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SCREENING OF ANTIRADICAL AND ANTIBACTERIAL ACTIVITIES OF ESSENTIAL OILS OF *ARTEMISIA CAMPESTRIS* L., *ARTEMISIA HERBA* ALBA ASSO, & *THYMUS CAPITATUS* HOFF. ET LINK. GROWING WILD IN THE SOUTHERN OF TUNISIA

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Abstract
The present study was conducted to evaluate in vitro antibacterial and antiradical activities of essential oils extracted from air-dried leaves of *Artemisia campestris Artemisia herba alba* and *Thymus capitatus* growing wild in the southern of Tunisia. The principle compounds *of Artemisia campestris* oil were β-pinene (45.8%) and α-pinène (12.5%), the major constituents of *Artemisia herba alba* oil were β-thujone (30.0%) and α-thujone (25.7%) whereas the *Thymus capitatus* oil was mainly composed of carvacrol (68.8%) and p-cymène (11.1%). The determination of the antiradical activity by DPPH method showed that *Thymus capitatus* oil exerted the highest activity with (0.15 µl/ml), followed by *Artemisia herba alba* (1.0 µl/ml) and *Artemisia campestris* (2.09 µl/ml). The screening of the antibacterial activity against seven bacteria using the disc diffusion method showed that *Thymus capitatus* oil strongly inhibited the growth of all bacteria studied (20 - 30 mm) except *Pseudomonas aerogunosa* which was resistant to all oils. The two other oils exhibited moderate and weak antibacterial activity. These results show and confirm that *Thymus capitatus* possesses strong antiradical and antibacterial activities, and therefore it could be used as a natural preservative ingredient in food and/or pharmaceutical industries.

Key Words: *Artemisia campestris*; *Artemisia herba alba*; *Thymus capitatus*; antradical activity; antibacterial activity.

Introduction

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infection diseases [1]. Word Health Organisation (WHO) noted that majority of world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants which are widely used as medicine constitute a major source of natural organic compounds.

Essential oils have been shown to possess antibacterial, antiviral, insecticidal and antioxidant properties [2-3]. Some others oils have been used in food preservation, aromatherapy and fragrance industries. Essential oils are a rich source on biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils [4-5].

Lipid peroxidation is a complex occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Formation of free radicals may play an important role in the origin of life and biological evolution, implying their beneficial effects on organisms [6]. Radicals are known to take part in lipid peroxidation, which causes food deterioration, aging of organisms and cancer promotion [7.] Reactive oxygen species are reported to be involved in asthma,

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inflammation, arthritis, neurodegenaration, Parkinson's disease, mongolism and perhaps dementia [8]. However, free radicals and other relative species cause the deterioration of biomolecules (e.g., prtein, amino, acids, lipid and DNA) which leads to cell injury and death [9].

Antioxidants act as radical-scavengers, and inhibit lipid peroxidation and other free radical-mediated processes: therefore, they are able to protect the human body from several diseases attributed to the reactions of radicals. The antioxidants are also an increasingly important ingredient in food processing. Their traditional role involves, as their name suggests, inhibiting the development of oxidative rancidity in fat-based foods, particularly meat and dairy products, and fried foods. The most widely used synthetic antioxidants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA) are very effective in their role as antioxidants. However, their use in food products has been failing off due to their instability, as well as due to a suspected action as promoters of carcinogenesis [10]. For this reason, there is a growing interest in the studies of natural healthy (non toxic) additives as potential antioxidants.

