

## Original article

Antibacterial activity of an essential oil and various extracts of the medicinal plant *Thymus hirtus* sp. *algeriensis* Boiss. & Reut

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## Abstract

Bacteria are most often implicated in cases of food poisoning and the extensive use of chemical, antibacterial agents in the medication leads to the selection of resistant bacterial strains. The essential oils of medicinal and aromatic plants can be used as an alternative for the treatment of infectious diseases and for the protection of food against any alteration. This study was carried out in order to examine *in vitro* the antibacterial activity of the essential oil of *Thymus*, ethanolic and aqueous extracts are tested on pathogenic bacteria: (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella enterica*), responsible for the toxins food infections in Algeria.

The essential oil revealed variable antibacterial activities against the bacterial strains tested except the *Pseudomonas aeruginosa* strain. However, the ethanolic extract shows particularly remarkable antibacterial activity on *Staphylococcus aureus* only with a inhibition zone of the order of  $(10.91 \pm 0.05)$ . The aqueous extract showed no antibacterial activity. The evaluation also of the association effect of the essential oil of an aromatic plant (*Thymus algeriensis*) and an antibiotic gentamycin on four bacterial strains, one gram-positive and the other three gram-negative, gives us very important results regarding the association between thyme essential oil and gentamycin which appeared more effective compared to the use of each separate compound.

**Key words:** Medicinal plants, traditional phytotherapy, antibacterial activity, *Thymus hirtus* sp. *algeriensis*

## 1. Introduction

For centuries and even millennia, our ancestors have used plants to relieve their pain, heal their ailments and heal their wounds. From generation to generation, they have passed on their simple knowledge and experiences, striving when they could to write them down (Tabuti *et al.*, 2003). Thus, even today, despite the phenomenal development of the pharmaceutical and chemical industry, popular interest in herbal medicine has never ceased to evolve.

Medicinal plants contain a large number of active molecules of multiple interests used in industry, food, cosmetology and dermopharmacy. Among these molecules are coumarins, alkaloids, phenolic acids, tannins, lignins, terpenes and flavonoids (Bahorun, 1997). Flavonoids potentially have biological, anti-inflammatory, anti-carcinogenic, antimicrobial and antioxidant activities (Atik Bekkara *et al.*, 2007).

In developing countries, between 70 and 95% of the population uses medicinal plants for primary care because of a lack of access to

prescribed medicines but also because the plants have been able to demonstrate real efficacy. It is estimated that at least 25% of all modern medicines are derived directly or indirectly from plants, thanks to the application of modern technologies to traditional knowledge.

According to the FAO (1996), Professor Auguste Chevalier, Burkina Faso's first botanist explorer, states that there is not a plant on earth that does not have some connection with human needs and serves some part of its table, its clothing, its roof, its pleasures, its remedies or at least its home. This statement proves that it is necessary to understand people's relationships with the environment and more specifically with plants. It is with this in mind that we are interested in a study of the antibacterial activity of the species *Thymus hirtus* sp. *algeriensis*.

The Algerian flora with its 3000 species belonging to several botanical families, 15% of which are endemic (Quezel and Santa, 1963), remains little explored both pharmacologically and phytochemically.

The valorization of medicinal plants of the national flora will be of great contribution for the Algerian pharmaceutical industry and will have a definite economic impact.

The genus *Thymus* includes a large number of species, subspecies and varieties of wild plants. Several of these species are characteristic of the Mediterranean area. They are used in a fresh or dry state as culinary plants. Their essential oils are widely used in alternative medicine due to their antiseptic, antispasmodic and antimicrobial properties (Van Den Broucke and Lemli, 1981; Panizzi *et al.*, 1993).

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Algerian thyme has several Arabic vernacular names, djertil, hamria, hamzoucha khieta mezouqach. Is a bitter astringent, stomachic, diaphoretic, antispasmodic and stimulating. It uses the best buds and young flowering branches. Thym contains essential oils whose main components are thymol and carvacrol, tannins, bitter principles, saponins and plant antiseptics (Beloued, 2009).

