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ORIGINAL ARTICLE

Phytochemistry, antioxidant and antibacterial activities of two Moroccan *Teucrium polium* L. subspecies: Preventive approach against nosocomial infections

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Abstract The aim of the present study was to determine the chemical composition and antioxidant activity of essential oils (EOs) from two *Teucrium polium* subspecies, to evaluate, also their antibacterial activities, against some nosocomial-bacteria. The phytochemical screening of essential oils was analyzed using gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry analysis (GC-MS). The antibacterial activities were assessed by disc diffusion method and minimal inhibitory concentration (MIC), against Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter koseri and Acinetobacter baumannii*) and Gram-positive bacteria *Staphylococcus aureus*. The antioxidant potential was evaluated in vitro by three assays, namely free radical scavenging activity against 1,1-diphenyl-2-picrylhydrzyl (DPPH), ferric reducing activity power (FRAP) and total antioxidant capacity. Twenty-six components were identified in the EO of *Teucrium polium* subsp. *aurum* representing. Its major component was Caryophyllene (19.13%) followed by γ-Muurolene (13.02%), τ-cadinol, (11.01%), α-Gurjunene (9.2%), Rosifoliol (8.79%), 3-Carene (7.04%). However, twenty two components were identified in the EO of *T. polium* subsp. *polium*. Its major components are 3-carene (16.49%), γ-Muurolene (14.03%), α-pinene (9.94%), α-phellandrene (6.93%) and

Abbreviations: EOs, essential oils; MIC, minimal inhibitory concentration; *T. polium*, *Teucrium polium*.

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Caryophyllene (7.51%). The antibacterial activity of both essential oils showed a higher activity against tested nosocomial bacteria especially against *S. aureus* and *A. baumannii*. The EO of *T. polium* subsp. *aureum* showed better antioxidant activity as measured by DPPH and FRAP assays with IC_{50} values of 3.7 ± 0.2 mg/ml and 2.31 ± 0.11 mg/ml, respectively. The total antioxidant capacity assay showed that *T. polium* subsp. *aureum* had a significant activity with value to 3308.27 mg equivalent to ascorbic acid/g of EO. The Moroccan *T. polium* essential oils could be exploited as an antimicrobial agent for the treatment of several infectious diseases caused by bacteria, especially, those who have developed resistance to conventional antibiotics.

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1. Introduction

Nosocomial infections dominated by bacterial strains represent a real problem in our modern health care system, and they are characterized by high morbidity and mortality rates (Jenkins, 2017). In Morocco, The incidence of nosocomial infections, in the reanimation units is high and dominated by multi-drugs resistant bacteria (Maoulainine et al., 2014). Utilization of medicament is crucial for treating these infections, but it is also under the important selection pressure affecting many bacteria and those who have developed resistance. The world health organization (WHO, 2017), published a report listing the most dangerous multidrug-resistant bacteria to which a new antibiotic should be discovered urgently. This is the reason why natural alternatives were established in order to overcome the incidence of resistant bacteria (Rossiter et al., 2017). Several reports have indicated potent antimicrobial activity of essential oils, extracted from aromatic and medicinal plants, against various microorganisms (Gherraf et al., 2017; Siddique et al., 2020). Against to antibiotics, essential oils are composed of secondary metabolites therefore, the microorganisms cannot resist in mutant (Moussaoui and Alaoui, 2016). Among these essential oils, the potential antimicrobial of Teucrium species has been documented (Belmekki et al., 2014; Djabou et al., 2013; Ricci et al., 2005). The *Teucrium polium* L. (Lamiaceae) is distributed throughout Morocco with its subspecies, among which *Teucrium polium* L. subsp. *Polium* and *Teucrium polium* L. subsp. *aurum* (Navarro and El oualidi, 1997). In Morocco folk medicine, *Teucrium polium* (Germander) locally called "Jaada", it is used for the treatment of a variety of diseases, including digestive disorders, liver problems (Fakchich and Elachouri, 2014) inflammation, hypertension, fever, diabetes, rheumatism, parasitic diseases such as amoebicide (Henchiri et al., 2009). Numerous studies showed therapeutic properties of *Teucrium polium* such as, anti-inflammatory, anti-cancer (Menichini et al., 2009), antibacterial (Djabou et al., 2013; Stanković et al., 2017), antidiabetic (Tabatabaie and Yazdanparast, 2017), anti-spasmodic, anti-nociceptive (Parsaee and Shafiee-nick, 2006) and antioxidant effects (Khaled-Khodja et al., 2014). Study on chemical composition of essential oils from Algerian *Teucrium polium subsp polium* have been previously reported, α-pinene, Germacrene, γ-Cadinene, and α-Cadinol were identified as their major constituents (Djabou et al., 2012). However, to our knowledge, this is the first report on the phytochemical of *T. polium* subsp. *aureum* essential oil and, there has not been any study on the antioxidant and antibacterial activities of EOs from *T. polium* subsp. *aureum* and *T. polium* subsp. *polium*. Therefore, the main goal of the present work was to determine the phytochemicals and antioxidant potential of *T. polium* subsp. *polium* and *T. polium* subsp. *aureum* essential oils. Moreover to evaluate their antibacterial activity, against bacterial responsible for the nosocomial infections contracted at patients in the University Centre Hospital of Fez Morocco.

