disc diffusion assay (mm).

Samples	Bacteria				Yeasts		Fungi					
	E. c.	*K. p.*	*S. a.*	*B. c.*	*C. a.* 10231	*C. a.* 26790	*A. fla.*		*A. fum.*		*F. o.*	
							a	*b*	*a*	*b*	*a*	*b*
B 1	6.0±0.0	6.0±0.0	14.3±0.6	6.0±0.0	11.0±1.0	10.3±0.6	8.00±0.0	9.0±0.0	13.0±0.0	10.0±0.0	7.0± 0.0	7.0±0.0
B 2	6.0±0.0	6.0±0.0	14.0±1.4	6.0±0.0	10.0±0.0	10.0±0.0	7.5±0.7	9.0±0.0	13.0±0.0	10.0±0.7	6.5±0.7	7.0±0.0
B 3	6.0±0.0	6.0±0.0	12.0±0.0	6.0±0.0	11.0±2.8	10.0±0.0	7.0±0.0	7.5±0.7	13.0±0.0	10.0±0.0	7.0±0.0	7.0±0.0
Ghardaïa	6.0±0.0	6.0±0.0	18.0±3.0	6.0±0.0	20.0±7.8	11.7±0.6	8.5±0.7	9.0±0.0	20.0±2.8	14.0±1.4	8.5±0.7	9.5±0.7
Biskra	8.7±1.2	8.0±0.0	16.0±0.0	6.0±0.0	15.3±2.1	17.7±1.5	14.0±1.4	10.5±0.7	----	24.0±2.1	14.0±1.4	10.5±0.7
Bouilef	8.7±1.2	8.0±0.0	14.5±0.7	6.0±0.0	11.7±2.1	17.0±3.6	11.5±0.7	10.0±0.0	----	27.0±0.0	11.5±0.7	9.0±1.4
Dj 1	8.0±0.0	6.0±0.0	16.0±3.5	6.0±0.0	10.0±0.0	11.0±0.0	12.5±0.7	11.0±0.0	----	23.0±0.7	12.5±0.7	10.0±0.0
Dj 2	6.0±0.0	6.0±0.0	14.3±0.6	6.0±0.0	15.0±0.0	16.6±0.6	13.5±0.7	10.0±0.0	----	21.0±0.7	13.5±0.7	10.0±0.0
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	6.0±0.0	6.0±0.0
CHL	25.0±0.0	21.3±0.6	25.5±0.7	29.5±0.7	----	----	----	----	----	----	----	----
VAN	6.0±0.0	6.0±0.0	17.0±0.0	6.0± 0.0	----	----	----	----	----	----	----	----
GMN	23.0±0.0	20.0±0.0	21.0±0.0	21.0±0.0	----	----	----	----	----	----	----	----
CIP	35.5±0.7	30.5±0.7	32.0±0.0	37.0±0.0	----	----	----	----	----	----	----	----
FLU	----	----	----	----	6.0±0.0	15.0±0.0	6.0±0.0		6.0±0.0		6.0±0.0	
NY	----	----	----	----	16.0±0.0	19.0±1.0	22.3±0.6		33.7±1.2		16.0±1.0	

CHL: Chloramphenicol, VAN: Vancomycin, GMN: Gentamicin, CIP: Ciprofloxacin, FLU: Fluconazole, NY: Nystatine were used as positive controls. ----: Not tested. B: Béchar, Dj: Djerma. Mean values of zones of growth inhibition in mm including disc diameter of 6 mm±Standard deviation. a, b: after 3 and 5 days of incubation, respectively.

Table 3. Minimum inhibitory concentrations (MIC) of aerial parts of *Pituranthos scoparius* essential oil against sensitive strains.

Strainstested	**Essential oil (µL/mL)**							
	B 1	**B 2**	**B 3**	**G**	**Bik**	**Bf**	**Dj 1**	**Dj 2**
Staphylococcus aureus	> 4	> 4	> 4	> 4	4	4	> 4	4
Candida albicans ATCC 10231	> 4	> 4	> 4	> 4	> 4	> 4	> 4	4
*Candida albicans*ATCC 26790	> 4	> 4	> 4	> 4	3	2	3	2
Aspergillus flavus	> 4[a]	> 4	> 4	> 4	> 4	4	> 4	> 4
	> 4[b]	> 4	> 4	> 4	> 4	> 4	> 4	> 4
Aspergillus fumigatus	> 2[a]	> 2	> 2	< 2	3	< 2	< 2	< 1
	> 4[b]	> 4	> 4	4	3	3	3	2
Fusarium oxysporum	> 4[a]	> 4	> 4	> 4	3	3	3	3
	> 4[b]	> 4	> 4	> 4	> 4	> 4	> 4	> 4

B: Béchar; G: Ghardaïa; Bik: Biskra; Bf: Bouilef;Dj: Djerma. a, b: after 3 and 5 days of incubation, respectively.

