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Antibacterial Activity of Essential Oil of *Tetraclinis articulata (Vahl) Masters* against Enterobacteriaceae Isolated from Hospital Infections

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ABSTRACT

The proliferation of resistant bacteria has become a major preoccupation in the field of health, which easily justifies the search for new treatments. Natural products constitute a good source for the discovery of new antibacterial agents. It is for this purpose, this work deals with the study of the antibacterial activity of essential oil of the leaves of Tetraclinis articulata (Vahl) Masters against bacterial strains of clinical origin by the disc diffusion method and the broth macrodilution method. The results revealed that the essential oil showed interesting antibacterial activity and is characterized by a bactericidal action against the tested bacteria.

Keywords: *Tetraclinis articulata (Vahl) Masters*, Essential oil, Antibacterial activity, Macrodilution, bactericidal

INTRODUCTION

The rarity of diseases in wild plants is explained by the development of a natural defense system, which enables them to fight against pathogens effectively. To protect against bacteria, fungi and viruses, plants synthesize, constitutively or inducedly, a multitude of antimicrobial molecules [1,2]. The originality of this defense system lies in the exceptional chemical variability of the molecules produced [3]. These last constitute, by the diversity of the structural and functional groups which they display, a vast reservoir of active substances. The geographical situation and the diversity of Morocco's bioclimatic stories offer rich and diverse vegetation, mainly concerning medicinal aromatic plants that grow spontaneously. Some plants give essential oils with antibacterial properties [4-6] and antifungal agents [7-11]. To this end, our work aims to highlight the aromatic and medicinal potential of Moroccan plants, through the study of the antibacterial activity *in vitro* of the essential oil of Barbary thuya, *Tertaclinis articulata (Vahl) Masters*, belonging to the order Pinales, family of Cupressaceae, under family of Callitroidae, genus *Tetraclinis*. It is the only species of this genus in the northern hemisphere [12]. It is a species endemic to North Africa (Morocco, Algeria and Tunisia) with the exception of a few islands in the South-East of Spain and on the island of Malta. In Morocco, this species covers a much wider area which represents 80% of North African *Tetraclinacies* [13]. In phytotherapy, different parts of the plant are used against childhood fevers, gastric pain, severe diarrhea, diabetes, hypertension, etc. [14,15]. This is done with the aim of searching for active natural substances constituting a draft of an in-depth study which would propose an alternative to the problems of resistance and toxicity of the usual antibiotics.

MATERIALS AND METHODS

Microorganisms studied

The bacterial strains tested were: 2 strains of *Escherichia coli*, 3 strains of *Klebsiella pneumoniae*, *Proteus penneri* and *Proteus mirabilis* (Table 1). All strains were provided by the microbiology laboratory of the Military Hospital Moulay Ismail Meknes and have been identified and tested for their sensitivity to antibiotics by the agar diffusion method.

Table 1: Profile of tested bacteria

Bacterial strains	Gram	Profile	Sex	Nature of sampling
Escherichia coli ESBL	Gram-negative bacilli	ESBL	Male	Cytobacterioligical urine exam
Escherichia coli 2	Gram-negative bacilli	Low-Level Penicillinace (LLP)	Female	Cytobacterioligical urine exam
Klebsiella pneumoniae ESBL	Gram-negative bacilli	ESBL	Male	Urinary catheter
Klebsiella pneumoniae 2	Gram-negative bacilli	High-Level Penicillinace Fos-R	Female	Cytobacterioligical urine exam
Klebsiella pneumoniae 3	Gram-negative bacilli	Low-Level penicillinace (LLP)	Female	Vaginal specimen
Proteus penneri	Gram-negative bacilli	High-Level Penicillinace (HLP)	Male	Pus
Proteus mirabilis	Gram-negative bacilli	High-Level Penicillinace (HLP)	Male	Cytobacterioligical urine exam

ESBL: Extended-spectrum Beta-lactamases

Tests for antibacterial activity

The evaluation of the antibacterial activity of the essential oil of the thuja leaves is carried out first by the disk diffusion method, due to its simplicity and its effectiveness in testing the sensitivity of bacteria. The Minimum Inhibitory (MIC) and Bactericidal (MBC) concentrations are estimated by the liquid dilution method to determine the nature of the antibacterial activity of the essential oil (bacteriostatic or bactericidal).

Disc diffusion assay

Isolated colonies were transferred to tubes containing sterile distilled water to have microbial suspensions having turbidity close to 0.5 McFarland (10^8 CFU/ml). Subsequently, the entire surface of the Mueller Hinton agar was inoculated with this bacterial suspension. The sterile disks impregnated with 10 µl of the essential oil were deposited on the surface of the agar. The dishes were incubated for 24 h at 37°C in the oven. Antibacterial activity was determined by measuring the diameter of the inhibition zone.