2. Materials and methods

2.1. Chemicals

Homologous series of n-alkanes (C8–C20), Butylated hydroxytoluene (BHT), 2,2-Diphenylpicrylhydrazyl radical (DPPH), ammonium molybdate, sodium phosphate, quercitin, vitamin C, iron III chloride (FeCL3), and 2,3,5-triphenyltetrazolium chloride (TTC) used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). While potassium ferricyanide ($K3Fe(CN)_6$), culture media and standard antibiotics were purchased from COGELAB (Morocco).

2.2. Selection and identification of plant material

Aerial parts of *T. polium* L. subsp. *Polium* and *T. polium* L. subsp. *aureum* were collected in April 2015 from the regions of Midelt (Morocco). They were identified by Professor Amina Bari, a botanist in the department of Biological Sciences, Faculty of Science, Sidi Mohammed Ben Abdellah University, Fez (Morocco). Plant material was dried for three weeks in the shade at room temperature. The samples dried were stored at 5 °C until the preparation of the plant essential oils.

2.3. Isolation of the essential oils

A portion (200 g) of the dried aerial parts was submitted for 3 h to water distillation, using a Clevenger-type apparatus, according to the method recommended by the European Pharmacopoeia (1975). Plant material (200 g) was distilled in 700 ml of water in a 2000 ml flask. The obtained essential oils were dried with anhydrous "sodium t sulfate" and stored in a refrigerator at 4–5 °C prior to analysis. Yields were calculated based on the dried weight of each sample.

2.4. Chemical analysis of the essential oils

2.4.1. Gas chromatography-flame ionization detector (CG-FID)

The isolated essential oils were diluted with hexane (dilution ratio 10:100), and 1 µl was sampled for the gas chromatographic analysis. Trace gas chromatograph (GC) (ULTRA

S/N 20062969, Thermo Fischer), gas chromatograph equipped with HP-5MS non polar fused silica capillary column (60 m × 0.32 mm, film thickness 0.25 μm) was used. Operating conditions: oven temperature program from 50 °C (2 min) to 280 °C at 5 °C/min and the final temperature kept for 10 min; 2 "split mode" ratio 1:20; Nitrogen (N_2)carrier gas, flow rate 1 ml/min; temperature of injector and detector (flame ionization detector) was fixed at 250 °C and 280 °C, respectively.

2.4.2. Gas chromatography-mass spectrometry analysis (GC-MS)

The *Teucrium polium* essential oils were analyzed run on a Thermo Fischer capillary gas chromatograph directly coupled to the mass spectrometer system (model GC ULTRA S/N 20062969; Polaris QS/N 210729), using an HP-5MS non polar fused silica capillary column (60 m × 0.32 mm, 0.25 mm film thickness). The operating condition of GC-MS oven temperature was kept as follows: initial temperature 40 °C for 2 min, programmed rate 2 °C/min up to final temperature 260 °C with isotherm for 10 min; injector temperature 250 °C. The helium was the carrier gas with a flow rate (1 ml/min). Essential oils were diluted in hexane with a dilution ratio of 10:100. The volume of the injected specimen was 1 μl of diluted oil, split injection technique; ionization energy 70 eV, in the electronic ionization mode; ion source temperature 200 °C, scan mass range of *m*/*z* 40–650 and interface line temperature 300 °C. Components characterization was made by determination of their retention indices (RI) relative to those of a homologous series of *n*-alkanes (C8–C20) (Fluka, Buchs/sg, Switzerland) and by matching their recorded mass spectra with those stored in the spectrometer database (NIST MS Library v. 2.0) and the bibliography (Adams, 2007).

