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THE COMPOSITION OF THE ESSENTIAL OIL AND THE ANTIMICROBIALAND ANTIFUNQAL ACTIVITIES OF *TETRACLINIS ARTICULATA* (VAHL) MASTERS FROM THE MOROCCAN CENTRAL PLATEAU (MOROCCO)

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Abstract

The Barbary Thuya, *Tetraclinus articulata (Vahl)* Masters, is a coniferous tree endowed with several pharmaceutical., medicinal and therapeutic proprieties and industrial. It is a medicinal and aromatic plant which occupies an area of 565720 ha in the field forrestier marocan recognized by his diversity and its flora and fauna richness. Then, and to especially develop this natural resource of the central plateauau of Morocco in the province of Khénifra (Aguelmous), this scientific study will focus on the chemical composition of oil essential of the leaves of *Tetraclinis Articulata (Vahl)* Masters, and on the antibacterial and antifungal activities.

Indeed, the essential oil of the leaves of Barbary Thuya of the area of Aguelmous is different from that of other areas of the Maghreb and Morocco. It is characterized by the α-Pinene prevalence with 29,5%, of cedrol with 19,22%, β-Phellandrene with 7,65% and of sabinene with 5.38% whereas Bomyl acetate and slightly present with 1,52%. It has another specificity compared to the literature which is the absence of Limonene and camphor. In addition, a study on the antibacterial and antifungal activities of the essential oil of the leaves of Barbary Thuya showed a positive effect on some bacterias Gram-positive and Gram-negative and some pathogenic fungis to humanity and the animals. Then, this resource can be used in pharmacology.

Keywords: *Tetraclinus articulata (Vahl) Master;, Morocco; leaves; Essentiel oil; chemical composition; antimicrobial and antifungal activities.*

Introduction

Tetraclinus articulata (Vahl) Masters occupies about 565720 ha in marocan forest recognized by his diversity and its richness of flora and fauna [1]. It is an endemic plant of the basin Mediterranean and according to Boukhriss et al. (2009) [2] it is endimic of the Western southern Mediterranean. It has a great socio-economic importance; its wood and used in craft industry, essential oils of its wood and its leaves are used in the chemical and pharmaceutical industry [3-4]. Present at the native state in the central plateau in Morocco (Province of Khenifra) various parts of this species are used in medicine traditional [5-6]. The present study will put forward the composition of the essential oil leaves and its antibacterial and antifungal activities.

Materiel and Methods

Plant material:

The vegetable material used in our study is the leaves of Tetraclinis Articulata (Vahl) Masters. It is collected in Mars 2021, of the trees of to the area of Aguelmous (Province of Khenifra) which belongs to the moroccan central plateau. The collected leaves are dried at room temperatue, during two week (15 days).

Essential oil extraction

The extraction of the essential oil of the leaves of *tetraclinis articulata (Vahl)* Masters was made by hydrodistillation by the use of an apparatus of the Clevenger type. Indeed, treatment of 100g of plant material is mixture with a volume of 2 liters distilled water. The volatile part is treated with anhydrous sodium sulphate to be well dried, essential oil then is recovered and stored with the refrigerator with 4°C on the darkness [7].

Chemical analysis of essential oil by chromatography GC-MS

The analysis of the chemical composition of the essential oil of *Tetraclinis articulata (Vahl)* Masters leaves is made by a chromatograph Beijing Elmer Autosystem GC, couple with an automatic injector with two columns polar and non-polar. Helium is the gas used with a flow of 1 ml per minute for each column. The temperature of injection is of 250 °C [8].

Antibacterial and antifungal activities

The method used is that of macrobroth i.e. the method of dilution per tube. It consists of the preparation of different concentration from antibiotic and antifungal with incorporation from various bacteria and fungis. The weakest concentration with which the bacterium or the mushroom appears that is retained equivalent to the MIC (Minimal inhibition of Concentration) [9-11].