The choice of this plant was based on the importance of its "Lamiaceae" family on the one hand and on its known and frequent traditional use in the population studied on the other.

## 2. Materials and Methods

### 2.1 Plant material

The plant *Thymus hirtus* sp. *algeriensis* was harvested in March 2019, in the commune of El Kantara (Wilaya of Biskra). Voucher specimen are deposited in the herbarium of the Department of Nature and Life Sciences, University of Biskra, Algeria under the code LAM-023-3-2019.



**Photo 1:** The plant *Thymus hirtus* sp. *algeriensis*

The aerial part of the plant (leaves, stems and flowers) dried in a dry and ventilated place, away from direct sunlight and at room temperature for a few days. After drying, the plant was manually crushed and stored in paper bags until it was used.

### 2.2 Extraction of essential oils

The extraction of essential oils was carried out by hydrodistillation in a Clevenger apparatus. A quantity of 100 g of dry plant material is placed in a flask heater with 1000 ml of distilled water and then brought to a boil (simple boiling has been used) for 4 h. The essential oils are recovered in a small opaque bottle and stored at 4°C.

#### 2.2.1 Calculation of essential oil yield

The essential oil yield is the ratio between the weight of oil and the weight of the plant (Afnor, 1986). It is expressed as a percentage and calculated by the following formula:

$$\text{Yield} = M'/M.100$$

Yield : Essential oil yield expressed as a percentage (%).

M' : Mass of the essential oil obtained in grams (g).

M : Mass of dry plant matter used in grams (g).

### 2.3 Preparation of extracts

#### 2.3.1 Ethanolic extract

It is a solid-liquid extraction. The principle consists in dissolving the active principle inside the solid and dragging it out using a solvent (Ribereau-Gayon, 1968). Ethanol was used as a solvent.

A test sample of 100 g of powder was macerated in 1000 ml of ethanol for 24 h; after filtration the extracts are dried with rotavapor, to remove the ethanol, the operation is repeated 3 times.

#### 2.3.2 Aqueous extract

An aqueous maceration was also carried out on 5 g of powder with 50 ml of distilled water and placed under agitation for 24 h. After filtration, the extracts are dried with rotavapor, to remove the water.

#### 2.3.3 Calculation of dry extract yields

The dry matter yield of the plants was determined by calculating the following ratio:

$$\text{Yield} = M'/M.100$$

Yield : Yield in extract expressed as a percentage (%).

M' : Mass of the extract obtained in grams (g).

M : Mass of dry plant matter used in grams (g).

### 2.4 Evaluation of antibacterial activity

Two different methods are used to evaluate the antimicrobial effect of the different crude extracts:

- The method of diffusion from a paper disc, that allows the detection of the antimicrobial activity of the different extracts.
- The micro-dilutions method, which aims to determine, MICs (minimum inhibitory concentrations) from a range of product concentrations, in the culture medium.

#### 2.4.1 Antibacterial activity

The principle of evaluating the antimicrobial activity of extracts consists in performing a microbial culture on a solid medium, in the presence of discs impregnated with different extracts. If the extracts have antibacterial activity, an inhibition zone around the disc will be observed. The diameter of this inhibition zone is proportional to the effectiveness of the antimicrobial activity of the test extract (Fattouch *et al.*, 2007).

The antibacterial activity of essential oils and extracts was carried out using the disc diffusion method on a solid culture medium (Ait-Ouazzou *et al.*, 2012).

#### 2.4.2 The bacterial strains used

Antibacterial tests were performed on clinical and reference strains.

#### Reference strains

*Escherichia coli* ATCC 25922

*Staphylococcus aureus* ATCC 25923

*Pseudomonas aeruginosa* ATCC 27853

#### Clinical strains

*Salmonella enteritica*

The bacterial strains used in this work got from the laboratory of the Batna Cancer Centre Hospital, Algeria.

#### 2.4.3 Replicating bacterial strains

The different bacterial strains were transplanted by the nutrient agar stripe method and incubated at 37°C for 24 h to obtain young and isolated bacterial colonies used to prepare the inoculum.