Artemisia campestris ("T'gouft"), *Artemisia herba alba* ("Chih") and *Thymus capitatus* ("Zaâtar") are medicinal plants commonly used by local population in the southern of Tunisia for several purposes. *Thymus capitatus* is used as spicy herb for flavouring cheeses, soups, stews, stuffings, meats, fishes, dressings, sauces, and honey. It is widespread in the Mediterranean area. The essential oil of *Thymus capitatus* are also used in the flavour and food industries. The oil is used in the flavouring of toothpaste, mouthwashes, cough medicines and in the manufacture of perfumes and cosmetics. As a medicinal plant, *Thymus capitatus* has traditionally been considered an anthelmintic, antispasmodic, carminative, emmenagogue, expectorant, rubefactient, sedative, stimulant, and tonic. The plant has been used as a folk medicine against asthma, arteriosclerosis, colic, bronchitis, coughs, diarrhea, and rheumatism. [11]. The essential oils of *Thymus capitatus* have been investigated by many researchers whose reported that the major components of these oils were thymol, carvacrol, linalool, γ-terpinene with the presence of p-cymene, borneol and β-bisabolene in relatively lower amounts [12-15]. *Thymus capitatus* essential oils exhibited antibacterial, antifungal and antioxidant activities [16-21].

Artemisia herba-alba Asso. known also as "desert wormwood" is used as aromatisant for tea and in folk medicine for treatment of colds, coughing, intestinal disturbances and as antidiabetic agent [11]. Investigations on the medicinal properties of *A. herba-alba* extracts reported anti-diabetic, leishmanicidal, antibacterial, antifungal, mutagenic, antimutagenic and antioxidant properties [22-28]. Over last decades, studies on *A.herba alba* were focused on its essential oils. Their composition in different parts of the world revealed a high level of polymorphism and led to the definition of several chemotypes. Studies from Spain [29-31] showed that monoterpene hydrocarbons and oxygenated monoterpenes are the most abundant skeletons in *A. herba-alba* oil, but large amounts of sesquiterpenes were found for some populations. Two oil types were found for plants grown in Israel and Sinai [32] those of cineole-thujane-borane type and the pinane type with monoterpene skeletons. In Jordan regular monoterpenes were predominant and the principal components were α- and β-thujones, classifyng the plant as being a thujone chemotype [33]. In Morocco, the market leader in *A.herba-alba* essential oil exports, 16 chemotypes were found [34-35], with 12 having monoterpenes as major components and for four, sesquiterpene skeletons represent the major fraction of the oil. For Algerian oil, monoterpenes were the major components, essentially camphor, α- and β-thujones, 1,8-cineole and chrysanthenyl derivatives [36-37]. Oxygenated monoterpenes were found to be the major components of *A. herba-alba* oil extracted from aerial parts of plants originated from arid regions of Tunisia [14,38]. Haouari and Ferchichi [39] reported that the main components of the essential oils hydrodistilled from the aerial parts of 18 individual *Artemisia herba-alba* Asso. plants collected from subcultured plants originated from different localities in sub-arid to Saharan were cineole, thujones, chrysanthenone, camphor, borneol, chrysanthenyl acetate, sabinyl acetate, davana ethers and davanone. Twelve samples had monoterpenes as major components, three had sesquiterpenes as major components and the last three samples had approximately the same percentage of monoterpenes and sesquiterpenes.

Artemisia campestris "Field sagewort" is a perennial scarcely aromatic herb or small shrub. The aerial part of this plant is used in popular medicine as anthelmintic, antiseptic, cholagogue, deobstruent, emmenagogue, stomachic, tonic, hypotensive and antivenin. The plant was used by some native North American Indian tribes as an abortifacient to terminate difficult pregnancies. The plant has been crushed and applied externally to rheumatic joints, eczema, bruises and sores. A poultice of the crushed leaves has been applied to sore eyes. An infusion of the roots has been used, especially on children, as a hair tonic and to treat scalp infections. It has been taken internally to promote urination and bowel movements. *Artemisia campestris* L. is widespread in the south of Tunisia. The leaves of this plant collected in