Broth macrodilution method

Serial dilutions of essential oil in Mueller-Hinton Broth (MHB)-Tween 80 (0.01%, v/v) were prepared in sterilized test tubes, so as to obtain a concentration range of between 80 $mg.ml^{-1}$ and 0.3 $mg.ml^{-1}$. The inoculum to be tested (13 µl, 10^8 CFU/ml) was added to each of the test tubes, which were incubated at 37°C for 24 h and finally centrifuged at 5000 g for 5 min at 20°C. The MIC was then determined from the first test tube devoid of bacterial growth. All determinations were performed in triplicate and a control consisting of MHB with 0.01% (v/v) Tween 80 was included. After determination of the MIC, the samples showing no bacterial growth and the control tube were streaked on MHA agar, which were incubated for 24 h at 37°C. The MBC (%, v/v) of the essential oil is deduced from the first box free of bacteria. The MBC/MIC ratio defines the bacteriostatic or bactericidal character of an essential oil. When this ratio is less than 4, the oil is considered bactericidal [16].

RESULTS AND DISCUSSION

The results of the antibiograms performed for the clinical isolates are shown in Table 2. There are a number of strains that react differently to antibiotics, which shows the mutagenic character of these strains that enables them to acquire antibiotic resistance.

Table 2: Antibiotic susceptibility testing of clinical strains

	***Escherichia coli* ESBL**	***Escherichia coli 2***	***Klebsiella pneumoniae* ESBL**	***Klebsiella pneumoniae* 2**	***Klebsiella pneumoniae 3***	***Proteus penneri***	***Proteus mirabilis***
ClG	R	R	R	R	S	R	R
IMP	S		R				
NFX	R	S	R	S	S	S	S
CIP	S	S	R	S	S	S	S
CTX	R		R				
AMX	R	R	R	R	R	R	R
ATM	R		R				
TCC	R		R				
FOX	R		R				
FEP	R		R				
CAZ	R		R				
AMC	R	R	R	R	S	R	R
CS	S	S	S	S	S	R	R
SXT	R	S	R	S	S	S	S
AN	S	S	S	S	S	S	S
G	R	S	R	S	S	S	S
CRO		S	R	S	S		S
PIP	R	S	R	R	R		R
TIC	R	S	R	R	R	R	R
TOB	R	S	R	S	S	S	S
ETP	S	S	R	S	S	S	S
CXM		R		S	S	S	S
CFM		S		S	S	S	S
CPD		S		S	S		S
FOS	R	S	S	R	S	S	R

ClG: Céfalotine, IMP: Imipénème, NFX: Norfloxacine, CIP: Ciprofloxacine, CTX: Céfotaxime, AMX: Amoxiciline, ATM: Aztreonam, TCC: Co-Ticaclave, FOX: Céfoxitine, FEP: Céfépime, CAZ: Céftazidime, AMC: Amoxi-clavulanate, CS: Colistine, SXT: Co-trimoxazole, AN: Amikacine, G: Gentamicine, CRO: Ceftriaxone, PIP: Pipéracilline, TIC: Ticarciline, TOB: Tobramycine, ETP: Ertapénème, CXM: Céfuroxime, CFM: Céfuxime, CPD: Céfpodoxime, FOS: Fosfomycine, S: Sensitive, R: Resistant

The disc diffusion method allowed us to demonstrate the antibacterial power of the essential oil of the leaves of *Tetraclinis articulata* (Vahl) Masters. The zones of inhibition are shown in Table 3. According to the Sheng-Hsien classification [17], the zones of inhibition indicate that most strains are sensitive to the essential oil of the leaves of *T. articulata* Masters. Disc diameter included: ≥ 8 mm: good activity; 7.5-7.9 mm: average activity; 7-7.4 mm: moderate activity; 6.5-6.9: low activity; ≤ 6.4 mm: no activity [17].

Table 3: Diameters (mm) of the zones of inhibition

Bacterial strains	Inhibition zone (mm)
Escherichia coli ESBL	16
Escherichia coli 2	17
Klebsiella pneumoniae ESBL	9
Klebsiella pneumoniae 2	13
Klebsiella pneumoniae 3	22
Proteus penneri	8.5
Proteus mirabilis	20

In general, the pathogenic germs used in this bio test were sensitive to the essential oil of the leaves of *T. articulata*. Antibacterial activity thresholds are between 20 $\mu l.ml^{-1}$ and 2.5 $\mu l.ml^{-1}$ (Table 4).