#### 2.4.4 Preparation of bacterial inoculum

The inoculum was prepared by sampling well isolated and identical bacterial colonies from a tube containing 9 ml of sterile physiological water. The inoculum density was adjusted to 0.5 Mc Farland or an optical density of 0.08 to 0.10 at 625 nm (corresponding to approximately 108 CFU/ml) (Casfm, 2015).

#### 2.4.5 Seeding of bacteria

Seeding is carried out in Petri dishes containing Mueller-Hinton MH agar (the most commonly used medium for antibacterial agent susceptibility testing) and left for 15 min to solidify, and with a swab, soaking it in the bacterial suspension, then seeding the entire surface of the stippled dishes from top to bottom, in tight streaks using a swab (Kechkar, 2008).

#### 2.4.6 Preparation of the various extracts

The essential oil, ethanolic extract and aqueous extract have been prepared using DMSO which is inert on bacterial activity.

#### 2.4.7 Disk deposits

After sowing, diffusion discs (Watman N°3) paper sterilized by autoclaving at 120°C for 15 min are placed on the surface of the boxes sown with sterile pliers; these discs are injected by 20 microlitres of essential oil and extracts (Gulluce *et al.*, 2003). Two controls were performed: A DMSO impregnated disc serves as a negative control and a gentamicin disc serves as a positive control. All tests were repeated three times.

The boxes are kept at laboratory temperature for 30 min to allow for pre-diffusion. Then they are incubated at 37°C for 24 h.

#### 2.4.8. Reading the result

The diameters of the inhibition zones are measured using a caliper (mm), outside the closed box, the manipulations are repeated 3 times to ensure that the method runs smoothly (Joffin and Layeral, 2006).

### 3.2 Inhibition zones

The results of the inhibition zones of the strains tested are shown in the following Table.

**Table 1:** Diameter (mm) of the inhibition zones

strain	inhibition zone			
	Essential oil	Ethanolic extract	Aqueous extract	Gentamycin
<i>S. aureus</i>	11.52 ± 0.41	10.91 ± 0.05	-	33.37 ± 0.59
<i>E. coli</i>	11.53 ± 0.43	-	-	26.47 ± 1.09
<i>P. aeruginosa</i>	-	-	-	32.53 ± 0.22
<i>S. enterica</i>	12.51 ± 0.19	-	-	27.66 ± 2.22

(-) means that there is no inhibition zone

These values represent the mean ± standard deviation

#### 2.4.9 Liquid micro-dilutions method

Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) were determined using the micro-dilution technique with Muller Hinton broth (Nccls, 2000).

##### 2.4.9.1 Determination of the Minimum Inhibitory Concentration (MIC)

Microdilution is generally performed in 96-well plates with a rounded bottom. The bacterial suspensions were diluted with broth and distributed in these plates (Kahlmeter and Turnidge, 2012).

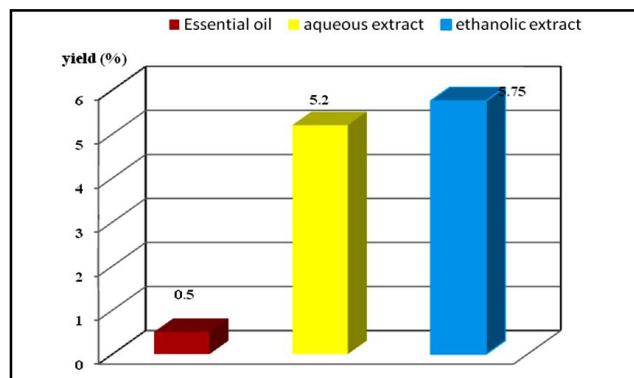
A stock solution of the essential oil and ethanolic extract was prepared in the DMSO at 10%, then serial dilutions of the essential oil and ethanolic extract were made in the 96-well microplate. 90 µl of Mueller Hinton broth was incorporated and then inoculated with 10 µl of standardized bacterial inoculum. All tests are repeated three times. A positive control containing 10 µl of inoculum and 190 µl of Muller Hinton Broth, and a negative control containing 100 µl of essential oil or ethanolic extract dissolved in 10% DMSO and 100 µl of Muller Hinton Broth without inoculum). The plates were covered and incubated at 37°C for 24 h.

