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Chemical composition and antibacterial activity of essential oil of *Tetraclinis articulata* (Vahl) Masters branches of eastern Morocco

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Abstract

Background: Some pathogenic microbial species are becoming less sensitive to antibiotics and developing resistance multiples. The use of essential oils is a serious substitute for treatment with antibiotics in infectious diseases. The present study aims to investigate the chemical composition and to evaluate the antibacterial activity of the essential oil of branches of *Tetraclinis articulata* (Vahl) Masters (Cupressaceae).

Methods: The extraction of the essential oil was realized by hydrodistillation, and the analysis was carried out by gas chromatography coupled with mass spectroscopy (GC–MS/FID). The antibacterial test was carried out using two different methods: the disc diffusion method and the macrodilution method which aims to determine the minimum inhibitory concentration (MIC).

Results: The yield of essential oil was 0.84%. Chromatographic analysis revealed that the major constituents were α-pinene (38.75%), limonene (13.24%), bornyl acetate (8.78%) and camphor (7.68%). The results of the antibacterial activity show that the essential oil exerts a powerful effect to inhibit some strains tested.

Conclusions: The antibacterial activity demonstrated in this study could justify the traditional uses of this plant and could contribute to value its potential in order to develop new bioactive compounds.

Keywords: *Tetraclinis articulata* (Vahl) Masters, Antibacterial activity, MIC, Camphor

Introduction

In the field of anti-infectives, the discovery of new substances has always been the goal of Human, since the main cause of death in the past was infectious diseases [1]. When penicillin therapy was first performed, Human thought he had won the battle against pathogenic bacteria. However, with the appearance of penicillin-resistant bacteria, he had the impression of regression, and with the extraction and synthesis of new powerful antibiotics, the fear dispersed. Nevertheless, like any drug, antibiotics are not totally harmless to the body and can cause many side effects [2]. These antibiotics, which have saved lives and relived the sufferance of millions of people for years to date, are also threatened by the emergence and spread of multi-resistant germs.

In order to overcome the problem of resistance of microorganisms to antibiotics, most of the work is currently oriented towards other antimicrobial agents with a very specific mode of action. Thus, researchers and scientists are trying to find effective and accessible alternatives from natural products extracted from medicinal and aromatic plants, which are known to be endowed with antimicrobial properties [3–11].

Among the aromatic plants, *Tetraclinis articulata* (Vahl) Mast. Synonyms (Cupressaceae), Barbary thuja synonyms of Callitris quadrivalvis Rich and A. Rich., Thuja articulata Vahl, resinous species belonging to the order Pinales, known in Morocco as the "Araar", is characterized by its resistance to destructive agents and the

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use of its wood in crafts, carpentry and cabinetry. It is endemic in the south western Mediterranean and especially the Maghreb [12]. In Morocco, forests cover about 566.000 ha, and are divided into 6 major biogeographic units at the Rif, oriental, eastern Middle Atlas, valleys of the central plateau and the western plateau, Western Middle Atlas and High Atlas. In traditional medicine, the different parts of the tree, particularly the leaves and branches, are used in the treatment of intestinal and respiratory infections [13].

A research in the scientific literature indicates that there are few reports of studies on the antibacterial properties and chemical composition of essential oil extracted from the branches of the Thuja. In this context, this study was conducted in order to put under scrutiny the chemical composition and to evaluate the antibacterial activity of this essential oil.

Materials and methods

Plant material and extraction

The branches of *Tetraclinis articulata* were harvested in April (2016) in the forest of Tafoughalt-zegzel (Latitude: 34°50′20″N; Longitude: 2°24′49″W), region in East of Morocco. The extraction of essential oil was carried out by hydrodistillation in a Clevenger-type apparatus [14]. Three distillations were performed for 3 h. The essential oil was stored at 4 °C in the dark before proceeding with chromatographic analysis and biological activities.

Chromatographic analysis

The analysis of the essential oil was performed using Agilent 6890 GC–MS system, equipped with a split/splitless injector. GC–MS conditions were line/detector temperature 250 °C, carrier gas (helium, 1 mL/min), split ratio 100:1, and capillary column DB5MS (30 m × 0.25 mm; film thickness 0.25 µm, Agilent, Palo Alto, CA, USA). The temperature program was established to grow with 10 °C/min up to 280 °C. The injected volume was 0.30 µL with a total scan time of 32 min. The GC-FID analysis used an Agilent type 6890 GC connected to a FID detector and all method and analysis parameters were the same as those described above.

Microorganisms studied

Eight clinical bacteria were chosen for their pathogenicity, and they have been isolated from the various samples (Table 1). Isolation and identification of clinical strains were performed at the microbiology laboratory of Military Hospital Moulay Ismail in Meknes.