2.5. Antibacterial activities of essential oils

2.5.1. Bacterial strains

In this study the antibacterial activity of *T. polium* L. subsp. *Polium* and *T. polium* L. subsp. *aureum* was tested against, Gram-positive *Staphylococcus aureus* (*S. aureus)* and Gram-negative bacteria included *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa), Acinetobacter baumannii* (*A. baumannii*) *and Citrobacter koseri (C. koseri)*. All strains tested were isolated in a hospital environment from clinical patients in reanimation service (CHU, Morocco). The inoculum suspension was obtained by taking colonies from 24 h cultures. The colonies were suspended in sterile 0.9% aqueous solution of NaCl and shacked for 20 s. The density was adjusted to the turbidity of a 0.5 McFarland Standard (10^8 CFU/ml) (Mello et al., 2014).

2.5.2. Agar disc diffusion assay

The agar disc diffusion assay was determined in triplicate according to the experiment described by Furtado and Medeiros (1980). The suspensions of microorganisms (1–5 10^8 CFU/ml) were flood inoculated onto the surface of Mueller Hinton (MH) agar plates. Sterile 6 mm diameter filter discs (Whatman paper N° 3) were impregnated with 10 μl/disc of the compound and were put on to the surface of the inoculated Mueller Hinton agar. The plates were incubated at 37 °C for 18 h. Antibacterial effect was evaluated by measuring the inhibition zones against the tested bacterial strains. The standard drugs for comparison were the discs antibiogram of Imipenem (IMP) Vancomycyn (VA), Cefaclor (CEC), Nifrofurantoin (F), Kanamycin (K).

2.5.3. Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was performed using a microdilution assay in 96-well plates according to the experiment of the *National Committee for Clinical Laboratory Standards* (NCCLS, 1999) with some modifications. The different concentrations of essential oils are prepared in a suspension containing 0.2% agar in sterile distilled water in order to disperse the compounds without adding solvent or detergent (Remmal et al., 1993). They are carried out by successive dilutions 1/2 ranging from 100 to 0.09 mg/ml. The concentrations obtained in the well were between 25 and 0.02 mg/ml. Bacterial suspensions were prepared in the same manner described previously and diluted in MH broth and plated in 96 well plates at a density of 1–5 × 10^6 CFU/ml. Finally, the plates were incubated at 37 °C for 18 h, bacterial growth was visually by adding to each well 20 μl of 2,3,5-triphenyltetrazolium chloride (TTC) aqueous solution (1%), with additional incubation for 1 h. MIC was defined as the lowest concentration that does not produce a red color (Mello et al., 2014).

2.6. In vitro antioxidant activities of essential oils

2.6.1. DPPH radical scavenging activity

The DPPH method was introduced 50 years ago by Blois (1958). The ability of the essential oil to scavenge the DPPH radical was measured using the method described by Wu et al. (2003). 0.1 ml of various concentrations of the essential oil or standard was added to 1.5 ml of the ethanolic solution containing 0.1 mmol of DPPH (2, 2-diphenyl-1 picrylhydrazyl). The absorbance of the mixture was measured at 517 nm by a spectrophotometer (Jasco V-530) after 30 min of incubation time at room temperature in dark. The percentage inhibition was calculated by the following equation:

$$I\ (\%) = \left(1 - \left(\frac{As}{A0}\right)\right) * 100$$

where A0 is the absorbance of the negative control, and A_S is the absorbance of the sample. BHT served as a positive control. The IC_{50} values were calculated as the concentration of causing a 50% inhibition of DPPH radical.

2.6.2. Reducing power capacity (FRAP)

The reducing power capacity of the tested oils was determined in accordance with the procedure of Oyaizu (1986). 200 μl of the essential oils were mixed with 500 μl of phosphate buffer (0.2 M, pH 6.6) and 500 μl of potassium ferricyanide [K3 Fe (CN)6] 1%. The obtained solution was incubated at 50 °C for 20 min. The mixture was acidified with 500 μl of trichloracetic acid (TCA) 10%. Which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with 500 μl of distilled water and 100 μl of FeCl3 (0.1%), and the absorbance was measured at 700 nm (Jasco v-530). Quercetin and BHT were used as a standard. The results were expressed as IC_{50} (mg/ml). IC_{50} (concentration

corresponding 0.5 of absorbance) was calculated by plotting absorbance against the corresponding concentration.

2.6.3. Total antioxidant capacity

The assay was based on the reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate Mo (V) complex in acid pH (Prieto et al., 1999). A total volume of 25 µl of essential oil was added to 1 ml of reagent solution (0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). The mixtures were incubated at 95 °C for 90 min and then cooled to room temperature. The absorbance was measured at 695 nm (Jasco v-530). The total antioxidant activity was expressed as the number of equivalence of ascorbic acid (mg AAE/g EO).

