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Antibacterial effects of *Thymus algeriensis* extracts on some pathogenic bacteria

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ABSTRACT. Despite the presence of many antibiotics, bacterial resistance is growing steadily and some of these antibiotics have become ineffective, which poses a major challenge to the health sector. In this context, this work has demonstrated, *in vitro*, the inhibitory action of the bacterial growth resulting from methanolic and ethanolic extracts of *Thymus algeriensis* Boiss. & Reut., a medicinal plant species harvested from the Algerian South-west area, as well as the determination of the phenolic content of those crude extracts. The methanolic extract of *Thymus algeriensis* showed a significant antibacterial effect with 16.5 and 19 mm against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. *Klebsiella pneumoniae* was not inhibited by both tested extracts. Besides, ethanol extract has not prevented the growth of the *Enterobacter cloacae*. This biological activity can be explained by the appreciable rates noted for both of the plant extracts in terms of total phenolic levels, which ranged between 79.45 and 67.13 mg GAE g⁻¹ dry weight.

Keywords: Antibacterial effect; Extracts; Medicinal plant; Phenolic content; *Thymus algeriensis*.

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Introduction

All over the world and for many centuries, the herbaceous plants are an integral component of everyday life and culture. These plants were used in pharmaceuticals, cosmetics and in food technology as antioxidants. Medicinal plants and herbal medicines form an important part of the treatment in the indigenous medicine systems. A wide variety of modern drugs have been used for treating illnesses including constipation and cancer, which were derived from the plant kingdom.

This is why many countries are actively engaged in bio-mining Medicinal plants for therapeutically precious and biologically active phytochemicals (Kumar & Jnanesha, 2016).

The *Thymus* sp. (Thyme) are small permanent therapeutic botanical herbs native of the Mediterranean basin, south of Italy, and Asia. They belong to the Lamiaceae family, which is one of the largest families among flowering plants, practically, with almost a range of 220 genera and 4000 species in the world (Ameen, 2013, Colpaert 2006, Javed, Erum, Tabassum, & Nikolić et al., 2014).

The thyme has also become one of the most important medicinal plants used for food purposes as a spice for its taste qualities. *Thymus algeriensis* Boiss. & Reut. (Synonym *Thymus hirtus* Willd. subsp. *algeriensis*) is an aromatic plant under the common name of “Mazoukcha”. This species is the most widespread North African species. It is characterized by curved stems and white or pink flowers (see Figure 1), essentially used in Algeria both as a popular herb and as a spicy herb. (Hazzit & Baaliouamer, 2007) (Jayari et al., 2018).

According to annual estimates, the market demand for thyme is steadily expanding to around 500 tons in the United States and 1000 tons in Europe (Roby, Sarhan, Selim, & Khalel, 2013, Nezhadali et al., 2014). Among many biological activities of *Thymus* are those can be listed as follows: antibacterial, antifungal, analgesic, carminative, antioxidant, spasmolytic and antimutagenic. (Dapkevicius et al., 2002, Giordani et al., 2004, Babovic et al., 2010, Festy, 2014, Goetz & Ghedira, 2012, Soni, 2012, Regnier, Combrinck, Veldman, & Du Plooy, 2014, Gavarić et al., 2015).



Figure 1. *Thymus algeriensis* Boiss. & Reut.

In order to detect new sources of antibacterial agents, we report, in this study, the results of antibacterial effects using the crude extracts obtained. Non volatile hydrophilic fractions were extracted by maceration of *Thymus algeriensis* aerial parts with two common solvents, ethanol, and methanol.

Therefore, the objectives of this study were (a) to determine the effect of different solvents (methanol and ethanol) on extraction by means of measuring the total phenolic content (TPC), the total flavonoid content (TFC) and the total anthocyanin content (TAC); and (b) to evaluate the antibacterial properties of *T. algeriensis*.

Material and methods

Chemicals

Folin-Ciocalteu reagent, Aluminum chloride (AlCl_3), gallic acid, Quercetin, Cyanidin-3-glucoside provided by Caque lab. (Bechar, Algeria) & Gitalab. (Tlemcen, Algeria); Mueller-Hinton agar and nutrient broth obtained from Biology research lab. (Saida, Algeria) and were purchased from Merck (Darmstadt, Germany). Methanol, ethanol, sodium carbonate, antibiotics were supplied by Boudjemaa Tourabi Hospital (Bechar, Algeria) and were purchased from Merck (Darmstadt, Germany).