The Minimum Inhibitory Concentration (MIC) has been defined as the minimum concentration of extract for which no growth visible to the naked eye is observed.

### 3. Results

#### 3.1 Yield

According to the results shown in (Figure 1), it has been observed that the yield of essential oils of the species *Thymus hirtus* sp *algeriensis* is 0.5035%, While the yield of the aqueous extract of this species is 5.20% which is lower than the ethanolic extract which is 5.75%.



**Figure 1:** The plant *Thymus hirtus* sp. *algeriensis*

### 3.3 Sensitivity of bacterial strains

The reading is made by measuring the diameter of the inhibition zone around each disc using a caliper in (mm) (Table 2). The results are expressed by the diameter of the inhibition zone and can be symbolized by signs based on the sensitivity of the strains (Ponce *et al.*, 2003).

Not sensitive or resistant (-): diameter < 8 mm.

Sensitive (+): diameter between 9 and 14 mm.

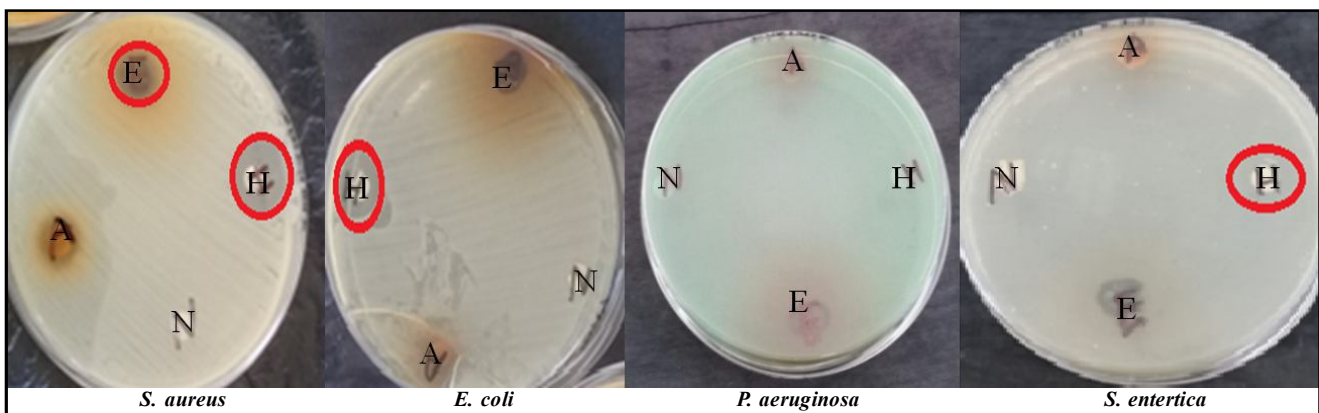
Very sensitive (++) : diameter between 15 and 19 mm.

Extremely sensitive (++++): diameter > 20 mm.

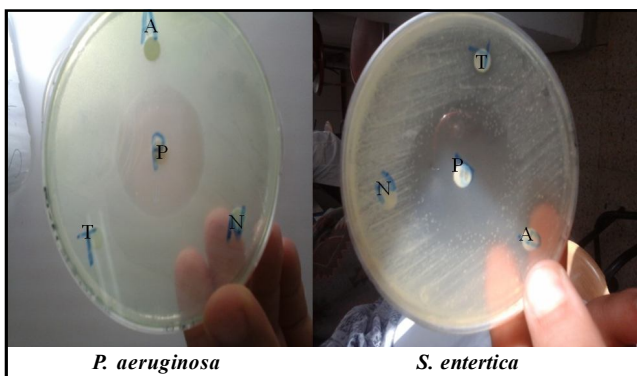
The sensitivity results of bacterial strains are shown below:

**Table 2:** Sensitivity of bacterial strains

strain	sensitivity			
	Essential oil	Ethanolic extract	Aqueous extract	Gentamycin
<i>S. aureus</i>	+	+	-	+++
<i>E. coli</i>	+	-	-	+++
<i>P. aeruginosa</i>	-	-	-	+++
<i>S. enterica</i>	+	-	-	+++



**Photo 2:** Aromatogram result of the different extracts



**Photo 3:** Gentamycin inhibition zone.

According to (Tables 1 and 2), which showed the inhibition zones of *T. hirtus*, the antibiotic gentamycin has distinct effects on the bacteria tested, compared to the other extracts of the plant studied.

For the results of the essential oil, it was observed that the susceptible strains, *S. aureus*, *E. coli* and *S. enterica* have inhibition zones of 11.52, 11.53, and 12.51 (mm), respectively. On the other hand, the *P. aeruginosa* strain has been shown to be resistant.

The ethanolic extract reacted positively only on the *S. aureus* strain with a diameter of  $10.91 \pm 0.05$ , so this strain is sensitive.

For aqueous extracts, the results showed that these extracts have no antibacterial effect against all bacterial strains, tested either gram-positive or gram-negative.

### 3.4 Minimum inhibitory concentration

From Table 3 which presents the Minimum Inhibitory Concentrations (MICs) of *T. hirtus*. The oil was found to be active to varying degrees against all bacterial strains tested, except *P. aeruginosa*, *E. coli* and *S. enterica* had MICs of 50 mg/ml, while *S. aureus* had an MIC of 25 mg/ml. The ethanolic extract reacted positively only against *S. aureus* at a MIC of 25 mg/ml.

**Table 3:** The minimum inhibitory concentration (MIC)

strain	MIC (mg/ml)	
	Essential oil	Ethanolic extract
<i>S. aureus</i>	25 ± 0.33	25 ± 0.22
<i>E. coli</i>	50 ± 0.25	-
<i>P. aeruginosa</i>	-	-
<i>S. enterica</i>	50 ± 0.45	-

### 3.5 Minimum bactericidal concentration

According to Table 4 which presents the minimum bactericidal concentrations (MBCs) of *T. hirtus*, it has been shown that the oil was only active against *S. enterica* strain by CMB of 100 mg/ml and *S. aureus* strain by a CMB concentration of 50 mg/ml.

The ethanolic extract reacted positively only against *S. aureus* with a CMB of 50 mg/ml.

**Table 4:** The Minimum bactericidal concentration (MBC)

strain	MBC	
	Essential oil	Ethanollic extract
<i>S. aureus</i>	50 ± 0.23	50 ± 015
<i>E. coli</i>	-	-
<i>P. aeruginosa</i>	-	-
<i>S. enterica</i>	100 ± 048	-

**Table 5:** Inhibition zone of mixture between essential oil and gentamycin

		inhibition zone			
		<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. enterica</i>
mixture between essential oil and gentamycin	<b>MS Mother Solution</b>	22.52 ± 0.36	25.91 ± 0.14	10.78 ± 0.11	18.37 ± 0.59
	1/2	18.21 ± 0.43	16.82 ± 0.05	8.92 ± 0.14	15.47 ± 10.08
	1/4	15.23 ± 0.425	14.32 ± 0.10	/	12.53 ± 0.12
	1/8	13.28 ± 0.14	10.23 ± 0.09	/	10.23 ± 0.06

According to Table 5, it was found that the mixture between *T. hirtus* essential oil and gentamycin antibiotic gave a stronger activity compared to the use of each single compound, regardless of the bacterial strain treated. Regarding the effect of essential oil on the

### 3.6 Test of the effect of the combination of essential oil of *Thymus algeriensis* and gentamycin and two

In order to test the effect of the combination of the essential oil of *T. algeriensis* and the antibiotic gentamycin on the four bacterial strains, the two extracts were mixed, concentration-by-concentration (each dilution with the corresponding one). The results of this test are shown in Table 5.

bacterial strain, *P. aeruginosa* which appeared resistant, becomes sensitive in the case of use of mixture, especially in the use of the mother solution (Table 6).