2.7. Statistical analysis

The mean values, ±standard deviations were calculated by using GraphPad Prism 5 (Microsoft Software). The results were compared by one-way ANOVA followed by Tukey-test, using the same software. Differences at $P < 0.05$ were considered to be significant.

3. Result and discussion

3.1. Essential oils yield

The essential oils obtained from the aerial parts of *T. polium* subsp. *aureum* and *T. polium* subsp. *polium* were transparent yellow in color, yielded 0.9% and 0.75%, respectively. This yield is higher than that of *T. polium* studied in Algeria (0.27%) (Belmekki et al., 2014) and less than that of Saudi Arabia *T. polium* (1.65%) (Al-Ghamdi and Al-ghamdia, 2014).

3.2. Essential oils chemical composition

The chemical composition of essential oils from two Teucrium species was analyzed by GC-FID and GC–MS techniques, and their chromatographic profiles were summarized in Figs. 1 and 2. Constituents of EOs are listed in order of their elution on the HP-5MS column (Table 1). Twenty six components were found in the essential oil of *Teucrium polium* subsp. *aurum* representing 93.16% of the total oil. Its major component was Caryophyllene (19.13%) followed by γ-Muurolene (13.02%), τ-cadinol, (11.01%), α-Gurjunene (9.2%), Rosifoliol (8.79%), 3-Carene (7.04%), with a prevalence of Sesquiterpene hydrocarbons (64.11%). However, Twenty two components were identified in the oil of *T. polium* subsp. *polium* (94.49% of total oil). Its major components are 3-carene (16.49%), γ-Muurolene (14.03%), α-pinene (9.94%), α-phellandrene (6.93%) and Caryophyllene (7.51%). Sesquiterpene hydrocarbons (54.5%) and Monoterpene hydrocarbons (38.23%) constituted the most abundant fraction of this oil. Notice that certain components exist in the *T. polium* subsp. *aureum* EO (α-terpenyl acetate, β-Cubebene, Aristolene, Valencene, Cubenol and Rosifoliol) but, are absent in the *T. polium* subsp. *polium* EO. Similarly; α-phelandrene, Aromadendrene, and Ledol; exist in the essential oil of the *T. polium* subsp. *polium* but not in the oil of the *T. polium* subsp. *aureum*. Moreover, To our knowledge only one study has been reported on the phytochemical of *T. polium* subsp. *polium* that demonstrated that Germacene (14.8%) β-pinene (16.6%) and α-pinene (7.2%) are its main components (Djabou et al., 2012). However, other studies have determined the chemical composition of *T. polium* but they did not specify its subspecies. Cozzani et al. (2005) found that the main components of *T. polium* EO were α-pinene (28.8%), β-pinene (7.2%) and p-cymene (7.0%). Germacrene (25.81%), bicyclogermacrene (13%), ß-pinene (11.69%) were found to be the main components in *T. polium* EO (Belmekki et al, 2014). In Karpathos Island, Carvacrol (10.1%), Germacrene (3.1%), and γ-Cadinene (2.5%) are the major constituents of *T. polium* EO (Menichini et al., 2012). Furthermore, the major constituents of *T. polium* oil from Saudi Arabia were α-Cadinol (5.93%) and β –pinene, β-gurjurene, and α-pinene (Al-Ghamdi and Al-ghamdia, 2014). These differences in the composition of essential oils can be dependent on different factors, including the location of the plant species and method of extraction of the essential oil (Zhigzhitzhapova et al., 2014). In other hand, gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry analysis (GC-MS) have been extensively used in the determination of EOs composition, because of their advantages such as high efficiency and speed properties (Shabir, 2005). Moreover, owing to the widespread use of GC in routine essential oils analysis, it is necessary that good GC methods are developed and that these are thoroughly validated (Sousa and Brancalion, 2011). For example, Wang et al. (2014) developed an automatic gas chromatograph system equipped with a mass spectrometer and a flame ionization detector (GC-MS/FID) for online measurements of volatile compounds in ambient air, such as C2–C12 hydrocarbons, C3–C6 carbonyls, halocarbons, and alkyl nitrates (Wang et al., 2014).

