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Antibacterial Effect of Essential Oil of *Thymus hyemalis* against Five Bacterial Strains Isolated from Hospital Infections

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Abstract: *This work aims to study the antibacterial activity of the essential oil of aerial parts of Thymus hyemalis in order to search for new bioactive products of natural origin with antibacterial activities. Five bacterial strains of clinical origin were tested: Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and Staphylococcus aureus. Two methods have been used to evaluate the antibacterial effect of this essential oil: the disc diffusion method and the macrodilution method which aims to determine the minimum inhibitory concentration. Our results indicate that Thymus hyemalis oil has shown significant activity against the Escherichia coli strain, and is characterized by a bactericidal action against the inhibited germs.*

Keywords: *Thymus Hyemalis, Essential Oil, Klebsiella Pneumoniae, Proteus Mirabilis, Pseudomonas Aeruginosa*

INTRODUCTION

The therapeutic virtues of aromatic and medicinal plants have been experimented for centuries, and their precious virtues have been transmitted orally from generation to generation or recorded in old writings. The reputable remedies have prevailed despite the development of modern medicine that has marginalized the use of natural medicinal techniques. In all regions of the world, the history of peoples shows that these plants have always occupied an important place in medicine. Indeed, even nowadays, despite the progress of pharmacology, the therapeutic use of medicinal plants is very present in some countries of the world and especially in developing countries, in the absence of a modern medical system.

Microbial infections are diseases caused by the development, in humans or animals, of bacteria or yeasts. Over the last decade, there has been a great deal of interest in the study of microbes, from a biological, nosological and therapeutic point of view. This importance given to the study of microbial diseases follows the appearance of the resistance of the strains to the most commonly used drugs and the complications that these germs produce in patients with a particular clinical profile (Balkis *et al., 2002.*; Juj *et al.*, 2002; Ramage *et al.*, 2002, Rodero *et al.*, 2002). Thus microbial infections formerly described as ordinary, are now classified as serious infections that can cause a high rate of mortality and morbidity in immunocompromised patients and

diabetics. Antimicrobial chemotherapy has shown great efficacy against superficial infections. However, profound damage remains the most difficult to treat given the cytotoxicity of systemic antimicrobials.

The development of new therapeutic agents is essential to fight against the phenomenon of microbial resistance. For this purpose the investigation of plants represents an inestimable potential for the discovery of new antimicrobial substances. For this interest, essential oils considered bioactive natural substances are a good choice in the discovery of new therapeutic molecules, and attract the interest of many researches given the number of their countable biological properties.

They are being studied for their possible use as an alternative of synthetic products in the treatment of infectious diseases. Indeed, several studies have highlighted the different biological activities of essential oils, especially their antimicrobial powers (Sadiki *et al.*, 2017, 2018a, 2018b; Joshi *et al.*, 2018; Diao *et al.*, 2014; Calo *et al.*, 2015).

Thyme, a 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 of which 15 are endemic (Fennane et al., 2007). The whole plant is widely used in traditional medicine; their essential oils are widely used in alternative medicine due to their antiseptic, antispasmodic and antimicrobial properties (Panizzi *et al.*, 1993). *Thymus hyemalis* Lange, winter thyme, is normally present in siliceous and calcareous extensions, from sea level to 400-700m above sea level. It is able to resist long dry periods, but its winter flowering condition (November through May) does not allow it to grow in areas having very cold weather. The chemical compositions of essential oil of *T. hyemalis* has previously been reported (Rota *et al.*, 2008, Martinez RM et *al.*, 2005; Martinez S *et al.*, 2006; Goodner *et al.*, 2006; Saez, 1995; Jordan *et al.*, 2006; 2003), thymol, carvacrol, borneol and linalool were the most components abundant in this area.

The present work aims to determine the antibacterial activity of the essential oil extracted from the aerial part of *Thymus hyemalis* collected from the eastern region of Morocco. To the best of our knowledge, the antibacterial activity of the essential oil of *Thymus hyemalis* of Morocco has not been studied before.

Materials and Methods

Plant material

The aerial parts of *Thymus hyemalis* were collected in March 2015 from Ras El Ma (Qabouyawa) (35 ° 08 '10 "N, 2 ° 25' 30" W) located in the region of Rif, in the northeast of Morocco.

Extraction of essential oil

The extraction of the essential oils was carried out by hydrodistillation in a Clevenger type apparatus (Clevenger, 1928). The obtained collected essential oil was kept in the dark at 4 ° C until further use.

Microbial strains

The microbiological material consists of five bacterial pathogenic strains, responsible for certain serious infectious diseases: *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis Pseudomonas aeruginosa* and *Staphylococcus aureus*. The strains were isolated from samples of clinical origin; they were provided from the microbiology laboratory of Moulay Ismail military hospital.

Tests of antibacterial activity

- **Disc diffusion method**

The antibacterial properties of the essential oil were evaluated in vitro, first, by the agar diffusion 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 hours. After incubation,

an area or clear halo is present around a disc if the essential oil inhibits bacterial growth (Oumzil *et al.*, 2002). All tests were repeated three times.