summer (August) are widely used in traditional medicine as decoction for their antivenin anti-inflammatory, antirheumatic and antimicrobial properties [11]. The essential oils of *Artemisia campestris* have been studied by several authors and were found to contain different compounds such as alpha and beta-pinenes, p-cymène, caryophyllene oxide, spathulenol, limonene, dehydro-1,8-cineole, cadin-4-en-7-ol, gamma-terpinene, (Z)-beta-ocimene, aromadendrene, germacrene D, bicyclogermacrene, myrtenol, p-cymen-8-ol, gamma-cadinene, ar-curcumene, delta-cadinene, calamenene, alpha-muurolene, gamma-muurolene, gamma-cadinene, bisabolene and endoperoxide, (Z,E)-farnesol, cedrol and verbenone [38,40-49]. Solvent extracts and essential oils from *Artemisia campestris* have been shown to exhibit antioxidant, hepatoprotective, antibacterial, antiviral, insecticide and allelochemical activities [25,27,50-51].

The aim of this study was to determine the chemical composition, antiradical and antibacterial activities of the essential oils of *Artemisia campestris*, *Artemisa herba alba* and *Thymus capitatus* growing wild in the southern of Tunisia.

Materials and methods

Plant material

Aerial flowering parts of the studied species (*Thymus capitatus, Artemisia herba alba and Artemisia campestris*) were collected in 2007 from natural populations in Beni-Khedache (mountainous region in the southern of Tunisia) and identified taxonomically by Pr. M. Neffati, head of ecology laboratory, Institut des Régions Arides, Tunisia. The plant raw materials were cleaned and air-dried at room temperature (15-20°C) for two weeks. Then, the leaves were separated from the other parts and used for the analyses. Voucher specimens of air-dried leaves were deposited in the laboratory of water, soil and plants laboratory of Institut des Régions Arides referring TC2007 to *Thymus capitatus*, AHA2007 to *Artemisia herba alba* and AC2007 to *Artemisia campestris*.

Extraction of essential oil

The air-dried leaves of the studied plants were submitted for 4 hours to hydrodistillation using a French-type Clavenger apparatus. The obtained essential oils were dried over anhydrous sodium sulphate, then stored at 4°C until tested and analysed.

Determination of chemical composition of the essential oils

Gas Chromatography analysis (GC)

The GC analysis of the essential oils was performed using a Perichrom-2100 gas chromatograph fitted with flame ionization detector (FID) and SE-52 capillary column (30 m x 0.22 mm i.d., film thickness 0.20 µm) (5 % diphenyl 95 % dimethylsiloxane). Injector and detector temperatures were set at 220°C and 240°C respectively. Oven temperature was programmed from 40°C to 220°C at a rate of 5°C/min. Nitrogen was the carrier gas, at a flow rate of 1.0 ml/min. Diluted sample (1/100 in hexane, v/v) of 0.2 µl was injected manually and in splitless mode. Quantification data were obtained electronically from FID area percent data without the use of correction factors.

Gas chromatography/mass spectrometry analysis (GC/MS)

GC/MS analysis of the essential oils was performed using Agilent 6890N Network GC system combined with Agilent 5975 B Inert MSD detector (quadruple) in the electron impact mode (70 eV). A HP-5-MS capillary column (30m x 0.25 mm i.d., film thickness; 0.25 µm) was used. The column temperature was programmed from 50 to 280°C at a rate of 7°C/min. The carrier gas was helium adjusted to a linear velocity of 34 cm/s. Scan time and mass range were 2.2 s and 50-550 m/z, respectively. Samples (0.1 µl) were injected with a split ratio of 1:100.

Components identification

Identification of the individual components was assigned by comparison of their recorded mass spectra with those of a computer library (Wiley 275 library and NIST 98 Library) and authentic standards, and by comparing their calculated retention indices relative to (C_8-C_{22}) with literature values measured on columns with identical polarity [52]. The Identification of some components of the oils was also confirmed by co-injection of authentic standards under the same GC conditions as above.