Plant Extracts

An expert in traditional medicine collected *T. algeriensis* Boiss. et Reut., in May and June 2016 in the semi-arid area surrounding Bechar, southwest of Algeria (desert climate, latitude: $31^\circ 37' 0'' \text{ N}$, longitude: $-2^\circ 13' 0'' \text{ O}$; mean annual rainfall $< 100 \text{ mm}$, average temperature: Max./Min. ($4/42^\circ \text{ C}$)).

Plant species was identified by the Laboratory of Biotoxicology, Pharmacognosy and Biological Valuation of Plants, University Dr. Tahar Moulay Saida, Algeria, as target species of interest. Plant material stripped and air-dried at room temperature (not faced to direct sunlight).

The voucher specimen has been deposited at the Herbarium of the University Dr. Tahar Moulay; Saida, Algeria. (Code. T.A.B.R-2016).

Two solvents were used to extract polar fractions of the *Thymus algeriensis* aerial parts by maceration method. 50 g of sample (powdered) soaked under frequent agitation, in either 500 mL of 100% methanol or 500 mL of 100% ethanol. After they allowed standing at room temperature for a period of 3 days, extracts were filtered, concentrated and stored at 4° C until later analysis. (Azwanida, 2015)

Microbial Samples

The seven analyzed microbial species (Five gram-negative and two gram-positive bacteria) were provided by the Laboratory of Biotoxicology Pharmacognosy and Biological Valorization of Plants, University Dr. Tahar Moulay Saida, Algeria; were taken from international collections:

1-*Escherichia coli* ATCC 25922; 2-*Klebsiella pneumonia* ATCC 4352 ; 3-*Pseudomonas aeruginosa* ATCC 27853; 4-*Salmonella typhimurium* ATCC 13311; 5- *Enterobacter cloacae* ATCC 49452; 6-*Enterococcus faecalis* ATCC 49452; 7-*Staphylococcus aureus* ATCC 25923.

Phytochemical screening

Extract yield (%) was determined as described in Yihune and Yemata (2019), as follows:

$$\text{Extract yield (\%)} = \frac{\text{Weight of crude extract}}{\text{Initial weight of plant powder}} * 100$$

Then, to determine the total phenolic content, 200 μL of the sample extract was mixed with 1 mL of Folin–Ciocalteu's reagent. After 4 min., 800 μL of 7.5% Na_2CO_3 solution was added, and the mixture was incubated for 120 min. in the dark.

The reaction mixture absorbance was measured at 760 nm, and the reaction mixture without the sample was used as a blank. Gallic acid was used as a standard. The TPC was expressed as Gallic acid equivalents for dry powder (mg GAE g^{-1}) (Ćujić et al., 2016).

The TFC was determined with aluminum chloride test; 1 mL of diluted plant extract was mixed with 1 mL of 2% AlCl_3 methanolic solution. After incubation at room temperature for 10 min., the absorbance of the reaction mixture was measured at 430 nm. Quercetin was used as a standard, and the TFC was expressed as mg quercetin equivalents g^{-1} for dry weight (mg QE g^{-1}) (Ghedadba, Bousselsela, Hambaba, Benbia, & Mouloud, 2014).

The pH differential method used to deduce the TAC, which consists in measuring the absorbance of the mixture at two wavelengths: 510 and 700 nm via two buffer systems at pH 1.0 and at pH 4.5. Cyanidin-3-glucoside was chosen as a standard, and the TFC was expressed as mg cyanidin-3-glucoside equivalents g^{-1} for dry weight (mg C3G g^{-1}).

$$A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}$$

$$\text{TAC} = [(A \times \text{MW} \times \text{DF}) / \text{MA}] \times 100$$

Where: A: absorbance; MW: molar mass; DF: dilution factor; MA: molar absorption. All previous measurements were carried out in triplicate.

Assay for the antibacterial potential

To evaluate the susceptibility of bacterial strains to plant extracts the disc-diffusion method was used. The bacteria cultures were grown in nutrient broth liquid medium at 37°C. After 24 hours of growth, each bacterium, at a concentration of 10^6 cells mL^{-1} , was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, filter paper discs (6 mm in diameter) impregnated with extract (50 μL) were placed on the surface of each inoculated plate.