Results and discussion

Chemical composition

The chemical composition of the essential oil of the leaves of *Tetraclinis Articulata (Vahl)* Masters by chromatography was identification of 93.22% of the components highlights only two components were predominants α-Pinene and cedrol (Table 1).

The results obtained improve the edaphoclimatic effect on the chemical composition of essential oil of the leaves of *Tetraclinis Articulata (Vahl)* Masters. This resulted on the one hand in the absence of Limonene and Camphor in the essential oil of our settlement of Barbary Thuya of the area of Aguelmous (Khenifra), whereas they represent respectively between 5-23% and 17-19% in the area of Khemisset [2-12], 7% and 11% in the area of Ben Slimane [1], 0.5% and 19% in the area of Tetouan [13], 3% and 22% in the area of Marrakech [14] and 7.3% and 14.5% in the area of Tafoughalt Zegzel (Berkane) [15]. In Algeria the literature brings back same thing for Algeria 6.7% and 16.6% in the area of Ain Defla [16] and in Tunisia Limonene and absent on the other hand camphor represents between 1.43% and 5.6% according to the vegetative stage [17].

In addition, the percentage of the major components constitute the essential oil of the central plateau Moroccans differs from that report/ratio by literature in other areas of the large Maghreb. by the composition of the essential oil of the leaves of Barbary Thuya in major components. Indeed, for our study the present major elements are α-Pinene with 29.5%, cedrol with 19.22%, β-Phellandrene with 7.65% the sabinene evec 5.38% and Bomyl acetate with 1.52%, whereas the major components for other studies are different. For the area of Khemisset the major components are α-

Pinene and the acetate of bomyl with respectivemet 23.54% and 30.74% [12] and 16.8% and 30.6% [1,2] with the area of Ben Slimane found 10.84%, 35.05% and 6.79% for respectively α-Pinene, Bomyl acetate and borneol, of the approximate values to the area of Tetouan [13] with respectively 1.3%, 16.5 and 9.6%, whereas for the area of Marrakech El Jemli et al. (2017) [14] found respectively 7.16, %, 26.81% and 6.40%, and with the area of Tafoughalt Zegzel (Berkane) the study made by Sadiki et al. (2019) [15] shows percentage respective of 22.6%, 16.8% and 5.2%. With regard to Algeria there are a predominance of α-Pinene, Bomyl acetate with 25.3% and 20.6% [16] in Ain Defla. On the other hand, for Tunisia the results resemble those of Ben Slimane with respectively 21.52%, 26.30% and 6.04% [17]. But another study in Tunisia (Tunis) bring back the presence of α-Pinene accounts for 24.9% with one predominance of another component Linalool acetate with 21.44% [18]. Whereas Jlizi et al. (2018) [19], mentioned, with the area of Sidi Bou Said in Tunisia, the presence of cedrol with 13.17%, α-Pinene with 34.4%, 3-carene with 7.32% and Sabinene with 6.79%, whereas Bomyl acetate represents only one small percentage with 0.58% and total absence of camphor and limonene.

Antibacterial activity

The antibacterial activity of the essential oil of *Tetraclinis articulata (Vahl)* Masters leaves fairly weak in comparison with Gentamicin is used like bacteria of positive control. The study was carried out on five bacteria Gram-positive (*Bacillus Cereus, Cutibacterium Acnes, Staphylococcus Aureus, Staphylococcus Epidermidis and Streptococcus Pyogenes*) and two Gram-negative bacteria (*Pseudomonas Aeruginosa and Serratia Marcescens*) (Table 2).

The essential oil of Barbary Thuya leaves has a relatively important inhibiting effect on Streptococcus Pyogenes with the MIC of 174.70 µg/ml compared to the other bacteria studied. On the other hand it has a weak effect on Staphylococcus Epidermidis with the MIC of 312.63 µg/ml. Whereas in against part, our study revealed considerably an important inibitor effect on the bacteria Gram-negatif compared to the bacteria Gram-positive. On the bacteria Gram-negatif study revealed considerably important a inibitor effect

Another study made in Egypt by Ibrahim and Al (2017) [20], carried out in the area of Giza, the reverse of the results of our study shows. Indeed, the essential oil of the aerial part of *Tetraclinis articulata (Vahl)* Masters has a more important ihibitor activity on *Staphylococcus epidermidis* by report/ratio *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Antifungal activity

The antifungal activity of the essential oil of *Tetraclinis articulata (Vahl)* Masters leaves is fairly weak in comparison with Amphotericin B used like fungis of positive control. The study was carried out on Seven fungis (Table 3).