Antiradical activity

The antiradical activity of essential oils in question was tested according to the method employed by Sokmen et al. (2004) [53], with slight modifications, using the stable radical 2,2'-diphenylpicrylhydrazyl (DPPH). The hydrogen atom or electron donation abilities of the corresponding infusions and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of the radical DPPH. Briefly, 50 µl of various concentrations of oils in methanol water were added to 3 ml of a 0.004% methanol solution of the radical DPPH. After a 30 min incubation period at room temperature (15-20°C), the absorbance was read against

a blank at 517 nm with SHIMADZU UVmin1240 UV-Vis spectrophotometer. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$I\% = 100 \times [(A_{blank} - A_{sample}) / A_{blanc}]$

Where A_{blank} is the absorbance of the control reaction (containing 50 µl methanol and 3 ml DPPH methanol solution) and A_{sample} is the absorbance of the test compound (essential oil or standard).

The antiradical activity was defined as the amount of essential oil to decrease the initial DPPH· concentration by 50% (IC_{50}) which was calculated from the graph plotting inhibition percentage (I%) against essential oil concentration (µl essential oil/ml).

The antiradical activities of the essential oils were compared with that of standard ascorbic acid evaluated using the same procedure. Tests were carried out in triplicate.

Antibacterial activity

Microorganisms

The essential oils were individually tested against microorganisms including *Escherichia coli (ATCC 25922)*, *Seratia marcescens*, *Klebsiella pneumoniae (ATCC 15320), Staphylococcus aureus (ATCC 25923)*, *Citrobacter fruendii* ATCC 8090, *Enterobacter amnigenus ATCC* 33072 and *Pseudomonas aeruginosa (ATCC 27853)*.

Antibacterial activity assay

The agar disc diffusion method was employed for the determination of antibacterial activity of the essential oils in question according to the method employed by Yadegarinia, Gachkar, Rezaei, Taghizadeh, Astaneh, & Rasooli (2006) [54] with some modifications. Briefly 0.1 ml of 10^8 cfu/ml bacterial suspension was spread on the Mueller Hinton agar (MHA) plates. Sterile filter paper discs (5 mm in diameter) were impregnated with 10 µl of the oil and were placed on the inoculated plates. A standard disc containing gentamicine (10 µg/disc) was used as reference control. These plates, after remaining at 4 °C for 2 h, were incubated for 24 h at 37 °C. The diameters of the inhibition zones were measured in millimetres. All tests were performed in triplicate.

Results and Discussion

Chemical composition of essential oils

The chemical composition of the essential oils of *Artemisia campestris*, *Artemisia herba alba* and *Thymus capitatus* determined by GC and GC-MS are presented in table 1. Oil yields of the plants were determined as 1.2, 1.0 and 2.6% (v/w) respectively.

Table 1. Major compounds (in %) of studied essential oils

Compounds	RI (HP-5)	*Artemisia campestris*	*Artemisia herba alba*	*Thymus capitatus*
α-Pinene	951	12,5	-	2,2
camphene	974	-	0,8	-
β-Pinene	990	45,8	-	-
Sabinene	998	1,7	1,4	0,7
Myrcene	1005	3,3	-	2,2
p-cymene	1027	4,6	1,5	11,1
Limonene	1030	7,7	-	0,6
1-8,cineole	1035	-	6,0	-
(Z)-β-Ocimene	1053	3,0	-	-
(E)-β-Ocimene	1057	2,4	-	-
γ-Terpinene	1060	3,6	1,1	8,6
α-thujone	1111	-	25,7	-
β-thujone	1129	-	30,0	-
chrysanthenone	1143	-	0,5	-
camphor	1155	-	4,5	-
trans-pinocarveol	1168	-	1,3	-
borneol	1182	-	1,0	-
Terpinen-4-ol	1200	1,1	2,8	-
α-Terpineol	1220	0,8	-	-
bornyl acetate	1294	-	5,7	-
Carvacrol	1310	-	-	68,8
trans-jasmone	1396	-	0,7	-
(E)-Caryophyllene	1418			2,3
γ-Muurolene	1468	0,5	-	-
Germacrene D	1489	0,3	0,7	-
Geranyl propanate	1498	1,8	-	-
δ-Cadinene	1538	0,6	-	-
Spathulenol	1587	1,2	0,6	0,8
β-Eudesmol	1646	1,0	-	0,7