**Table 6:** Sensitivity of bacterial strains

		inhibition zone			
		<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. enterica</i>
mixture between essential oil and gentamycin	<b>MS Mother Solution</b>	+++	+++	+	++
	1/2	++	++	+	++
	1/4	++	+	-	+
	1/8	+	+	-	+

## 4. Discussion

Recent scientific research has focused on the study of novel plant-derived antioxidants and antibacterials, particularly polyphenols that replace synthetic substances (Bougatef *et al.*, 2009).

Similarly, treatment with conventional antibiotics is associated with several effects, adverse events such as increased risk of bleeding linked to linezolid, the emergence important bacterial resistant species as well as the disadvantages associated with the treatment with antibiotics incompatible with the external administration of the patient such as extension of hospital stay and exposure to infections nosocomial (Song, 2008).

Concerning the yield of aqueous extracts, it was found in this study that it is lower than those mentioned in the work of Zeghib (2013), (Asmaa and Laaribyia, 2017) who obtained a yield equal to 10.52%. While the yield of ethanol extract is higher than those mentioned in the work of Tamert (2007), who obtained a yield equal to 4.33%.

Many factors influence the yield, grade, physicochemical characteristics and chemical composition of essential oils such as species, environmental conditions, extraction technique, drying period and harvesting medium, cultural practices and the age of plant material (Aberchane *et al.*, 2001; Bourkhiss *et al.*, 2009).

Based on results from (Hazzit *et al.* 2009), on the same species, the *S. aureus* strain is extremely sensitive with an inhibition zone of 51 ± 3.4 mm while *E. coli*, *P. aeruginosa* and *S. enterica* strains are very sensitive with inhibition zones of 17.8 ± 1.7, 15.2 ± 1.0 and 15.6 ± 2.4 (mm), respectively.

The difference in inhibition diameters could be due mainly to the chemical composition of the essential oils and other extracts and the antibacterial activity of the latter could mainly be due to the majority compounds.

The results obtained are comparable to those obtained by (Amarti *et al.*, 2010), in a study on the antimicrobial activity of essential oils of *T. algeriensis* and *T. ciliatus* from Morocco on four bacterial strains including *E. coli* and *S. aureus*, showing that the essential oil of *T. algeriensis* has a high activity on both strains but remains low compared to the essential oil of *T. ciliatus*. This low activity may be due to the presence in the HE of *T. algeriensis* from  $\alpha$ -pinene (20.5%), from  $\beta$ -pinene (8.02%) and limonene (4.85%) known for their low antibacterial activities (Knobloch, 1989).

Thymus oils show an interesting antibacterial activity compared to the concentration used (1.5 l), especially against the bacteria *Staphylococcus aureus*. Indeed, *S. aureus*, highly pathogenic strain, has a high sensitivity to this oil. This great antibacterial activity can be related in the case of the Thymus oil to the presence of thymol which is the majority. This phenolic compound is indeed known for its antimicrobial properties (Ettayebi *et al.*, 2000).

In a study by Osman *et al.* (2009), the ethanolic extract of *T. algeriensis* showed remarkable activity against the *S. aureus* strain, with an inhibition zone of 11 mm.

The results found in our work are superior to those reported in the work of (Hazzit *et al.*, 2009), which obtained an oil MIC of 1 mg/ml for *E. coli* and *S. enterica*. On the other hand, *S. aureus* had a MIC of less than 0.5 mg/ml.

Other studies have pointed out that the higher the phenol content, the greater the antimicrobial efficacy; this applies to ethanolic extracts which have a higher antimicrobial capacity compared to the aqueous extracts (Mohammedi *et al.*, 2010). Studies by the World Health Organization and other authors (Dorman and Deans, 2000) have also shown that this constituent has strong antibacterial and antifungal activity against many species, including *S. aureus*, *E. coli* and *Aspergillus* sp.