3.3. Antibacterial activities of essential oils

The antibacterial activities of essential oils from *T. polium* subsp. *polium* and *T. polium* subsp. *aureum* were studied, for the first time, using agar disk diffusion test and minimal inhibitory concentration (MIC) methods against six bacteria strains (*E. coli*, *K. pneumonia*, *P. aeruginosa S. aureus*, *C. koseri* and *A. Boumanii*) responsible for nosocomial infections in Centre Hospital University of Fez, Morocco. Table 2 revealed that the tested EOs showed a wide antibacterial spectrum, against tested strains with the inhibition zone diameters varying from 8 to 23 mm, we notice that *T. polium* subsp. *aureum* and *T. polium* subsp. *polium* EOs had similar and comparative activities. Interestingly, these diameters were sometimes higher than those obtained with standard antibiotics used as controls. The gram-positive *S. aureus* and the Gram-negative *A. baumannii* were the most sensitive of the strains tested to both oil samples. However, these EOs showed a low activity against the Gram-negative *P. aeruginosa*. Table 3 summarized the MIC values of the essential oils against the tested strains. Oils from *T. polium* subsp. *aureum* and *T. polium* subsp. *polium* exhibited significant antimicrobial activity against *S. aureus* (MIC 0.17 mg/ml) and K. pneumonia (MIC 1.4 and 0.7 mg/ml). Compared to C. koseri (MIC 2.81 mg/ml) and A. baumannii (MIC 2.81 and MIC > 1.4 mg/ml), respectively. While, these EOs have low activity against *P. aeruginosa,* which was only inhibited at high concentration (5.62 mg/ml).

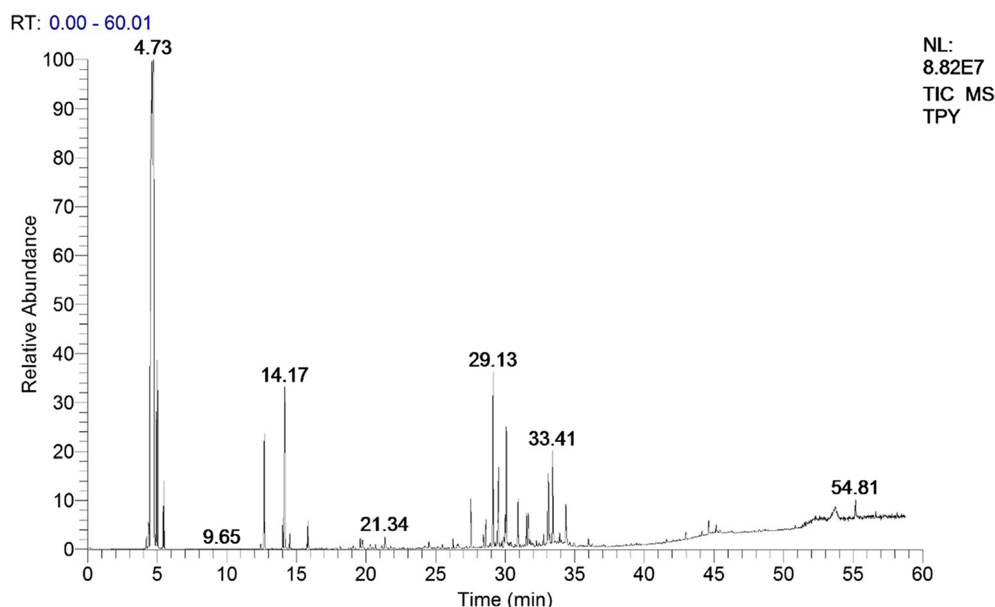

Fig. 1 Chromatographic profile by GC-MS of *T. polium* subsp. *polium*.