Empty standard antibiotic disks were used as a negative control. The plates were incubated at 37°C for 24 hours.

Antibacterial activity was determined by measuring the zone of inhibition in mm (Murray, Rosenthal, & Pfaller, 2016, El Abed, Guesmi, Mejri, Marzouki, & Ahmed, 2014). The qualitative results were converted in a semi-quantitative scale as absence of halo (0.0 mm); weak halo (3.0 - 7.0 mm); moderate halo (8.0 - 10.0 mm), and strong halo greater than 11.0 mm. When used as controls, solvents applied for extraction, showed no inhibitions in preliminary studies.

The antibacterial properties of *Thymus algeriensis* extracts were compared with those of the following positive controls: Rifampicin (RF); Gentamicin (GN) and Ampicillin (AP).

The extracts that showed antibacterial effect were tested to determine the Minimal Inhibitory Concentration (MIC) defined as the lowest concentration capable of inhibiting the growth of bacterial strains, where standard bacteria strains were used in the modulation (Santos et al., 2019).

To do this, a dilution method in solid medium was used. It consists of putting a standardized bacterial inoculum in direct contact with a range of plant extracts increasing concentrations. (Burnichon & Texier, 2003).

Seven bacterial samples of 10^6 cells mL^{-1} (*Escherichia coli*; *Klebsiella pneumonia*; *Pseudomonas aeruginosa*; *Salmonella typhimurium*; *Enterobacter cloacae*; *Enterococcus faecalis*; *Staphylococcus aureus*) were inoculated in plates with Mueller Hinton medium supplemented with different concentrations of the extracts 100, 200 and 300 μL .

After 24 hours of incubation at 37°C, the MIC of each sample was determined by the absence of bacterial growth in each plate, comparing the sample readout with the was not inoculated nutrient medium. Analyses were done in triplicate.

Statistical analysis

Data were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by the least significant difference test of Fisher (LSD) was employed and the differences between individual means and each means used were deemed to be significant at $p < 0.05$.

Results and discussion

Phytochemical screening

The extraction yields observed for methanolic and ethanolic extracts were 9.42 and 7.26 %, respectively. The total phenolic, total flavonoid and total anthocyanins of *Thymus algeriensis* extracts are summarized in Figure 2

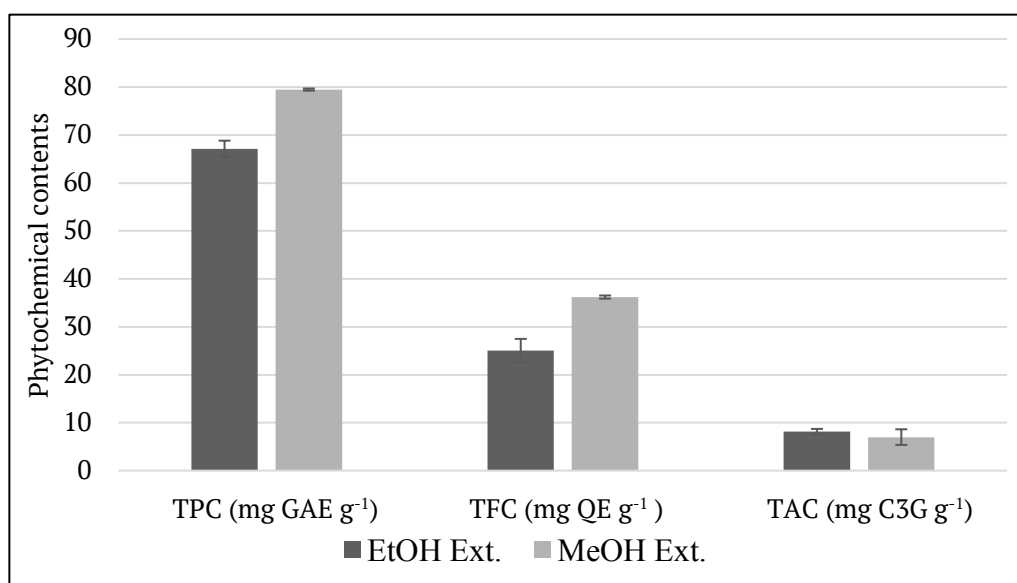


Figure 2. Results of phytochemical screening of *Thymus algeriensis* methanolic and ethanolic extracts. TPC (mg GAE g⁻¹), TFC (mg QE g⁻¹) and TAC (mg C3G g⁻¹)

MeOH Ext.: Methanolic extract; EtOH Ext.: Ethanolic extract.