The main compounds of the essential oil of *Artemisia campestris* were β-pinene (45.8%) and α-pinene (12.5%) followed by limonene (7.7%), p-cymene (4.6%), γ-terpinene (3.6%) and myrcene (3.3%). Monoterpene hydrocarbons constitute the major fraction of the oil (84.0%) while sesquiterpene hydrocarbons accounted for 1.4%. oxygenated monoterpenes and oxygenated sesquiterpenes amounted to 1.9% and 4.0% respectively. Similar composition of Tunisian *Artemisia campestris* essential oil has been reported in previous studies [38,40-41]. Other chemotypes have been ascribed containing the following major compounds: spathulenol, β-pinene and α-pinene [42]; γ-terpinene, capillene, 1-phenyl-2,4-pentadiyne, spathulenol, methyleugenol, *p*-cymene and β-pinene [43]; α-pinene (15.3%), β-pinene (9.8%), caryophyllene oxide (18.2%) and spathulenol (9.3%) [44]; beta-pinene (17.8%), cadin-4-en-7-ol (16.4%), gamma-terpinene (8.7%) and (Z)-beta-ocimene (7.4%) [45]; α-pinene (6.9-57.2%), germacrene D (0.4-28.6%), myrene (1.7-11.2%) and bicyclogermacrene (1.0-14.5%) [46].

The major compounds of *Artemisia herba alba* oil were β-thujone (30.0%) and α-thujone (25.7%) whit some amounts of 1,8-cineole (6.0%), bornyl acetate (5.7%), camphor (4.5%) and terpinene-4-ol (2.8%). The majority of components of this oil were oxygenated monoterpenes

(76%) of which 60.7% were ketones. This oil could be considered as thujone chemotype oil. This thujone chemotype oil was previously reported in some samples collected in the southern of Tunisia [39], Morocco [34-35], Algeria [55] and Jordan [33]. On the other hand, the principle components of this oil differed from that reported by several authors for *Artemisia herba alba* oils, in which camphor (15-68.2%) [32,36-37,56], davanone (18.1-51.2%) [30-31,39], chrysanthenone (17.4-77.0%) [34-35,39,55], 1,8-cineole (3-50%) [32,39], cis-chrysanthenol (24.5-30.0%) [31,56], cis-chrysanthenyl acetate (69%) [56] and sabinyl acetate (17.1-22.5%) [14,39] were found to be the most abundant components in addition to the other components with a relatively lower or equal amounts such as thujones, trans pinocarveol, etc. As demonstrated by several studies, this species exhibited different chemotypes and polymorphism of essential oils which show that the chemical composition of this oil is very sensitive to geographical, environmental and morphological parameters.

Carvacrol (68.8%) was the main component of the oil of *Thymus capitatus* followed by p-cymene (11.1%) and γ-Terpinène (8.6%). This chemical composition is in concordance with the carvacrol chemotype growing in Tunisia [12,14-15,18,21,57], Morocco [20] and Greece [58]. The Sardinian *Thymus capitatus* oil differed from Tunisian oil by its low content of carvacrol (10.8%) and its high amount of thymol (29.3%) and p-cymene (26.4%) [16]. The Turkish oil is dominated by carvacrol (35.6%) in addition with thymol (18.6%), p-cymene (26.4%) and γ-terpinene (12.3%) [59].

Antiradical activity

Antiradical activities or free radical-scavenging capacities of the corresponding oils were measured by DPPH method. The reduction ability of DPPH radicals' formation was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The results of antiradical activities of studied oils were presented in Table 2. *Thymus capitatus* oil exerted the highest antiradical activity with an IC_{50} value of 0.15 µl/ml DPPH solution, followed by *Artemisia herba alba* oil (1.00 µl/ml DPPH solution) and *Artemisia campestris* oil (2.08 µl/ml DPPH solution), but these activities is too low comparatively to that of ascorbic acid with an IC_{50} value of 2.54 µg/ml DPPH solution.

