Antimicrobial Organic and Aqueous Extracts of the Leaves of Thymus Numidicus, Collected from Bouira (Algéria)

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***Abstract*—** Aromatic and medicinal plants are an important source of antimicrobial molecules, especially in volatile extracts. The genus Thymus is considered among the richest in essential oils. In this context, this work focuses on the extraction of the organic compound by steam soxlhet of a species endemic; Thymus numidicus L. from Bouira. On the other hand, the determination of the antimicrobial activity of this organic compound. In this study, we have used these microorganisms; Echerichia coli, Pseudomonas fluorescence, Staphylococcus aureus, Aspergillus fumigatus, Candida albicans, and Aspergillus niger. The antimicrobial activity was tested by agar diffusion technique and calculation of the minimum inhibitory concentrations (MICS). The results showed a very good antimicrobial activity on the bacteria and no effect on yeasts and molds tested.

***Index Terms*—** Antimicrobial Activity, Microorganisms, Organic Compound, Thymus Numidicus.

I. Introduction

A large number of plants known to have very valuable biological properties is applied in pharmacy, cosmetology and agriculture [1]. According to the same author, these plants are a new source of active compounds called metabolites such as alkaloids and derivatives, terpenes, steroids, polyphenolic compounds and essential oils. According to [2], over 5000 natural substances have revealed to be useful in traditional medicine for the prophylaxis and treatment of diseases. In order to enhance the use of Algerian plants, interest was given to in T. numidicus L. This work aims to study the antimicrobial activity of different organic and aqueous extracts of T. numidicus.

II. Experiment

A. Material and Methods

MA non-microbiological material (glasses) as well as a biological one are used.

The part of the plant taken into account is the aerial one, which are rich in natural substances [3]. The antimicrobial effect of extracts of T. numidicus. is evaluated on many microorganisms. It was tested on tree bacterial strains: Echerichia coli, Pseudomonas fluorescence, Staphylococcus aureu. It was studied using one kinds of yeast: Candida albicans. Finally, one mould is used.

The methods used in the study concerns the obtaining of plant extracts as well as the antimicrobial power of these extracts.

Concerning the extraction of the plant substances from T. numidicus, we have adopted the method of Soxhlet advocated by [4]. It permits to obtain excellent results and has a remarkable reputation when it is applied in the vegetable field. This extraction is done using different solvents which are water, ethanol, methanol, chloroforme, hexane, ether of petrol, acetate of ethyle, and butanol.

The antimicrobial activity of extracts taken from T. numidicus. seeds is determined by the method of diffusion in an agar environment cited by [5, 6].The first step is the preparation of the microbial strains. It is followed by an antibiogram. This method has the advantage of being very flexible in the choice of the tested antibiotics, to be applied on a big number of bacterial species, and to be largely evaluated during 50 years of world usage [7].

III. Results And Discussion

Evaluation of the antimicrobial activity of plant extracts

Qualitative study of the antimicrobial effect of extracts

The results of the measurement of the diameters of the inhibition zones are summarized in the tables ranging from (1 to 8).

TABLE I
RESULTS OF THE QUALITATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACT WITH REGARD TO THE TESTED STRAINS

Strains tested	Gram	D. of 1st test	D. of 2nd test	Average D.
E. coli	Gram(-)	11	12	11,5
P. fluorescence	Gram(-)	11	10	10,5
S. aureus	Gram(+)	13	9	11
A. fumigatus	/	9	9	9
A. niger	/	9	9	9
C. albicans	/	9	9	9

According to table 1, it is noted that the water extract of T. numidicus has a slight activity on all the tested bacterial strains. Thus, it has no effect on the studied yeasts and molds.

From table 2, the methanol extract of T. numidicus has a slight activity on E. coli. While P. fluorescence is moderately inhibited by the action of methanolic extract. While S. aureus, is strongly inhibited by the action of this extract. Thus, no effect is mentioned on the studied yeasts and molds.

TABLE II
RESULTS OF THE QUALITATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT WITH REGARD TO THE TESTED STRAINS.

Strains tested	Gram	D. of 1st test	D. of 2nd test	Average D.
E. coli	Gram(-)	11	12	12
P. fluorescence	Gram(-)	11	20	12
S. aureus	Gram(+)	13	19	25
A. fumigatus	/	9	9	9
A. niger	/	9	9	9
C. albicans	/	9	9	9

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TABLE III
RESULTS OF THE QUALITATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF HEXANIC EXTRACT WITH REGARD TO THE TESTED STRAINS.