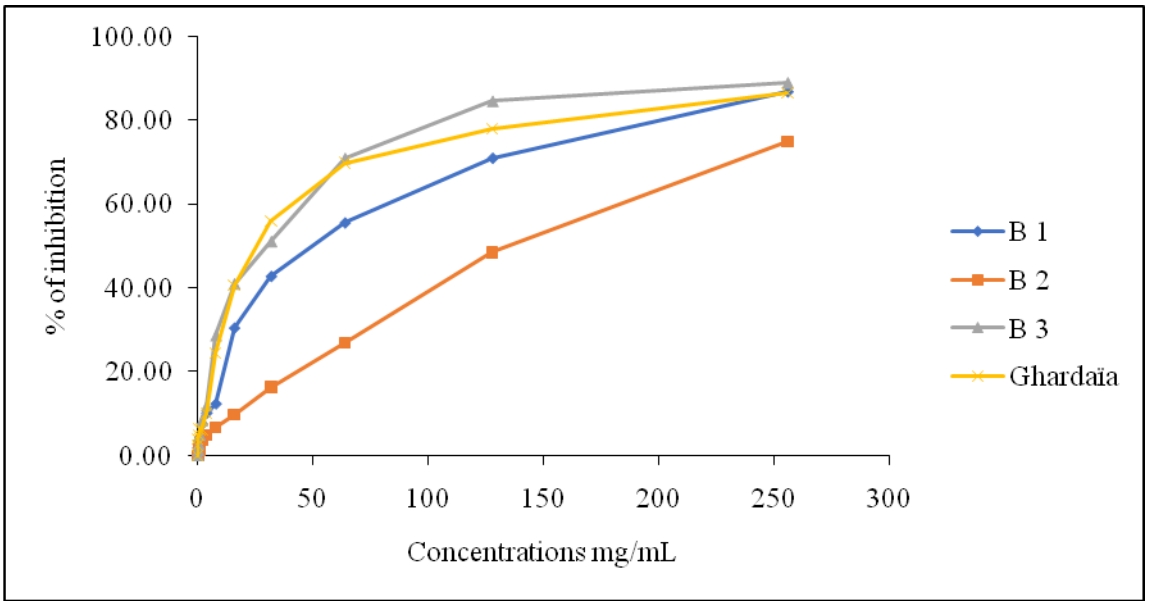
3.4. Antioxidant activity (DPPH Radical Scavenging Activity)

The anti-free radical activity profiles obtained reveal that the essential oils tested have dose-dependent activity, with higher percentages of inhibition, at a lower concentration (Figure 2). Indeed at 256 mg/mL, the sample of Béchar (B 3) showed the highest percentage of inhibition of the order of 88.99±0.10%, followed by the sample of Ghardaïa (86.60±0.14%), Béchar (B 1) (86.78±0.10%) and finally that of Béchar (B 2) (74.89±0.59%). Based on those results, we find that the essential oil from the aerial parts of *Pituranthos scoparius* exhibited very low antioxidant activity compared to standard antioxidants tested.

The IC_{50} values, presented in Table 4, allow us to evaluate and compare the effectiveness of essential oils. Thus, the IC_{50} values obtained for the four samples tested confirm those

obtained with the inhibition percentages. Indeed, the essential oils from the samples of Béchar (B 3) and Ghardaïa were the most active with IC_{50} of the order of 23.65±0.77 and 25.95±2.13 mg/mL, respectively. However, in comparison with standard antioxidants (IC_{50} ≈ 0.02±0.001 mg/mL), the essential oils of the different samples tested were found to be less active. The best antioxidant activity of Béchar (B 3) and Ghardaïa samples could be attributed probably to the presence of limonene and germacrene D in larger amounts.

To our knowledge, there are very few studies on the antioxidant activity of the essential oil of *Pituranthos scoparius*. The results obtained by Ksouri et al. in 2017 for the DPPH test, show that at an essential oil concentration of 20 mg/mL, the DPPH radical was fixed at 84.7%, with an IC_{50} of the order of 11.21±0.26 mg/mL, a lower IC_{50} in comparison with our results, however a lower antioxidant activity than the standards they tested, namely: ascorbic acid (IC_{50} = 4 µg/mL), α-tocopherol (IC_{50} = 9.55 µg/mL) and BHT (IC_{50} = 72.16 µg/mL).



B: Béchar.

Figure 2. Percentage of DPPH free radical inhibition according to different concentrations of *Pituranthos scoparius* essential oils.

Table 4. DPPH free radical scavenging capacity of essential oils of the aerial parts of *Pituranthos scoparius* expressed in IC_{50}.

Samples	IC_{50} (mg/mL)
Béchar (B 1)	41.59±0.81
Béchar (B 2)	156.23±1.26
Béchar (B 3)	23.65±0.77
Ghardaïa	25.95±2.13
Ascorbic acid	0.02±0.001

4. Conclusion

Various compositions of theessential oil of *Pituranthos scoparius* have been reported and a large chemical variability has been observed in Algeria.The results indicated that the essential oil was rich in hydrocarbons monoterpenes and phenylpropanoids compounds. The present study showed that the essential oil of *P. scoparius* has significant antimicrobial activity and

low antioxidant power. The antimicrobial activity of those essential oils may well be probably due to the presence of synergy between myristicine and terpinen-4-ol.

Author Contribution Statement

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

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