The strong activity of *Thymus capitatus* essential oil could be attributed to its high content of carvacrol (68.8%) and γ-terpinene (8.6%) [60]. *Artemisia herba alba* and *Artemisia campestris* oils exhibited weak antiradical activities because they are not contain high amounts of antioxidant compounds. The activity of *Artemisia herba alba* is higher than that of *Artemisia campestris* because of its high contents of oxygenated monoterpenes. *Artemisia campestris* oil exhibited the lowest activity despite its relatively high content of γ-terpinene comparatively to *Artemisia herba alba* oil, because of its high contents of monoterpene hydrocarbons [60]. The strong antioxidant activities of *Thymus capitatus* and other species oils with high carvacrol, thymol and γ-terpinene contents has been previously reported [18,21,53,61-62].

Table 2. Antiradical activity expressed as EC_{50} (in µg HE/ml DPPH solution) of studies essential oils.

Samples	IC_{50} (µl HE/ml DPPH)	IC_{50} (µg HE/ml DPPH)
Thymus capitatus	0,15	135
Artemisia herba alba	1	900
Artemisia campestris	2,083	1874.7
Ascorbic acid (standard)	-	2,45

In addition, it should be noted that the absolute values of EC_{50} of the studied oils were different from those reported in the literature. Indeed, the value of EC_{50} depends on several parameters: (i) the ratio between the amount of the essential oil and the amount the DPPH solution used in the mixture, (ii) the concentration of the DPPH solution, (iii) the time of incubation, (iv) the way of expressing the unit of IC_{50}. Therefore, the direct comparison of IC_{50} reported in various works is not realistic. Different IC_{50} values were reported for synthetic antioxidant such as ascorbic acid and BHT [1,18,53,61,63-64]. It seems to be more rational to express the H-donating capacity as the amount of DPPH, which may be scavenged by a sample tested (the stoichiometric coefficient for individual antioxidants) or as the amount of extract (in mg or µg) per mg DPPH. This approach makes it possible to compare the data obtained in one work with those of another work [65]. It was described that radical scavenging ability of some compounds can be influenced by their different kinetic behaviour [66]. For slow reacting compounds the influence was attributed to the complex reacting mechanism. The use of different methods is necessary in antioxidant activity assessment. No single testing method is sufficient to estimate the antioxidant activity of a studied sample. The combination of three methods such as β-carotene bleaching method (BCB), DPPH radical scavenging method and thiobarbituric acid reactive species method (TBARS) was a good choice to evaluate the antioxidant activity of essential oils [61].

The antioxidant power depends on the chosen method, on the concentration and on the nature and

phytochemical properties of studied antioxidants. It was confirmed that the same antioxidant samples exhibit different antioxidative values depending on the concentration and the measured antioxidant parameter. It is important to achieve a multiple concentration measurements to avoid incorrect conclusion in these cases [61,66].

Antibacterial activity

The three essential oils tested showed various degrees of inhibition against the seven bacterial strains using the disc diffusion method as presented in Table 3.

Table 3. Antibacterial activity of studied essential oils and Gentamicine (standard) expressed as diameter of inhibition zone in mm (including disc diameter of 5 mm) against selected bacterial strains.

