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Study of The Antibacterial Activity of the Essential Oil of *Thymus Pallidus* Against Bacteria Isolated from Hospital Infections

F.Z. Sadiki[1*], M. El Idrissi[1], M. Sbiti[2]
[1]*Laboratory of Molecular Chemistry and Natural Substances, Department of Chemistry, Faculty of Sciences Meknes, Moulay Ismail University, Bp. 11201, Zitoune, Meknes, Morocco.*
[2]*Microbiology Laboratory, Military Hospital Moulay Ismail, Meknes, Morocco.*
* Corresponding Author's Email : fatizahrasad@gmail.com

Abstract: Morocco, for its geographical location, offers rich and diverse vegetation. A large number of aromatic and medicinal plants grows spontaneously. The interest in these plants has grown steadily in recent years. Their properties, due in particular to the fraction essential oil, can be used to treat bacterial infections. In the context of the search for natural antibacterial substances, we tested the in vitro antibacterial effect of the essential oil extracted from the aerial part of *Thymus pallidus* against different bacteria responsible for many infections. A strong antibacterial activity of this essential oil against *Escherchia coli* and *Acinetobacter baumanni* was detected.

Keywords: Morocco, *Thymus pallidus*, *Escherchia coli, Acinetobacter baumanni*

INTRODUCTION

The history of medicinal and aromatic plants is closely linked to the evolution of civilizations. In all regions of the world, history shows that man has always used plants for food, clothing, shelter, hunt and treat (Cock, 2011). The knowledge of medicinal preparations and their toxic potential has been transmitted through generations by oral tradition and sometimes transcribed into literature of herbal remedies.

With the appearance of the side effects of synthetic drugs and the increasing resistance of pathogenic microorganisms to conventional antibiotics, much of the scientific research are moving towards the way of the use of active biological extracts Aromatic and medicinal plants.

In addition to viruses and parasites, bacteria are the most frequently implicated microorganisms in food poisoning, and their treatment with chemical antibacterial agents regularly leads to the selection of resistant bacterial strains (Espié *et al.*, 2005; François-Xavier, 2009).

Essential oils, natural products extracted from medicinal and aromatic plants, which are known to be endowed with antiseptic and antimicrobial properties (Haddouchi & Benmansour, 2008). Indeed, many of them have anti-toxic, anti-viral, antioxidant and anti-parasitic properties. More recently they are known to have anti-cancer properties (Valnet, 2005).

Thyme is one of the most widely used herbs as spices and extracts with strong anti-bacterial and anti-inflammatory properties in the traditional pharmacopoeia. Thyme, spontaneous aromatic plant belonging to the Lamiaceae family, is found mainly in the Mediterranean region, Asia, Southern Europe and North Africa (Maksimovic *et al.*, 2008). In Morocco, there are twenty-one species of thyme, 15 of which are endemic (Fennane *et al.*, 2007). Some species of thyme are widely used for their aromatic and medicinal values, and the uses of thyme species largely depend on the composition of the essential oil (Senatore, 1996; Mockute & Bernotiene,

1999; Van den Broucke & Lemli, 1981; Biondi *et al.*, 1993; Panizzi *et al.*, 1993; Janssen *et al.*, 1987; Juliano *et al.*, 2000; Zygadlo *et al.*, 1995; Teissedre & Waterhouse, 2000; Karpouhtsis *et al.*, 1998; Isman *et al.*, 2001).
Thymus pallidus is an endemic plant. It is found in brush, vegetation maquis, on limestone and siliceous substrates and well-drained soils. It populates the High Atlas Oriental. The decoctate of the plant is used to treating gastric disorders, pulmonary affections and chills. Despite their importance, very few studies have been conducted on *Thymus pallidus* species. This study aims to determine antibacterial activity of essential oil of aerial parts of *Thymus pallidus*.

2. Material and methods

2.1. Plant material

Samples of the aerial part (stems, leaves and flowers) of *Thymus pallidus* were collected from the natural population located in the region of Rif, Ras El Ma (Qabouyawa), northeast of Morocco on March 2015. The species was identified by Pr. Abdelilah Rahou, Professor in the Department of Biology, Faculty of Sciences Meknes, Moulay Ismail University, Meknes, Morocco.

2.2. Extraction of essential oil

Extraction of the essential oil of the aerial part *Thymus pallidus* was carried out by hydrodistillation in a Clevenger-type apparatus.

2.3. Antibacterial Activity

2.3.1. Bacterial strains tested

Seven bacterial strains isolated from samples of patients with different infections were tested in vitro, including five Gram negative bacteria (*Escherchia coli*, *Klebsiella* pneumonia, Proteus *mirabilis*, *Acinetobacter baumann*, and *Pseudomonas aeruginosa)* and two Gram positive bacteria (*Staphylococcus Aureus* and *Staphylococcus epidermidis*). They were collected at the Laboratory of Microbiology of the Military Hospital Moulay Ismail of Meknes. These bacteria are pathogenic and are known for their strong antibiotic resistance and invasiveness and toxicity in humans. They are frequently encountered in many infections in Morocco and pose a clinical and therapeutic problem.