Strains tested	Gram	D. of 1st test	D. of 2nd test	Average D.
E. coli	Gram(-)	11	13	12
P. fluorescence	Gram(-)	19	11	15
S. aureus	Gram(+)	23	18	20.5
A. fumigatus	/	9	9	9
A. niger	/	9	9	9
C. albicans	/	9	9	9

It is noted that S. aureus is strongly inhibited by the action of the extract. E. coli is slightly inhibited by the action of the hexanic extract. P. fluorescence is moderately inhibited by the action of this solution. This extract has no effect on the yeasts and molds.

TABLE IV
RESULTS OF THE QUALITATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF CHLOROFORMIC EXTRACT WITH REGARD TO THE TESTED STRAINS.

Strains tested	Gram	D. of 1st test	D. of 2nd test	Average D.
E. coli	Gram(-)	11	13	12
P. fluorescence	Gram(-)	19	19	15
S. aureus	Gram(+)	28	27	27.5
A. fumigatus	/	9	9	9
A. niger	/	9	9	9
C. albicans	/	9	9	9

It is noted that S. aureus is strongly inhibited by the action of the extract. E coli is slightly inhibited by the action of the chloroform extract. And P. fluorescence is moderately inhibited by the action of this solution. This extract has no effect on the yeasts and molds

TABLE V
RESULTS OF THE QUALITATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF PETROLEUM ETHER EXTRACT WITH REGARD TO THE TESTED STRAINS.

Strains tested	Gram	D. of 1st test	D. of 2nd test	Average D.
E. coli	Gram(-)	13	12	12
P. fluorescence	Gram(-)	15	15	15
S. aureus	Gram(+)	30	10	20
A. fumigatus	/	9	9	9
A. niger	/	9	9	9
C. albicans	/	9	9	9

According to the table cited above, E. coli and P. fluorescence are slightly inhibited by the action of the According to the table cited above, E. coli and P. fluorescence are slightly inhibited by the action of the extract of petroleum ether. While S. aureus is highly inhibited by the action of the extract. Thus, it has no effect on the studied yeasts and molds. While S. aureus is highly inhibited by the action of the extract. Thus, it has no effect on the studied yeasts and molds.

TABLE VI:
RESULTS OF THE QUALITATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF ETHYL EXTRAT EXTRACT WITH REGARD TO THE TESTED STRAINS.

Strains tested	Gram	D. of 1st test	D. of 2nd test	Average D.
E. coli	Gram(-)	11	12	11.5
P. fluorescence	Gram(-)	14	15	14.5
S. aureus	Gram(+)	22	21	21.5
A. fumigatus	/	9	9	9
A. niger	/	9	9	9
C. albicans	/	9	9	9

S. aureus is strongly inhibited by the action of the ethyl acetate extract. While P. fluorescence and E. coli are slightly inhibited by the action of the acetate ethyle extract. This solution has no effect on the yeasts and molds.

TABLE VII
RESULTS OF THE QUALITATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRAT WITH REGARD TO THE TESTED STRAINS.

Strains tested	Gram	D. of 1st test	D. of 2nd test	Average D.
E. coli	Gram(-)	13	13	13
P. fluorescence	Gram(-)	23	21	22
S. aureus	Gram(+)	15	14	14.5
A. fumigatus	/	9	9	9
A. niger	/	9	9	9
C. albicans	/	9	9	9

Through table 7, P. fluorescence is strongly inhibited by the action of the ethanol extract. While S. aureus and E. coli are slightly inhibited by the action of ethanolic extract. Thus, no effect is mentioned on the studied yeasts and molds.

TABLE VIII
RESULTS OF THE QUALITATIVE EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF BUTANOLIC EXTRAT WITH REGARD TO THE TESTED STRAINS.

Strains tested	Gram	D. of 1st test	D. of 2nd test	Average D.
E. coli	Gram(-)	10	12	11.5
P. fluorescence	Gram(-)	11	15	13
S. aureus	Gram(+)	27	23	25
A. fumigatus	/	9	9	9
A. niger	/	9	9	9
C. albicans	/	9	9	9

D: Diameter D: mm

S. aureus is strongly inhibited by the action of this extract. Thus E. coli and P. fluorescence are slightly inhibited by the action of the extract. Thus, no effect is mentioned on the studied yeasts and molds.