Tests for antibacterial activity

Determination of the inhibition diameter (solid method)

The diffusion method from a solid disc was used to demonstrate the antibacterial activity. A bacterial suspension was diluted and adjusted to turbidity equal to that of the McFarland standard 0.5 (10^8 CFU/mL). The Mueller–Hinton Agar was poured into Petri dishes 90 mm in diameter. The agar surface was seeded with the microbial suspension. Paper dishes, of 6 mm diameter impregnated with 10 µL of the essential oil, were deposited on the surface of the agar. The dishes were then incubated at 37 °C for 24 h. After incubation, an area or clear halo is present around a disc if the essential oil inhibits bacterial growth. All tests were repeated three times.

Broth dilution method (determination of MIC and MBC)

A cascade dilution was performed in Mueller–Hinton Broth-Tween 80 (0.01%, v/v), so as to obtain a concentration range between 80 µL/mL and 0.3 µL/mL. 13 µL of a bacterial inoculum, equivalent to the standard density of 0.5 McFarland (10^8 UFC/mL), was deposited in each of the tubes of the range. A control of the bacterial growth, for which 13 µL of the standardized inoculum was deposited in MHB-Tween 80 medium (0.01%, v/v), was also carried out. The tubes were incubated at 37 °C for 24 h. The MIC was determined by the lowest concentration of essential oil giving a growth inhibition.

Table 1 Profile of tested bacteria

Bacterial strains	Gram	Profile	Sex	Nature of sampling
Escherichia coli	Gram-negative bacilli	ESBL	Male	Cytobacteriological urine exam
Klebsiella pneumoniae	Gram-negative bacilli	ESBL	Male	Urinary catheter
Proteus mirabilis	Gram-negative bacilli	Penicillinase High level	Male	Cytobacteriological urine exam
Pseudomonas aeruginosa	Gram-negative bacilli	Low level	Female	Coproculture (stool bacteria)
Acinetobacter baumannii	Gram-negative bacilli	Multi-resistant	Male	Cytobacteriological urine exam
Staphylococcus epidermidis	Gram-positive cocci	Multi-resistant	Male	Urethral
Staphylococcus non aureus	Gram-positive cocci	Multi-sensitive	Female	Pus
Staphylococcus aureus	Gram-positive cocci	Multi-sensitive	Male	Pus

ESBL extended-spectrum beta-lactamases

Table 2 Chemical composition of essential oil of *Tetraclinis articulata* branches

Compound	Area	RT	Formula
Tricyclene	0.92	7.92	$C_{10}H_{16}$
α-Pinene	*38.75*	8.24	$C_{10}H_{16}$
Camphene	1.48	8.52	$C_{10}H_{16}$
Sabinene	0.58	8.90	$C_{10}H_{16}$
β-Pinene	1.00	9.01	$C_{10}H_{16}$
Myrcene	2.52	9.19	$C_{10}H_{16}$
Δ-3-Carene	0.22	9.54	$C_{10}H_{16}$
α-Terpinene	0.17	9.69	$C_{10}H_{16}$
Limonene	*13.24*	9.99	$C_{10}H_{16}$
α-Ocimene	0.03	10.18	$C_{10}H_{16}$
Terpinolene	1.17	10.89	$C_{10}H_{16}$
(E)-Ocimene	0.35	11.12	$C_{10}H_{16}$
1,3,8-*p*-Menthatriene	0.27	11.34	$C_{10}H_{14}$
α-Campholenal	0.84	11.59	$C_{10}H_{16}O$
p-Mentha-1,3,8-triene	0.41	11.81	$C_{10}H_{14}$
Camphor	*7.68*	12.03	$C_{10}H_{16}O$
α-Toluenol	0.31	12.20	C_7H_8O
Borneol	2.95	12.39	$C_{10}H_{18}O$
γ-Terpinene	0.74	12.49	$C_{10}H_{16}$
p-Cymenene	0.49	12.58	$C_{10}H_{12}$
Allo-Ocimene	1.59	12.72	$C_{10}H_{16}$
Verbenone	0.68	12.92	$C_{10}H_{14}O$
Carveol	0.88	13.08	$C_{10}H_{16}O$
Methyl carvacrol	0.62	13.28	$C_{11}H_{16}O$
Carvone	0.45	13.43	$C_{10}H_{14}O$
Bornyl acetate	*8.78*	14.07	$C_{12}H_{20}O_2$
Carvacrol	0.94	14.28	$C_{10}H_{14}O$
α-Copaene	0.97	15.37	$C_{15}H_{24}$
β-Elemene	0.48	15.54	$C_{15}H_{24}$
Aromandendrene	1.36	15.78	$C_{15}H_{24}$
β-Caryophyllene	1.11	16.03	$C_{15}H_{24}$
Germacrene D	0.06	16.14	$C_{15}H_{24}$
(Z)-β-Farnesene	0.15	16.39	$C_{15}H_{24}$
α-Humulene	0.54	16.53	$C_{15}H_{24}$
γ-Muurolene	0.10	16.76	$C_{15}H_{24}$
γ-Cadinene	0.61	16.89	$C_{15}H_{24}$
β-Selinene	0.19	17.01	$C_{15}H_{24}$
α-Selinene	0.31	17.10	$C_{15}H_{24}$
γ-Cadinene	0.58	17.40	$C_{15}H_{24}$
(Z)-α-Farnesene	0.04	18.15	$C_{15}H_{24}$
α-Humulene	0.26	18.84	$C_{15}H_{24}$
Cadina-1(2),4-diene	1.23	19.25	$C_{15}H_{24}$