Microorganisms	*Artemisia campestris*	*Artemisia herba alba*	*Thymus capitatus*	Gentamicin (Standard)
Escherchia coli	18	12	30	20
Klebsielle pneumoniae	10	0	20	-
Serratia marcescens	5	20	30	20
Pseudomonas aerogunosa	0	0	0	-
Enterobacter amnigenus	0	0	30	-
Citrobacter frendii	10	15	10	-
Staphylococcus aureus	10	30	30	15

The results revealed that *Pseudomonas aerogunosa* was resistant to all oils tested whereas *Enterobacter amnigenus* was sensitive to only *Thymus capitatus* oil. The most potent oil was *Thymus capitatus*, followed by *Artemisia herba alba* and *Artemisia campestris*. The essential oil of *Thymus capitatus* exhibited pronounced antibacterial activity against *Escherchia coli*, *Serratia marcescens*, *Enterobacter amnigenus* and *Staphylococcus aureus* (30 mm), high activity against *Klebsielle pneumoniae* (20 mm) and low activity against *Citrobacter frendii* (10 mm). The *Artemisia herba alba* essential oil showed pronounced activity against *Staphylococcus aureus* (30 mm), high activity against *Serratia marcescens*, moderate activity against *Citrobacter frendii* and *Escherchia coli* (15 and 12 mm respectively), and inactive against *Klebsielle pneumoniae*, *Pseudomonas aerogunosa* and *Enterobacter amnigenus*. The essential oil of *Artemisia campestris* was less efficient than other oils against all bacteria tested except *Escherchia coli* which showed high sensitivity (18 mm). Indeed, this oil exhibited low activity against *Klebsielle pneumoniae* (10 mm), *Serratia marcescens* (5 mm) and *Citrobacter frendii* (10 mm) and inactive against *Pseudomonas aerogunosa* and *Enterobacter amnigenus*. The essential oil of *Thymus capitatus* was found to be more effective than 10 µg Gentamicin against *Escherchia coli*, *Serratia marcescens*, *Enterobacter amnigenus* and *Staphylococcus aureus*. The essential oil of *Artemisia herba alba* was found to be more effective than 10 µg Gentamicin against *Staphylococcus aureus* and as effective as 10 µg Gentamicin against *Serratia marcescens*. *Artemisia campestris* was found to be less effective than 10 µg Gentamicin against all bacteria tested.

The pronounced antibacterial activity of *Thymus capitatus* essential oil against several bacteria has been reported by other authors [16-18]. This strong activity is attributed to the high content of carvacrol known by its high antibacterial activity [4,16,67-68]. Dorman [4] reported that the antibacterial activity of the essential oils is related to their respective composition, the structural configuration and the functional groups of their constituents and the possible synergistic interaction between components. They announced that the oil with the widest spectrum of activity was found to be *Thymus vulgaris*, followed by *Origanum vulgare*, *Syzygium aromaticum*, *Myristica fragrans*, *Piper nigrum* and *Pelargonium graveolens*, and that the component with the widest spectrum of activity was found to be thymol, followed by carvacrol, α-terpineol, terpinen-4-ol, eugenol, (±)-linalool, (-)-thujone, δ-3-carene, cis-hex-3-an-1-ol, geranyl acetate, (cis + trans)-citral, nerol, geraniol, menthone, β-pinene, R(+)-limonene, α-pinene, α-terpinene, bornéol, (+)-sabinene, γ-terpinene, citronellal, terpinolene, 1,8-cineole, bornyl acetate, carvacrol methyl ether, myrcenen, β-caryophyllene, α-nisabolol, α –phellandrene, α-humulene, β-ocimene and p-cymene. The resistance of *Pseudomonas aerogunosa* and the pronounced sensitivity of *Escherchia coli and Staphylococcus aureus* against essential oils have been also reported by Bouhdid et al. [62].

It has been reported that the bacteria demonstrating the biggest inhibition zones by diffusion method are not always the ones that present the lowest MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values. In fact, the diameter of the growth inhibition zone is affected by the oil solubility and volatility [69]. The majority of the essential oils assayed for their antibacterial properties showed a more pronounced effect against the Gram (+) bacteria [62,70]. The resistance of Gram (-) bacteria to essential oils has been ascribed to their hydrophilic outer membrane which can block the penetration of hydrophobic compounds into target cell membrane [71]. Carvacol can destabilizes the cytoplasmic membrane and acts as a proton exchanger, thereby reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death [72]. Furthermore, it has been reported that essential oils rich on phenolic components possesses high levels of antimicrobial activity. However, the compounds present in the greatest proportions are not

necessary responsible for the total activity, the involvement of less abundant constituents should also be considered [73]. Therefore, the activity could be ascribed to the presence of other components such as p-cymene, linalool and β-pinene also known to possess an antibacterial activity [74-76].