The essential oils of *T. articulata* showed good inhibitory effects on some tested microorganisms. The essential oil showed a strong activity against *K. pneumoniae 2* and *P. mirabilis* two bacteria that have a high level of resistance. This essence has an anti-bacterial effect on *E. coli* ESBL. However, *K. pneumoniae* ESBL and *P. penneri* resist to this essential oil. The *in vitro* MIC value indicated that concentration 2.5 µg/ml of essential oil is sufficient to inhibit *E. coli* BLSE, *K. pneumoniae 3* and *P. mirabilis.* The MBC/MIC ratio defines the bacteriostatic or bactericidal character of essential oil. The MBC/MIC ratios for all strains were less than four. It is therefore apparent that the essential oil of the leaves of *T. articulata* has a bactericidal power (Table 5).

Table 4: MICs in µl/ml of the essential oil of *Tetraclinis articulata (Vahl) Masters* leaves on the different bacterial isolates in liquid Miller Hinton

	µl/ml									
	80	40	20	10	5	2.5	1.25	0.6	0.3	C
Escherichia coli ESBL	-	-	-	-	-	-	+	+	+	+
Escherichia coli 2	-	-	-	-	-	-	+	+	+	+
Klebsiella pneumoniae ESBL	-	-	-	+	+	+	+	+	+	+
Klebsiella pneumoniae 2	-	-	-	-	-	+	+	+	+	+
Klebsiella pneumoniae 3	-	-	-	-	-	-	+	+	+	+
Proteus penneri	-	-	-	+	+	+	+	+	+	+
Proteus mirabilis	-	-	-	-	-	-	+	+	+	+

(+): Growth, (-): Inhibition, C: Control

Table 5: Antibacterial parameters (MIC and MBC) of the essential oil of the leaves of *Tetraclinis articulata (Vahl) masters* and their interpretation

Bacterial strains	MIC ($\mu l.ml^{-1}$)	MBC ($\mu l.ml^{-1}$)	MBC/MIC	Interpretation
Escherichia coli ESBL	2.5	2.5	1	Bactericidal
Escherichia coli 2	2.5	2.5	1	Bactericidal
Klebsiella pneumoniae ESBL	20	40	2	Bactericidal
Klebsiella pneumoniae 2	5	5	1	Bactericidal
Klebsiella pneumoniae 3	2.5	5	2	Bactericidal
Proteus penneri	20	20	1	Bactericidal
Proteus mirabilis	2.5	2.5	1	Bactericidal

The antibacterial activity of essential oils depends mainly on their chemical profile. It should be noted that our essence is characterized by the predominance of α-pinene (23.54%), camphor (24.21%), bornyl acetate (17.37%) and borneol (10.32%) [18]. Moreover, all these compounds are known for their antimicrobial properties. Moreover, Camphor the major component of *Lavandula stoechas* essential oil analyzed has antibacterial properties [19,20]. In another study, Felice et al. [21] concluded that camphor and its derivatives can be considered antimicrobial constituents of *Achillea teretifolia* essential oil. In fact, a study by Angioni et al. [22] on the essential oil of the leaves of *Juniperus oxycedrus* showed that it exhibits good inhibitory activity, this essential oil is characterized by the abundance of α-pinene (about 86%). Moreover, bioassays carried out on the essential oils rich in borneol have proved that this compound is endowed with a great antimicrobial power [23]. In addition, borneol and other terpene alcohols are known for their strong antimicrobial power due to their high solubility in water and therefore this gives them a high ability to penetrate the walls of the bacterial and fungal cells [24]. On the other hand, bornyl acetate, can also participate in the registered antibacterial effect, based on the work of Tzakou et al. [25]. Results of the studies previously reported by Bourkhis et al., A. Chikhoune et al. and Djouhari et al., on the same essential oil, they reported the same major compounds (α-pinene, camphor, bornyl acetate and borneol) and proved that these compounds are endowed with a great antibacterial power [26-28]. However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Secondly there is some evidence that minor components have a critical part to play in antibacterial activity, possibly by producing a synergistic effect between other components [29,30].

CONCLUSION

This study shows that the essential oil of the leaves of *T. articulata* exhibits significant antibacterial effects against the microorganisms tested. The different isolated strains do not have a similar sensitivity vis-a-vis the essential oil, most have appeared susceptible. These results can be considered as an important source of information on the antibacterial properties of the essential oil of the leaves of *T. artiulata* of Eastern Morocco, and demonstrate the potential of essential oils for the search for new antibiotics in order to fight the recurring problem of bacterial resistance to antibiotics.

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