The essential oil of Barbary Thuya leaves has a relatively important inhibiting effect on *Microsporum canis*, *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans* with one MIC respectively of 132.88 µg/ml, 144.82 µg/ml, 154.21 µg/ml and 155.10 µg/ml compared to the other studied fungis. The weakest inhibtrice activity is that on *Microsporum gyspseum*.

Conclusion

The present study was to realize in order to develop this natural resource of the area of Aguelmous province of Khenifra (Morocco), *Tetraclinus articulata (Vahl)* masters. The essential oil of the leaves of Barbary Thuya contains components different from those of the other area of Morocco by the Cedrol prevalence with 19.22% in addition to α-Pinene with 29.5% and the absence of Limonene and Camphre which are present for the other studied areas of Morocco. This difference can be justified by the effect of the edaphoclimatic conditions of the area, the genetic effects of the plants and the ecological influence of the ecosystem. In addition, a study on the antibacterial and antifungal activities of the essential oil of the leaves of Barbary Thuya showed an effect on some bacteria Gram-positive and Gram-negative and some pathogenic fungis to humanity and the animals. Then, this resource can be used for the therapeutic treatment. On those, it is recommended to carry out the valorization of *Tetraclinis articulata (Vahl)* Masters and to make experiments in vivo on laboratory animals in order to determine the level of toxicity of the essential oil of the leaves of this

resource and the period and the amount of treatment.

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Table 1. Chemical composition of essential oil leaves of *Tetraclinis articulata (Vahl)* Masters.

Peak	Compounds	Percentage (%)
1	α-Thujene	2.45
2	α-Pinene	29.5
3	α-Fenchene	0.61
4	β-Phellandrene	7.65
5	β-Pinene	0.88
6	β-Myrcene	3.21
7	α-Phellandrene	0.45
8	3-Carene	1.32
9	4-Carene	2.65
10	o-Cymene	0.87
11	Sabinene	5.38
12	α-Terpinene	1.32
13	α-Terpinolene	2.54
14	Bornyl acetate	1.52
15	Terpinolene	3.97
16	Geranyl acetate	0.44
17	Naphtalene	0.46
18	Caryophyllene	2.87
19	α-Caryophyllene	3.81
20	β-Cubebene	0.74
21	δ-Cadinene	0.46
22	α-Farnesene	0.57
23	Cedrol	19.22
24	Decahydronaphtalene	0.33
Total identified (%)		93.22

Table 2. Antibacterial activities, MIC (µg/mL), of essential oil leaves of *T. articulata (Vahl)* Masters

Bacteria type	Bacterias	MIC (µg/ml)
Gram-positif	*Bacillus Cereus*	245.24
	Cutibacterium Acnes	210.22
	Staphylococcus Aureus	291.46
	Staphylococcus Epidermidis	312.63
	Streptococcus Pyogenes	174.70
Gram-negatif	*Pseudomonas Aeruginosa*	217.15
	Serratia Marcescens	212.76
Positive control	Gentamicin	11.27

Table 3. Antifungal activities, MIC (µg/mL), of essential oil leaves of *Tetraclinis articulata* (Vahl) Masters

Fungi	MIC (µg/ml)
Aspergillus Fumigatus	154.21
Aspergillus Niger	144.82
Microsporum Canis	132.88
Microsporum Gypseum	206.53
Trichophyton Mentagrophytes	184.14
Trichophyton Rubrum	170.92
Candida Albicans	155.10
Amphotericin B	6.74