Italic values indicate the percentage of the major compounds of the essential oil

The minimum bactericidal concentration (MBC) was determined from the MIC. The tubes showed no visible growth with the naked eye after incubation and the control tube, were streaked on MHA. The inoculated dishes were incubated for 24 h at 37 °C. The MBC is the lowest concentration that shows no bacterial growth.

Results and discussion

The yield of essential oil

The extraction of essential oil by hydrodistillation from the branches of *Tetraclinis articulata* enables us to obtain a yield of the order of 0.84 ± 0.01%. This rate is relatively higher than that reported by Bourkhis et al. (0.41%) [15]. This shows that certain factors such as the nature of the soil, the climate and quality of the plant material used can influence the secretion of essential oils in a plant.

Identification of chemical composition of essential oil

Forty-two compounds were identified, corresponding to 98.89% of the oil, mainly represented by monoterpenes. The main constituents were α-pinene (38.75%), limonene (13.24%), bornyl acetate (8.78%) and camphor (7.68%). The analysis results are shown in Table 2 and the chromatographic profile is shown in Fig. 1.

Our results are similar to those reported by Bourkhiss et al. [15] who studied the essential oil of branches of *Tetraclinis articulata* (Vahl) Masters of Khemisset region (Northwester Morocco), and have identified thirty-three compounds representing more than 80% of the total chemical composition, the main compounds of which were α-pinene (30.22%), limonene (22.29%), widdrol (5.41%) and bornyl acetate (4.76%).

Similarly, Buhagiar et al. [16] reported that the essential oil of the terminal branches (woody and non-woody) of Thuja from Malta was characterized, respectively, by α-pinene (31.0%; 46.4%), followed by bornyl acetate (19.1%; 7.3%), camphor (18.1%; 7.3%) and limonene (3.8%; 6.2%).

Furthermore, Ait Igri et al. [17] have shown a slightly different composition than ours, bornyl acetate (31.10%), camphor (20.20%) and limonene (7.10%), which were identified as the major compounds of the essential oil of the branches of Thuja collected in the region of Essaouira (south-west Morocco).

This difference was also seen in the work of Tekaya-Karaoui et al. [18], where the main components in the essential oil of the terminal branches (non-woody) of Thuja were muurolene (29.0%) and 4, 6-dimethyl-octane-3,5-dione (22.4%), whereas camphene (43.2%) and (Z)-β-ocimene (11.7%) were the major compounds in the terminal branches (woody).

These differences in chemical composition of essential oil may be attributed to several factors: seasonal and environmental, soil, geographic area, harvest period, extraction method [19–24].

