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Evaluation of the Bacteriostatic and Bactericidal Activity of Essential Oil of *Thymus Satureioides*

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Abstract: *Essential oils have significant antimicrobial activities and can successfully replace antibiotics that show their inefficiencies against resistant microorganisms [1]. The objective of this study is to evaluate the antibacterial activity of essential oils* extracted *from the leaves and flowering tops of Thymus satureioides against three gram-negative bacteria (Escherichia coli, Acinetobacter baumannii and Enterobacter cloacae) and two gram-positive bacteria (Bacillus cereus* and *Staphylococcus aureus*). *The extraction is carried out by hydrodistillation using a Clevenger-type apparatus with an average yield of 3.4%. The antibacterial activity is highlighted by the diffusion method on agar medium. A Strong inhibitory activity against bacteria isolated was recorded. However, the bactericidal activity is remarkable against all strains tested except Bacillus cereus.*

Keywords: *Essential oil, Thymus satureioides, Antibacterial activity*

1. INTRODUCTION

Microorganisms are the concealed enemies to the mankind. They are small but cause a very profound damage in human body as well as other living organism. The agents, which have the capacity to kill the microbes or arrest the multiplication, are called the antimicrobial agents or drugs. There are a lot of antimicrobial drugs of which some are discovered or established and some are hidden in the nature. Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management [2, 3].

However, the development of resistance of pathogenic human bacteria to drugs commonly antibiotics required a search for new antimicrobial substances from other sources, including plants [4]. Among the extracts of natural plants are essential oils obtained from many aromatic and medicinal plants. These have many biological activities.

In Morocco, *Thymus satureioides*, known as "za-itra" is one of the most popular herbs, used extensively in traditional medicine. It is considered as an important flavoring agent, commonly used in herbal tea, very much appreciated for its tonic and stimulant properties [5]. In addition, the species is used in the cosmetic and perfume industries, and also for the preservation of many food products [5]. *T. satureioides* is widespread in the arid and semi-arid parts of the Moroccan mountains, at altitudes ranging from 600 to 2100 m [6].

In terms of its medicinal properties, it has been reported that *T. satureioides* possesses numerous pharmacological and biological properties including analgesic [7], anti-inflammatory [8], antispasmodic [9], microbicide [10, 11]; larvicide, and antioxidant activities [12].

The main objective of this study is to examine the antimicrobial activities of *Thymus satureioides* essential oils on the growth of bacterial strains known by their effects on urinary system, intestinal, respiratory and other.

2. MATERIAL AND METHODS

2.1. Plant Material

The leaves and flowering tops of *Thymus satureioides* were harvested at blooming stage in Taltemssen region (south region of Agadir) during the month of May, then they are air-dried at room temperature in the shade for a week.

2.2. Microorganisms Used and Growth Conditions

In this study, the used microorganisms are: *Escherichia coli, Staphylococcus aureus, Acinetobacter baumannii, Enterobacter cloacae* and *Bacillus cereus*. The selected strains in this study are the cause of several diseases (urinary, intestinal, respiratory, etc ...). The bacterial strains are grown and maintained in nutrient agar for 24h in the dark at 37°C.

2.3. Extraction of Essential Oils

The essential oils were obtained by steam distillation in a Clevenger-type apparatus, 100 g of vegetal material accurately weighed and transferred to 1 liter distillation flask (Clevenger Apparatus) together with 600ml of distilled water and heated to boiling during 3-4 hours. The obtained oils were dehydrated using Sodium sulfate (Na2SO4) and stored in a refrigerator at 4 ° C in dark bottles to protect them from light and heat. .The essential oil yield is estimated according to the dry vegetal matter.

2.4. Methods for Evaluating the Antibacterial Activity

Two methods were used for this study:

2.4.1. Diffusion disk method

In this method, Mueller Hinton is seeded with a bacterial suspension of 24h [13]. The sterile filter-paper discs of 6mm, previously impregnated with essential oils is deposited on the agar by means of sterile tweezers.

After 24 hours of incubation, if the essential oils inhibited the growth of the organisms, a clear halo is observed around the discs. More the zone around the disk is large, more the germ is sensitive [14]. All tests were repeated three times.

2.4.2. Agar dilution method

Dilutions of the essential oils are prepared 1/10, 1/25, 1/50, 1/100, 1/200, 1/300 and 1/500 in 0.2% agar solution. In each test tube containing 13.5 ml of Mueller-Hinton sterilized at autoclaved and cooled at 45 ° C, we add aseptically 1, 5 ml of each of the dilutions so as to obtain the final concentrations of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 (v / v). We properly agitate the tubes to disperse the essential oils in the culture medium before pouring into Petri dishes. The control cultures containing the culture medium (Muller Hinton) plus the agar solution at 0.2% alone are also prepared.

The lowest concentration of essential oils inhibiting any growth visible to the naked eye after 16 to 20 hours of incubation at 37 ° C is the minimum inhibitory concentration (MIC) [14].

We determine the minimum bactericidal concentration (MBC) by seeding a sample of the Petri dish having no growth of bacteria on agar Mueller-Hinton. The lowest concentration of essential oils to which 99.99% of the bacteria are killed after 24 hours of incubation at 37 ° C corresponds to the MBC [14]. Each test was repeated three times to minimize the experimental error.