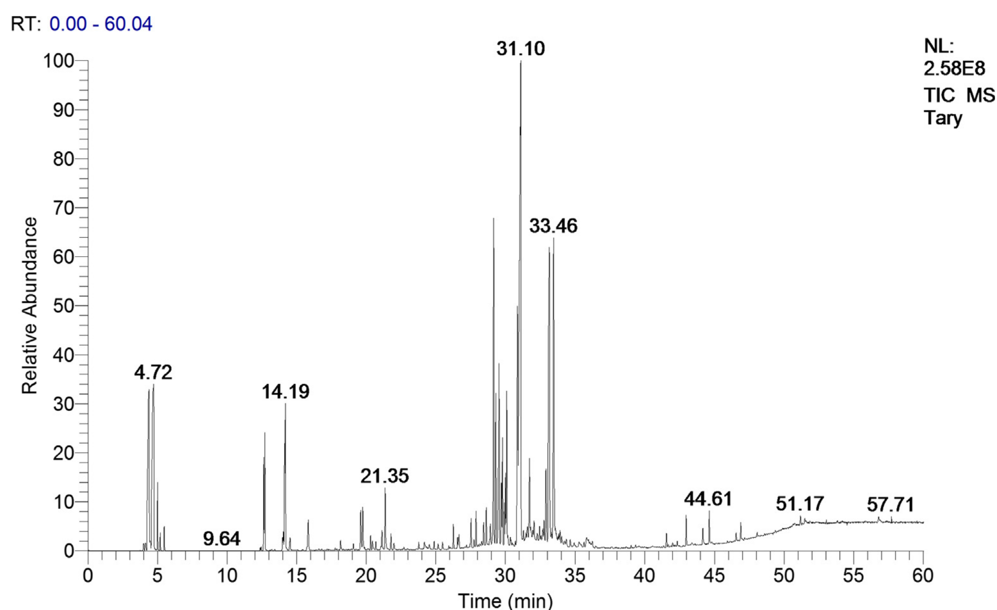

Fig. 2 Chromatographic profile by GC-MS of *T. polium* subsp. *aurum*.

Generally, the correlation between the antimicrobial activity of the EOs and their chemical composition suggests that the activity of the EOs could be attributed to the presence of high concentrations of the major compounds (Siddique et al., 2020). However, many reports have suggested that the synergistic or antagonistic action of the major and minor components from the EOs should be taken into consideration to explain their antimicrobial proprieties (Cutillas et al., 2018; Fadli et al., 2012). In the present study, we found that *S. aureus* appears to be more sensitive to both essential oils. It has been shown that Gram-negative bacteria are less sensitive than Gram-positive bacteria to the essential oils (Ruiz-Navajas et al., 2012; Tenore et al., 2011). This difference in susceptibility could be explained by the structure of cell envelope Gram-negative bacteria possess an outer lipopolysaccharides membrane, delineating the periplasmic space with the cytoplasmic membrane that can limit diffusion of hydrophobic compounds. Without this membrane, the cell wall from Gram-positive group can be permeated more easily and external agents can alter the cytoplasmic membrane (Ruiz-Navajas et al., 2012; Tenore et al., 2011). As we showed, *P. aeruginosa* was the most highly resistant to both essential oils and to the antibiotics. Numerous studies had shown that *P. aeruginosa* is the most resistant to EOs (Kamari et al., 2018; El Atki et al., 2019; Jalal et al., 2015). It reported that the resistance of *P. aeruginosa* may be due to the structure of its outer

Table 1 Percentage composition of essential oils from *T. polium* subsp. *polium* and *T. polium* subsp. *aurum*.

Compounds	RI	*T. polium* subsp. aureum area (%)	*T. polium* subsp. *polium area* (%)
α-Thujene	803	0.3	0.67
α-pinene	809	3.5	9.94
γ-Terpinene	824	0.5	1.01
b-Myrcene	971	0.59	0.12
α-phelandrene	993	–	6.93
3-Carene	1007	7.04	16.49
Trans-sabinol	1109	0.4	0.62
Myrtenol	1194	3.02	1.11
α-Terpinyl acetate	1230	1	–
Thymol	1244	0.4	0.57
β-Cubebene	1283	1.3	–
Aromadendrene	1343	–	1.3
Valencene	1391	0.56	–
Caryophyllene	1445	19.13	7.51
Aristolene	1416	0.9	–
Alloaromadendrene	1437	5.01	3.2
α-Himachalene	1452	1.32	5.56
β-elemene	1463	–	0.2
γ-selinene	1473	0.3	–
γ-Muurolene	1478	13.02	14.03
α-Gurjunene	1492	9.2	6.54
β-Eudesmol	1508	1.05	–
Ledol	1525	–	2.08
Germacrene-D-4-ol	1543	2.36	3.93
Spathulenol	1551	1.2	0.73
Caryophyllene oxid	1574	0.24	0.32
Cubenol	1583	0.7	–
τ-Cadinol	1595	11.01	5.1
Muurolol	1642	–	6.53
Rosifoliol	1654	8.79	–
Benzyl benzouate	1720	0.32	–
Monoterpene hydrocarbons		11.93	38.23
Oxygenated monoterpenes		3.82	2.3
Sesquiterpene hydrocarbons		64.11	54.3
Oxygenated sesquiterpenes		12.3	9.66
Total (%)		93.16	**94.49**

membrane particularly impermeable to EO and related to the action of efflux mechanisms, protecting the bacteria against the action of EO molecules (Utchariyakiat et al., 2016; Tenore et al., 2011). According to Fertout-Mouri et al. (2017), the Gram-positive microorganisms were found to be more sensitive to essential oils from *T. polium* than Gram-negative bacteria. Antimicrobial activity of essential oil from *T. polium* has been investigated against five multi-drug resistant ATCC bacteria (*P. aeruginosa, E. coli, S. aureus, Enterococcus faecalis,* and *Bacillus cereus*). The results showed that *T. polium* oil is active against all strains except for *P. aeruginosa* (Belmekki et al., 2014).