TPC: Total phenolic Content. TFC: Total Flavonoids Content. TAC: Total Anthocyanins Content. mg GAE g⁻¹: acid gallic equivalents. mg QE g⁻¹: quercetin equivalents. mg C3G g⁻¹: cyanidin-3-glucoside equivalents.

Both of the plant extracts had higher total phenolic content, but the *T. algeriensis* methanol extract had significantly ($p < 0.05$) higher TPC than its ethanol extract, the TPC was 79.45 and 67.13 mg GAE g⁻¹ dry weight. TFC and TAC recorded for ethanol extract were 25.04 (mg QE g⁻¹) 8.14 (mg C3G g⁻¹), respectively. Conversely, they were 36.18 (mg QE g⁻¹) and 6.98 (mg C3G g⁻¹) methanol extract.

The extraction yield of *Thymus vulgaris* L. using methanol/water (80:20, v v⁻¹) was 6.98. This extract had presented TPC of 88.59 mg g⁻¹ and TFC of 54.60 mg g⁻¹, as obtained by using higher polarity solvents. (Martins et al., 2015a). Another study on the methanolic extract yield of the same plant was determined as 9.0%, while TPC was 69.44 mg GAE g⁻¹ DW. (Albayrak, Aksoy, Albayrak, & Sagdic, 2013).

A large value of *Thymus algeriensis* TPC was detected in another study in the order of 81.5 mg GAE g⁻¹ (Brahmi et al., 2015). In addition, TPCs estimated for leaves polar fraction (Methanolic water⁻¹) of *Thymus algeriensis* and *Thymus capitatus* were 248.8 and 240.3 (mg GAE g⁻¹ DW), respectively.

However, TFCs were 15.36 and 14.94 catechin equivalents (mg CE g⁻¹ DW), in the mentioned order. (Megdiche-Ksouri et al., 2015). Moreover, our results are in accordance with the results obtained from other studies and which were conducted on Lamiaceae family (*Salvia officinalis* L.).

These have revealed that the methanol/water extract also had high TPC and TFC with 323.47 mg g⁻¹ and 218.59 mg g⁻¹ of dried extract (Martins et al., 2015b).

TPC resulted through the study of Skendi, Irakli, and Chatzopoulou (2017) were ranged between 34.3 and 70.4 mg GAE g⁻¹ DW of TPC for Lamiaceae family plants methanol extracts.

In the study of Sökmen et al. (2004) about *Thymus spathulifolius*, the amount of TPC in polar subfractions (methanol water⁻¹) and non-polar subfractions of the extract was estimated as 141 mg GAE g⁻¹ DW and 102 mg GAE g⁻¹ DW, respectively.

In contrast, other researchers reported TPC low values for *Thymbra spicata* L. methanol and ethanol extracts ranged between 13.14 and 13.13 (mg GAE g⁻¹ DW), and TFC ranged between 4.36 and 3.24 (mg QE g⁻¹ DW). Furthermore, methanol and ethanol extracts, obtained of *Thymus vulgaris* L., presented TPC that varied from 5.13 and 13.57 (mg GAE g⁻¹ DW), and TFC from 7.285 and 6.17 (mg QE g⁻¹ DW). (Gedikoğlu, Sökmen, & Çivit, 2019).

Besides, for *Thymus hirtus* sp. *algeriensis*, Guesmi, Ben Farhat, Mejri, & Landoulsi, (2014) reported TPC low levels from 7.05 to 8.81 mg GAE g⁻¹ DW. TPC found for *Thymus vulgaris*, was ranged from 4.75 to 8.10 mg GAE g⁻¹ DW (Roby et al., 2013), and were important as 83.31 mg GAE g⁻¹ for *Thymus argaeus* methanolic extract. (Sagdic, Ozkan, Aksoy, & Yetim, 2009).

All the *Thymus* 14 samples investigated by Tohidi, Rahimmalek, and Arzani (2017), exhibit a generally high TPC value (31.38–70.56 mg Tannic Acid Equivalents g⁻¹ DW), with the highest found in *Thymus daenensis* (70.56 mg TAE g⁻¹); while TFC announced between 1.89 - 8.55 mg QE g⁻¹.