3. Results and Discussion

3.1. Organoleptic Properties and Yield

The essential oils are yellow, with a liquid appearance and a very aromatic odor. The yields of essential oils from the dry material are 2.7% and 4.1% respectively for the leaves and flowering tops of *Thymus satureioides*. These yield of oils obtained, are considered significant compared to that given by El Bouzidi et al (2013) and Kasrati et al. (2014), obtained from the aerial parts 1.86% and 1.40% respectively [15,16].

The reasons for this variability can be explained by difference of the environmental conditions (climate and geographic location), the period of harvest and distillation technique [17, 18, 19].

3.2. Antibacterial Activity

The antibacterial activity was determined by measurement of inhibition zones (diameter in mm) produced around the discs after incubation.

The experimental results presented in the table 1 show that the essential oils of *Thymus satureioides* are active on all the tested strains. The largest zone of inhibition is observed in case of *Staphylococcus aureus* by the essential oil of flowering tops (45mm).

Table1. *Antibacterial activity of Thymus satureioides essential oils*

Organisms	Zone of inhibition (diameter in mm)	
	Essential oil of leaves	Essential oil of flowering tops
Staphylocoque aureus	30,7	45
Esherichia coli	15,5	20
Enterobacter cloacae	20	22
Acinetobacter baumannii	19	35
Bacillus cereus	16	38

According to the diameters of inhibition produced by these two oils, the inhibitory effect was confirmed by determining the minimum inhibitory concentration (MIC), the results obtained are summarized in Tables 2 and 3.

This method shows that both essential oils of *Thymus satureioides* has significant antibacterial activity on the tested strains. However, the studied microorganisms did not show the same sensitivity against the used essential oils.

In the case of essential oil of leaves, the strains *Enterobacter cloacae, Acinetobacter baumannii* and *Bacillus cereus* showed the same degree of inhibition 1/500 v/v/. While *Escherichia coli* and *Staphylocoque aureus* were inhibited at concentrations of 1/100 v/v and 1/250 v/v respectively.

In return, for the essential oil of the flowering tops, the concentration of 1/3000 v/v was sufficient to stop the growth of *Enterobacter cloacae* (gram -), followed by *Acinetobacter baumannii* (gram -) and *Bacillus cereus* (gram +) that were inhibited at the minimum inhibitory concentration of 1/2000 v/v. In the contrary, *Staphylococcus aureus* (gram +) and *Escherichia coli* (gram -) were inhibited by the essential oil at concentration of 1/1000 v/v.

Table2. *The minimum inhibitory concentration (MIC) of the essential oil from the leaves of Thymus satu reioides*

Organisms/Concentration (v/v)	1/100	1/250	1/500	1/1000	1/2000	1/3000	1/5000	C
Staphylocoque aureus	_	_	+	+	+	+	+	++
Esherichia coli	_	+	+	+	+	+	+	++
Enterobacter cloacae	_	_	_	+	+	+	+	++
Acinetobacter baumannii	_	_	_	+	+	+	+	++
Bacillus cereus	_	_	_	+	+	+	+	++

C: control cultures (-): inhibition (+): growth

Table3. *The minimum inhibitory concentration (MIC) of the essential oil from the flowering tops of Thymus satureioides*

Organisms/Concentration (v/v)	1/100	1/250	1/500	1/1000	1/2000	1/3000	1/5000	C
Staphylocoque aureus	_	_	_	_	+	+	+	++
Esherichia coli	_	_	_	_	+	+	+	++
Enterobacter cloacae	_	_	_	_	_	_	+	++
Acinetobacter baumannii	_	_	_	_	_	+	+	++
Bacillus cereus	_	_	_	_	_	+	+	++

C: control cultures (-): inhibition (+): growth

The results of the minimum bactericidal concentration presented in Table 4 shows that the essential oils has a bactericidal power against all studied strains except *Bacillus cereus*.

In the case of *Escherichia coli*, the action of the essential oil is directly bactericidal (MIC = MBC).

Table4. *Minimum bactericidal concentrations (MBC) of Thymus satureioides essential oils*

Organisms	*Staphylococcus*	*Esherichia coli*	*Enterobacter*	*Acinetobacter*
MBC (V/V) of leaves essential oil	1/100	1/100	1/100	1/250
MBC (V/V) of flowering tops essential oil	1/500	1/1000	1/1000	1/500

4. CONCLUSION

This work is devoted to determine the yield, antibacterial properties of essential oils extracted by hydro-distillation from leaves and flowering tops of *Thymus satureioides,* harvested in the region Taltemssen.

The average yield of essential oils of the studied plant was 3.4%. This is a very interesting result. The chosen technique is correct. The yield is however significant and can be profitable on an industrial scale.

The study of the antibacterial activity of essential oils on gram-positive bacteria and gram negative bacteria showed the presence of a strong antibacterial activity especially against *Enterobacter cloacae, Acinetobacter baumannii* and *Bacillus cereus.*

The results obtained in this study show that the essential oil of flowering tops of *Thymus satureioides* inhibits bacterial growth more than its leaves.

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