In addition, the sensitivity test of the four strains tested against gentamycin showed very strong activity on *S. aureus* and these results in inhibition zones with a diameter of 33.37 mm, however, this antibiotic exerts an activity different from one strain to another which is explained by a certain degree of resistance developed by the other bacterial strains.

Thymic drying and milling conditions can also be the cause of the absence of antibacterial activity. It is reported by Seidel (2005) that if the plant is known by its content in volatile or thermolabile compounds, it is advisable to freeze the plant material as soon as possible after its collection. It is also recommended to grind it subsequently in a mortar with liquid nitrogen as the grinding is also at the origin of the heat generation responsible for the loss of volatile molecules as well as the decomposition and oxidation of thermolabile molecules (Jones and Kinghorn, 2005).

So, the low activity of the ethanolic extract may be due to the small fraction of essential oils which solubilized in ethanol, the large proportion being lost due to drying and extraction conditions. Finally, the activity of an extract is probably due to the presence of synergy between a number of components, which, when separated, become inactive individually (Sarker *et al.*, 2005). This is interpreted by the fact that plants produce a huge variety of small antibiotic molecules with a wide spectrum of structures such as terpenoid, glycoesteroid, flavonoid and polyphenol. However, most of these Small molecules have low antibiotic activity compared to common antibiotics produced by bacteria and fungi.

In general, the essential oil and gentamycin tested are more active against gram positive bacteria than gram negative bacteria, this result can be explained by the richness of the wall of gram positive bacteria in proteins while in gram negative, it is mainly assembled in lipopolysaccharides (LPS), the outer membrane of the latter constitutes an effective permeability barrier. The LPS, thanks to its negative surface charges, prevents the diffusion of hydrophobic molecules, and proteins exclude the passage of hydrophilic molecules of high molecular weight. While gram bacteria positive are less protected against antibacterial agents (Hogan and Kolter, 2002). This seems to have a clear correlation with our results. The results of this study confirmed that it is preferable to use the mixture between the essential oil of *T. algeriensis* and the antibiotic gentamycin in order to have a potential synergistic effect or synergistic additive.

## 5. Conclusion

Algeria, by its geographical location, offers rich and diverse vegetation. A many aromatic and medicinal plants grow spontaneously. To this great diversity of flora, is added a centuries-old tradition of use of the plants. In Despite the progress made in medicine, several populations, particularly those of rural areas, use plants to treat themselves, either by inaccessibility to drugs prescribed by the modern medicine, either because these plants have given very encouraging therapeutic results during their use.

The use of phytotherapy can be dangerous due to the toxicity of certain plants, which requires precautions to be taken when using it. As well as in this work, the antibacterial effects of the essential oil, ethanolic and aqueous extract of the aerial part of *T. hirtus* sp. *algeriensis* were investigated. *T. hirtus* sp. *algeriensis* extracts were tested *in vitro* to reveal their inhibitory power against four bacterial strains.

The essential oil revealed variable antibacterial activities against the bacterial strains tested. However, the ethanolic extract shows a particularly remarkable antibacterial activity on *S. aureus* only. The aqueous extract showed no antibacterial activity. The difference in the inhibition zone between extracts is explained by the influence of several factors: The structure of the bacteria walls, the concentration of extracts, the nature and structure of the active substances in the extracts.

The results we have obtained confirm the powerful antibacterial activity of the essential oil of this species as well as the antibiotic Gentamycin. The combination effect of the essential oil of *T. algeriensis* and Gentamycin, which has been the objective of our work, shows a very strong activity against the four bacterial strains tested, with an important activity than those of the fractions tested separately.

Despite the encouraging results of this survey concerning phytotherapy, its practice in the EL Kantara region remains limited. Medicinal plants, like medicines, must have strict standard rules that only an herbalist can meet. Thus, more importance must be given to the cultivation, exploitation and marketing of these plants, which can be an important source of external income.

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## Conflict of interest

The author declared that there is no conflicts of interest in the course of conducting the research. The author has final decision regarding the manuscript and decision to submit the findings for publication.

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