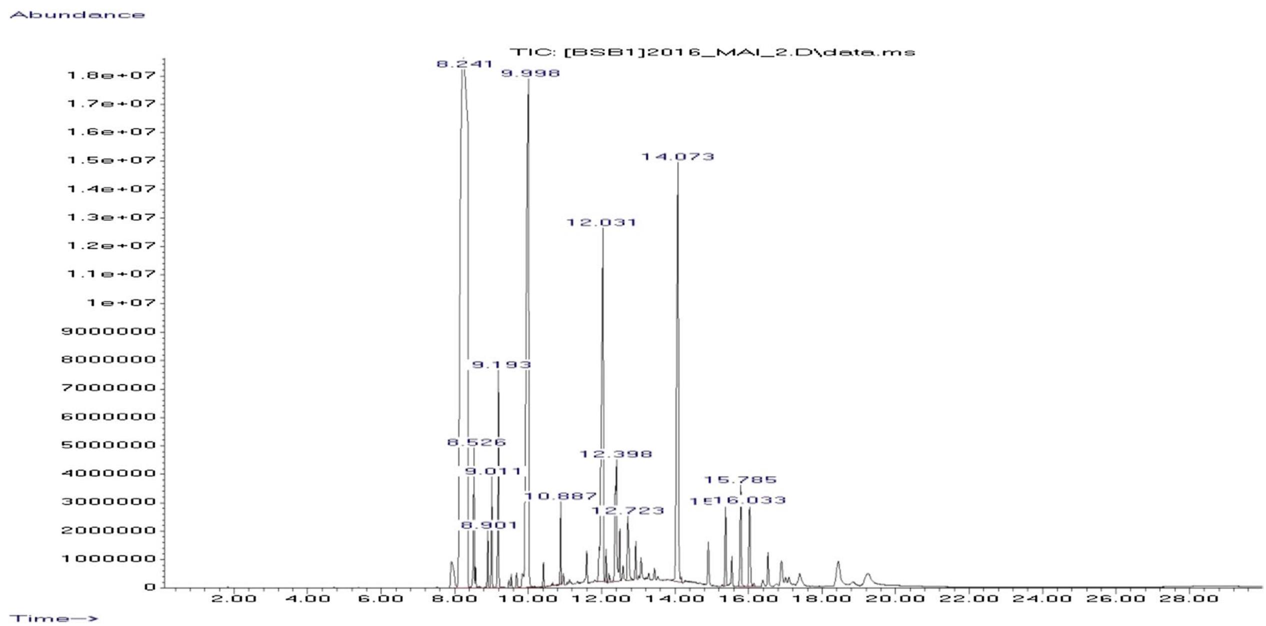

Fig. 1 Chromatographic profile of the essential oil of *Tetraclinis articulata* branches

Table 3 Antibacterial activity of the essential oil tested by disc diffusion assay

Bacterial strains	Zone of inhibition (mm)
Escherichia coli	19.16 ± 0.57
Klebsiella pneumoniae	6.66 ± 0.76
Proteus mirabilis	NA
Pseudomonas aeruginosa	NA
Acinetobacter baumannii	17.16 ± 0.28
Staphylococcus epidermidis	NA
Staphylococcus non aureus	NA
Staphylococcus aureus	9.26 ± 0.30

NA : no activity found

Antibacterial activity

According to the results of the aromatogram shown in Table 3, the essential oil exhibited moderate inhibitory power against *Escherichia coli* and *Acinetobacter baumannii*, which are antibiotic-resistant strains. The essential oil exhibited low activity against *Klebsiella pneumonia* and *Staphylococcus aureus*. The strains *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus epidermidis*, and *Staphylococcus non aureus* showed no sensitivity for the essential oil of branches of *Tetraclinis articulata*.

The MIC values are presented in Table 4. The minimum inhibitory concentration for the two bacteria *Escherichia coli* and *Acinetobacter baumannii* is 2.5 µL/mL. These strains are considered to be the most sensitive by comparison with the other bacteria studied.

The low antimicrobial activity of the essential oil can be explained by its low chemical profile in compounds known for their antimicrobial power, such as certain oxygenated monoterpenes [25, 26].

Our results are in agreement with those obtained by Bourkhiss et al. [15] who tested the same essential oil in vitro against four bacterial and two fungal strains, and only *Staphylococcus aureus* and *Micrococcus luteus* were found sensitive.

The MBC/MIC ratio defines the bacteriostatic or bactericidal character of an essential oil; if this ratio is less than 4, then the oil is considered bactericidal [27].

The MBC/MIC ratio of the essential oil of Thuya branches is between 1 and 2, which confirms that the oil exerts a bactericidal effect (Table 4).

Conclusions

In the present work, we studied the chemical composition and the in vitro antibacterial activity of the essential oil of the branches of *Tetraclinis articulata* (Vahl) Masters of the Eastern Morocco against pathogenic strains of clinical origin. The extraction of the branches by hydrodistillation results in a yield of 0.84 ± 0.01%. For the determination of chemical composition, the essential oil was analysed by gas chromatography coupled with mass spectroscopy. The results show that this oil contains mostly α-pinene (38.75%), limonene (13.24%),

Table 4 Antibacterial parameters (MIC and MBC) of the essential oil of the *Tetraclinis articulata* and their interpretation

Bacterial strains	MIC (μL/mL)	MBC (μL/mL)	MBC/MIC	Interpretation
Escherichia coli	2.5	2.5	1	Bactericidal
Klebsiella pneumoniae	80	80	1	Bactericidal
Acinetobacter baumannii	2.5	5	2	Bactericidal
Staphylococcus aureus	40	80	2	Bactericidal

bornyl acetate (8.78%) and camphor (7.68%). The study of in vitro inhibitor power shows that the essential oil tested has significant antibacterial activity against *Escherichia coli* and *Acinetobacter baumannii*, two strains that are resistant. Generally, the results obtained are promising and open new perspectives in the field of natural applications which can be a viable alternative to synthetic products.

Abbreviations
MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; ESBL: extended-spectrum beta-lactamases.

Author's contributions
Not applicable.

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