3.4. Antioxidant activities of essential oils

DPPH test, FRAP, and phosphomolybdenum assays were used to evaluate antioxidant activities of *Teucrium polium* oils. The free radical scavenging activity of investigating essential oils was evaluated by the DPPH test. $DPPH^{\bullet}$ is a stable free radical that can receive hydrogen or electron from an antioxidant to become a stable molecule. As depicted in Fig. 3, the both essential from *T. polium* oils presented a potent antiradical effect in a concentration dependent manner. The concentrations of the tested samples needed to remove 50% of the DPPH (IC_{50}) are calculated and presented in Table 4. The results indicate that the *T. polium* subsp. *aureum* showed the higher DPPH radical scavenging activity than *T. polium* subsp. *polium* with IC_{50} values of 3.7 and 7.2 mg/ml, respectively. But was significantly ($p < 0.001$) lower than that of pure reference antioxidant BHT (0.12 mg/ml).

The reductive capacity is generally associated with the presence of antioxidant agents which exert their effect by breaking the free radical chains via hydrogen atom donation (Blažeković et al., 2010). Therefore, the ferric reducing power of investigating essential oils was evaluated by the FRAP assay. The reducing power assay is often used to evaluate the ability of an antioxidant to transform the Fe^{3+} to Fe^{2+}. Fig. 4 showed the reducing power of different concentrations of two essential oil *T. polium* subspecies in comparison to quercetin and BHT. The results demonstrated that the both essential oils possessed the ability to reduce Fe^{3+}, and their reducing power increased with a concentration. *T. polium* subsp. *aurum* had a significant ferric reducing power than

Table 2 Inhibition zone diameter (mm) of *T. polium* subsp. *polium* and *T. polium* subsp. *aurum* essential oils.

Bacterial species	Inhibition zone (mm)						
	Essential oils		Antibiotics				
	T. polium subsp. *polium*	*T. polium* subsp. *aureum*	VA	F	CEC	IMP	K
K. pneumoniae	13 ± 0.5	11 ± 0.3	NI	22	NI	25	24
P. aeruginosa	8 ± 0.4	8 ± 0.2	13	20		12	NT
S. aureus	23 ± 1.4	21 ± 1	14	20	14	39	17
A. Boumanii	15 ± 0.8	20 ± 0.6	18	15	NI	NT	NT
C. koseri	12 ± 0.1	10 ± 0.01	16	16	9	12	15
E. coli	10 ± 0.5	11 ± 0.6	NI	19	NI	28	17

Inhibition zone includes diameter of disk (6 mm); NI: No inhibition; NT: Not tested; IMP: Imipenem; VA: Vancomycyn; CEF: Cefaclor; F: Nifrofurantoin; K: Kanamycin.

Table 3 Minimal inhibitory concentration (mg/ml) of *T. polium* subsp. *polium* and *T. polium* subsp. *aurum* essential oils.

Bacterial species	*T. polium* subsp. *polium*	*T. polium* subsp. *aureum*
K. pneumoniae	0.7	1.4
P. aeruginosa	5. 62	5.62
S. aureus	0.17	0.17
A. baumannii	2.81	1.4
C. koseri	2.81	2.81
E. coli	5.62	2.81

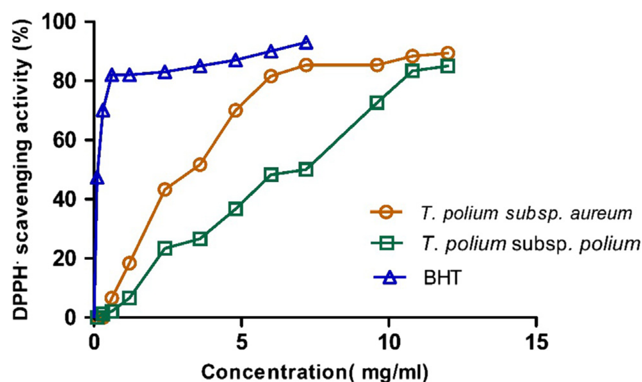

Fig. 3 DPPH free radical scavenging activity of *T. polium* subsp *aureum* and *T. polium* subsp *polium*. BHT was used as reference.