- **Broth dilution method (Determination of MIC and MBC)**

The minimum inhibitory concentrations (MIC), is the lowest concentrations capable of inhibiting any bacterial growth, were determined by the method of macro-broth dilution (Guinoiseau, 2011). A cascade dilution was performed in Mueller Hinton Broth-Tween 80 (0.01%, v/v), so as to obtain a concentration range between 80 $\mu L.mL^{-1}$ and 0.3 $\mu L.mL^{-1}$. 13 µL of a bacterial inoculum, equivalent to the standard density of 0.5 McFarland (10^8 $UFC.mL^{-1}$), 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 hours. The MIC was determined by the lowest concentration of essential oil giving a growth inhibition.

The minimum bactericidal concentration (MBC) was determined from the MIC (Guinoiseau, 2011). The tubes showing no visible growth with the naked eye after incubation and the control tube were streaked on MHA. The inoculated dishes were incubated for 24 hours at 37 °C. The MBC is the lowest concentration that shows no bacterial growth. The MBC/MIC ratio defines the bacteriostatic or bactericidal character of an essential oil; if this ratio is less than 4, the oil is considered bactericidal (Guinoiseau, 2011).

Results and Discussion

The results of the zones of inhibition shown by the essential oil of *Thymus hyemalis* are presented in Table 1.

The essential oil of *T.hyemalis* has been tested for its antibacterial properties against five pathogenic micro-organisms for humans; it has proved a strong inhibition against Escherichia coli, with a significant minimum inhibitory concentration value, which was 1.25 µL/mL (Table 2). The *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains were moderately inhibited. However, the essential oil of *Thymus hyemalis* is ineffective against *Klebsiella pneumoniae* and *Proteus mirabilis*, two bacteria with a resistant profile.

The MBC/MIC ratio of the essential oil of *Thymus hyemalis* is between 1 and 2, which confirms that the oil exerts a bactericidal effect (table 3).

Moreover, Tepe *et al.*, tested the antibacterial activity of the essential oil from aerial parts of *Thymus hyemalis* against a panel of microorganisms including *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Enterobacter aerogenes* ATCC 13048, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Listeria monocytogenes* ATCC 19115, *Pseudomonas fluorescens* ATCC 49838, and *Proteus mirabilis* ATCC 25933. No activity was observed against *E. aerogenes, K. pneumonia, P. aeruginosa, L. monocytogenes* and *P. fluorescens*. The most sensitive microorganisms were determined as *B. cereus* and *B. subtilis* with a MIC value of 31.25 mg/mL. This activity was followed by *E. faecalis, S. aureus* (62.50 mg/mL) (Tepe *et al.*, 2011).

Many herbs and their essential oils show antimicrobial activity that could prevent the growth of pathogenic microorganisms, thereby improving food safety.

The antimicrobial activity of essential oils is mainly related to their chemical composition, especially their major volatile compounds. So far, there is no study that can give us a clear and precise idea of the mode of action of essential oils.

Given the complexity of their chemical composition, there is every reason to believe that this mode of action is rather complex and difficult to determine from a molecular point of view. It is very likely that each of the constituents of the essential oils has its own mechanism of action.

Table 1: Antibacterial activity of the essential oil *Thymus hyemalis* by the disk diffusion method.

Bacterial Strains	Zone of inhibition (mm)
Escherichia coli	22.16 ± 0.57
Klebsiella pneumoniae	N.A
Proteus mirabilis	N.A
Pseudomonas aeruginosa	12.26 ± 0.30
Staphylococcus aureus	13.7 ± 0.6

N.A: no activity found.

Table 2. MICs of the essential oil of *Thymus hyemalis* in Mueller Hinton broth

[C] (µL/mL) Bacterial strains	80	40	20	10	5	2.5	1.25	0.6	0.3	C
Escherichia coli	-	-	-	-	-	-	-	+	+	+
Pseudomonas aeruginosa	-	-	-	-	-	+	+	+	+	+
Staphylococcus aureus	-	-	-	-	-	-	+	+	+	+

(+): Growth, (-): Inhibition, C: Control, [C]: Concentration

Table 3. Antibacterial parameters (MIC and MBC) of the essential oil of the *Thymus hyemalis* and their interpretation

Bacterial strains	MIC (µL/mL)	MBC (µL/mL)	MBC/MIC	Interpretation
Escherichia coli	1.25	2.5	1	Bactericidal
Pseudomonas aeruginosa	5	5	1	Bactericidal
Staphylococcus aureus	2.5	2.5	1	Bactericidal

Conclusion

Bacteria are responsible for many diseases. Their resistance to antibiotics is becoming more pronounced. To stop this process of synthesis-resistance, it is necessary to look for another approach to reduce or eliminate diseases without the use of synthetic products, so it is obvious to find solutions through the use of bioactive molecules that are herbal. It is in this context that we have studied the antibacterial activity of the essential oil of *Thymus hyemalis* from eastern Morocco. The evaluation of the antibacterial activity showed an effectiveness of the essential oil of the aerial parts against the bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, tested by the method of diffusion in solid medium and macrodilution in broth medium.

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