Through this study, it is mentioned the sensitivity of S. aureus to extracts organic either chloroform, hexamic, methanolic, ethanolic, petroleum ether and butanol. Thus, a slight inhibition is reported for Gram (-) E. coli and P. fluorescence. These Gram (-) have a high potential for resistance against the antimicrobial action of the organic extract of T. numidicus.

It should be noted that the plant extracts have antimicrobial properties more or less pronounced. Indeed, because of their lipophilic character, their constituents are linked to cell membranes of microorganisms inhibiting in particular electron exchange membrane. High concentration of organic extracts also leads to the membrane lysis [8]. Thus, the diameter of the zone of inhibition varies from one bacterium to another and from one sample to another, depending on the polarity of the solvents. This efficiency is due to the presence of phenolic compounds known for their antimicrobial effects [9], [10]. Similarly, it is important to note a significant effect of the butanol extract of T. numidicus on the strains studied. The effect of this extract is important compared to other extracts tested in particular S. aureus.

The extract in ethyl acetate has a broad spectrum of action covering Gram (+) and Gram (-). The extracted chloroform and hexane exert similar effects but with less significant inhibition zone values.

All the studied extracts inhibit the growth of S. aureus. Thus, the work of Sosa et al., (2006) using the extracts of T. numidicus on S. aureus are similar to those of the present study.

The aqueous extract is the most polar which means a low antimicrobial activity comparing to the extracts. So it is about polyphenols solubility in less polar solvents.

Several studies have demonstrated the high sensitivity of Gram (+) bacteria as compared to Gram (-) [11], [12], [13], [14], [15].

The sensitivity of S. aureus strains can be explained by the sensitivity of Gram (+) bacteria to external environmental changes such as temperature, pH, the nature of the extracts and the absence of the outer membrane [16]. This author note that different bacterial envelopes, walls and membranes, and other structures such as somatic, capsular and flagellar antigens are an essential architecture to adapt to environmental situations, the temperature, the osmosis, and pH; for attachment to the holders (cells).

The method used for assessing the antibacterial activity also affects the results. [17] found that the distribution method from wells on agar is more suitable for studying the activity of aqueous and organic extracts of aromatic plants.

P. fluorescence which is shown has a strong intrinsic resistance to biocides in relation to the nature of the outer membrane. The latter is composed of lipopolysaccharides which form an impermeable barrier to hydrophobic compounds. In the presence of permeable agents at the outer membrane. At this level, inactive substances become active against P. fluorescence [18]. While for the three tested yeasts, they are very resistant. So these yeasts have developed a strong resistance to the extracts of T. numidicus. Therefore, this can be attributed resisting ability of the antibacterial agent to uniformly diffuse into the agar.

Quantitative study of the inhibitory effect of the organic extract

Study of the minimum inhibitory concentration (MIC).

The results of the minimum inhibitory concentration (MIC) are presented in the tableau 9.

TABLE IX

RESULTS OF THE MIC .

Strains \ Concentrations	Extract	25%	50%	75%
E. coli	Butanol	+	-	-
S. aureus	Butanol	+	+	+
	Methanol	+	+	+
	Chloroforme	+	+	+
	Acetate of ethyle	+	+	+
P. fluorescence	Methanol	+	-	-

(-): inhibition, (+): growoth

It is noted that the growth of Gram+ bacteria is inhibited at a concentration of 25%. While that for bacteria to Gram-, it is limited to a concentration of 50%.

IV. CONCLUSION

This study is, initially, assessing the antimicrobial activity of organic and aqueous extracts recovered from the leaves of T .numidicus.

The extraction of organic and inorganic compounds in the plant by soxhlet gave different extracts.

The valuation of the antimicrobial activity of these extracts on the tested pathogens showed strong activity against the tested bacteria including S. Concerning the studied yeast and fungi, no activity is exercised by various organic and aqueous plant extracts.

Thus, the extract of T. numidicus inhibits the growth of Gram + at a concentration of 25%. Whereas for Gram- bacteria, it is limited to a concentration of 50%.

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