Table 4 Antioxidant activities of essential oils from *T. polium* subsp. *polium* and *T. polium* subsp. *aurum*.

	DPPH (mg/ml)	FRAP (mg/ml)	Total antioxidant capacity (mg AAE/g EO)
T. aureum	3.7 ± 0.2[b]	2.31 ± 0.11[b]	1774.42 ± 87[b]
T. polium	7.2 ± 0.55[a]	3.5 ± 0 .5[a]	3308.27 ± 100[a]
BHT	0.12 ± 0.01[c]	0.1 ± 0.01[c]	540.62 ± 40[c]
Quercetin	–	0.03 ± 0.00[d]	512.70 ± 15[c]

Values are giving as mean ± SD (n = 3). In each column, different letters are significantly different by the Tukey-test (P < 0.05).

T. polium subsp. *polium* with IC_{50} values of 2.31 mg/ml and 3.5 mg/ml, respectively, but it was still significantly ($p < 0.001$) less than that of the synthetic antioxidants quercetin and BHT (Table 4).

Total antioxidant capacity of investigating *T. polium* essential oils, and reference antioxidant (quercitin and BHT) was determined by phosphomolybdenum method, which is based on the reduction of Mo (VI) to Mo (V) in the presence of an antioxidant (Prieto et al., 1999). The results expressed as ascorbic acid equivalents (mgAAE/g EO) are given in Table 4. The total antioxidant capacity of *T. polium* subsp. *polium* was significantly better in comparison to *T. polium* subsp. *aureum,* BHT and quercetin with values of 30308.27; 1774.42; 540.62 and 512.70 mgAAE/g EO, respectively.

According to Abdillah et al (2015), An antioxidant agent is considered to be active against free radicals if IC_{50} less than

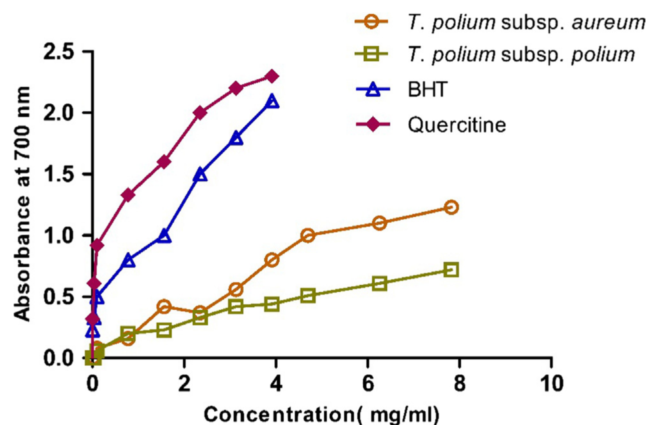

Fig. 4 Reducing power of *T. polium* subsp *aureum* and *T. polium* subsp *polium*. Quercetin and BHT were used as references.

5 mg/ml (Abdillah et al., 2015). The EO studied of *T. polium* subsp. *aureum* have $IC_{50} < 5$ mg/ml, therefore this EO is a possible good source of antioxidant compounds. The poor activity of the *T. polium* EO can be attributed to the weak ability of their main components to scavenge DPPH free radicals (Aazza et al., 2011). Moreover, previous studies showed significant free radical Scavenging activity of EOs from some Teucrium species (Ricci et al., 2005; Saroglou et al., 2007). It is noteworthy that the antioxidant activities of the two subspecies of *T. polium* essential oils are reported here for the first time.

4. Conclusion

This study is the first characterization of the chemical composition, antioxidant and antibacterial (against nosocomial infections) activities of two wild Moroccan *T. polium* subsp essential oils. Our results demonstrated that the major constituents are different in the essential oils of two subspecies. Moreover, the remarkable antioxidant effect and strong inhibitory activity against nosocomial-bacteria of *T. polium* essential oils suggest their possible use as a natural antibacterial drug for bacteria causing nosocomial infections in the intensive care rooms.

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Contribution of authors

We declare that this work was done by the authors named in this article, A. Abdellaoui conceived and designed the study.

Y. El atki and I. Aouam: carried out the laboratory work, collected and analyzed the data and writing the manuscript. F. El kamari and A. Taroq: supervised the work and assisted in data analysis, B. Oumokhtar who isolated and identified the nosocomial bacteria. While B. Lyoussi revised and corrected the manuscript. The manuscript was proof-read by all the authors and approved for publication.

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