2.3.2. Disc diffusion method

The antibacterial activity was determined by the method of diffusion on a solid medium (disk method or aromatogram) based on the principle of the antibiogram. The bacterial suspension (10^8 CFU / mL) is seeded on the surface by swab on Mueller-Hinton agar (MHA) in a Petri dish. Disks 6 mm in diameter were impregnated with 10 µl of the essential oil and placed in the petri dishes. Then, they were incubated for 24 h at 37 ° C. The diameters of the inhibition were measured in millimeters.

2.3.3. Determination of MIC and MBC

Serial twofold dilutions of each essential oil in Mueller-Hinton broth (MHB, Oxoid) were prepared in sterilized test tubes So as to obtain a concentration range between 80 mg. mL^{-1} and 0.3 mg. mL^{-1}. To enhance oil distribution, Tween 80 detergent was included in all assays at a final concentration of 0.01% (v/v). The inoculum to be tested (13 µL, 10^8CFU/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 were streaked on MHA agar plates, which were incubated for 24 h at 37°C. The MBC of the essential oil is derived from the first bacterium devoid of box.

The MBC / MIC ratio is used to distinguish the bactericidal effect (CMB / MIC <4) or bacteriostatic (CMB very distant from MIC) from the essential oil.

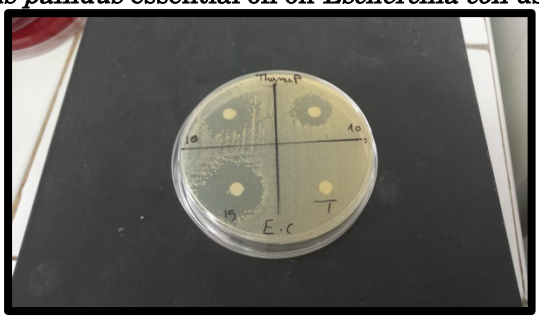
3. Results and discussion

The results of the antibacterial testing are summarized in Table 1. The oil of *Thymus pallidus* shows an interesting antibacterial activity especially against *Escherchia coli* and *Acinetobacter baumanni*. Indeed, *Acinetobacter baumanni*, a strain frequently resistant to numerous antibiotics and which is responsible for epidemics of nosocomial infections, has a high sensitivity to this oil. This great antibacterial activity can be linked due to the majority compounds.

Table 1: zone of inhibition (mm) of the essential oil of *Thymus pallidus*.

Bacterial strains	Inhibition zone (mm)
Escherchia coli	17
Klebsiella pneumoniae	13
Proteus mirabilis	12
Acinetobacter baumanni	19
Pseudomonas aeruginosa	10
Staphylococcus epidermidis	12
Staphylococcus aureus	10

Figure 1: Effect of *Thymus pallidus* essential oil on *Escherchia coli* using disc diffusion method



The MICs and CMBs determined for each of the tested microorganisms are summarized in Table 2. The essential oil of *Thymus pallidus* has been shown to be active on most bacterial strains.

In general, Gram-positive bacteria are more sensitive than Gram-negative bacteria, most of studies reported that Gram-negative organisms were slightly less susceptible than Gram-positive bacteria (Burt, 2004; Nedorostova *et al.*, 2009; Deba *et al.*, 2008). But in our case it was found that the most sensitive strain was *Acinetobacter baumanni* (Gram-) with MIC of 1.25 µl.ml^{-1} and *Escherchia coli* (Gram-) with MIC of 2.5 µl.ml^{-1}.

The MBC / MIC ratio defines the bacteriostatic or bactericidal character of an essential oil. When this ratio is less than 4, the essential oil is considered to be bactericidal (Éberlin, 1994). In this study, the MBC/MIC ratios of *Thymus pallidus* oil are less than 4 for all bacterial strains studied. This essential oil therefore seems to exert a bactericidal action against all the bacterial strains tested.

Table 2: MICs, MBCs and MBC/MIC ratios of essential oil of *Thymus pallidus*

Bacterial strains	MIC (µl.ml^{-1})	MBC (µl.ml^{-1})	MBC/MIC	Interpretation
Escherchia coli	2.5	2.5	1	bactericidal
Klebsiella pneumoniae	5	5	1	bactericidal

Proteus mirabilis	5	5	1	bactericidal
Acinetobacter baumanni	1.25	1.25	1	bactericidal
Pseudomonas aeruginosa	20	40	2	bactericidal
Staphylococcus epidermidis	5	5	1	bactericidal
Staphylococcus aureus	20	20	1	bactericidal

4. Conclusion

According to these results, the essential oil of *Thymus pallidus* showed an important antibacterial activity in vitro on highly pathogenic bacteria. Our results confirm the existence of increasingly recognized antibacterial properties of certain medicinal species of the traditional pharmacopoeia.
The demonstration of the performance of this essential oil on different bacterial strains can lead to a thorough study and prospects for their application as a phytomedication agent.

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