Moreover, the antibacterial activity of essential oils may be due to the presence of synergy between the major components and other constituents of the oils leading to various degrees of antibacterial activity. Accordingly, a synergistic effect against *B. cereus* vegetative cells has been observed between carvacrol and p-cymene at low concentrations. p-Cymene, which possesses relatively weak antibacterial activity, was responsible for the expansion of the bacterial cell membranes to a greater extent compared to carvacrol. By this mechanism, p-cymene acts synergistically with carvacrol probably by enabling it to be more easily transported into the cell [72]. In addition, it has been reported that the strains of *E. coli* that are not susceptible to the mixture of linalool-1,8-cineole are likely to be affected by linalol alone [77], which suggests that possible antagonistic and synergistic effects may occur according to the tested micro-organism.

The presence of high content of oxygenated monoterpenes (thujones, camphor and 1,8-cineole) in essential oil of *Artemisia herba alba* is the responsible of its pronounced activity against *Staphylococcus aureus* and its high activity against *Serratia marcescens*. Indeed. It has been reported that *Staphylococcus aureus* being the most significantly affected by monoterpene ketones such as thujones [4,68]. Since *Artemisia campestris* essential oil was mainly composed of monoterpene hydrocarbons, its antibacterial activity was found to be weaker than other oils. The moderate activity of this oil against *Escherchia coli* which is the most sensitive bacteria against essential oils is attributed to the presence of limonene, γ-terpinène, p-cymene, terpinen-4-ol and α-terpinéol even in relatively low amounts. It has been reported that these compounds exhibited a moderate to weak antibacterial against some bacteria [2,4,16,68]. Thus, indicating the significant role played by the minor components.

The presence of an oxygen function in the framework increases the antimicrobial properties of terpenoids. The bacteriostatic and fungistatic action of terpenoids was increased when carbonylated. Menthone and thujone were shown to have modest activity, *Cl. Sporogenes* and *S. aureus* being the most significantly affected. An increase in activity dependent upon the type of alkyl substituants incorporated into a nonphenolic ring structure appeared to occur in this study. An alkenyl substituant (1-methylethenyl) resulted in increased intibacterial activity as seen in limonene [1-methyl-4-(1-methylethenyl)-cycloheene], compared to an alkyl (1-methylethyl) substituants as in p-cymene [1-methyl-4-(1-methylethyl)-benzene]. The inclusion of a double bond increased the activity of limonene relative to p-cymene, which demonstrated no activity against the test bacteria. Furthermore, the stereochemistry had an influence on antibacterial activity. It was observed that α-isomers are inactive relative to β-isomers, e.g. α-pinene; cis-isomers are inactive contrary to trans-isomers, e.g. geraniol and nerol; compounds with methyl-isopropyl cyclohexane rings are the most active; or insaturation of the cyclohexane ring further increases the antibacterial activity, e.g. terpinolene and terpineol [4]. The relative of citronellal, thujones, p-cymene and 1,8-cineole has been associated with their low water solubility and hydrogen bonding capacity, this limiting their entry into the gram-negative organisms that possess inefficient hydrophobic pathways in the outer membrane [78].

Conclusion

The results of this study indicated the possibility of using the essential oil of *Thymus capitatus* collected from Beni-Kedache as natural antibacterial product against some bacteria and as natural antioxidant. These results should be confirmed by the determination of the CMI and CBI for the antibacterial activity and by the use of other methods for the evaluation of the antioxidant activity. The essential oil of Artemisia herba alba could be a potent antibacterial agent against *Serratia marcescens* and *Staphylococcus aureus* whereas *Artemisia campestris* essential oil could be active against *E. coli.*

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