The multiple studies that were conducted have established that the *Thymus* species are rich and promising sources of phenolics and flavonoids. The phenolic content of plant extract depends on various parameters such as genetic and ecological factors, the part of the plant used, the extraction method employed and even plant age (Amarti et al. 2010; Gharibi, Tabatabaei, & Saeidi, 2015).

Msaada et al. (2016), have revealed that both total phenols and flavonoids varied significantly among the region of collection, probably due to the dissimilarities in the soil, climate, solar lighting, humidity, and temperature, which could be affecting samples in each studied region.

TPC were ranged from 8.44 to 18.40 mg GAE g⁻¹ DW and from 26.83 to 63.64mg CE g⁻¹ DW, for TFC.

In addition to this, the aerial part extraction of *Haloxylon scoparium* with different solvents showed the highest yields for water and then for methanol. The high yield of extraction in polar solvents exhibited rich polar constituents of the plant aerial part (Lamchouri et al., 2012).

Assay for the antibacterial potential

The disc-diffusion method is the most common technique used to test the antibiotic properties of crude extracts. In this case, plant extracts were tested against bacterial strains. The data pertaining to the antibacterial potential of the plant extracts are presented in Tables 1 and 2.

Table 1. Antibacterial activity caused by *Thymus algeriensis* extracts through Agar Diffusion Method.

Bacterial Strains Tested	Inhibition zone diameter (mm)				
	<i>T. algeriensis</i>		Standard Antibiotics (10 µg mL ⁻¹)		
	Methanolic extract	Ethanol extract	AMP	GN	RF
<i>S. typhimurium</i> .	9 ^A	12 ^a	16	9	-
<i>E. coli</i> .	13	10	10	15	8
<i>K. pneumoniae</i> .	-	-	14	17	-
<i>P. aeruginosa</i> .	16.5	14	-	12	-
<i>E. cloacae</i> .	7	-	-	14	-
<i>E. faecalis</i> .	12.5 ^B	17 ^b	15	-	7
<i>S. aureus</i> .	19	15.5	18	13	10

GN: gentamicin; AMP: ampicillin; RF: rifampicin; Capital letters (A–B) and lowercase letters (a–b) indicate significant differences at $p < 0.05$.

Table 2. *Thymus algeriensis* Extracts' MICs (µg mL⁻¹).

Bacterial Strains Tested	MIC (µg mL ⁻¹)	
	Methanolic extract	Ethanol extract
<i>S. typhimurium</i> .	110	130
<i>E. coli</i> .	220 ^A	270 ^a
<i>K. pneumoniae</i> .	-	-
<i>P. aeruginosa</i> .	185	150
<i>E. cloacae</i> .	160	-
<i>E. faecalis</i> .	80 ^B	105b
<i>S. aureus</i> .	40	65

Note: MIC - Minimal Inhibitory Concentration; (-): Absence of activity; Negative controls did not show any activity. Capital letters (A–B) and lowercase letters (a–b) indicate significant differences at $p < 0.05$.

As can be noted from Table 1, assayed extracts showed antibacterial potential, especially against the highly pathogenic germs *P. aeruginosa*, *S. typhimurium*, and *E. coli*. Those estimates are promising since Gram-negative bacteria are generally more resistant than Gram positive ones (Siri et al., 2004).

Ethanol extract has no effect on the growth of *E. cloacae* while it inhibited widely the growth of both the bacterial strains tested *E. faecalis* and *S. aureus* inside zones with diameters 17 mm, 15.5 mm, respectively ($p < 0.05$).

This observation confirmed the evidence presented in a previous study that reported that plants synthesize an array of molecules with very diverse structures such as polyphenols, flavonoids, and terpenoids with low antibiotic activity compared to those produced by microorganisms (Sarker, 2005).

It can be concluded from Tables 1 and 2 that significant differences ($p < 0.05$) can be found in the antibacterial effects of the tested plant extracts. Besides, phenolic and flavonoid compounds of medicinal plant extracts have been reported to possess strong antibacterial activity (Hendra, Ahmad, Sukari, Shukor, & Oskoueian, 2011), (Farhadi, Khameneh, Iranshahi, & Iranshahy, 2018), (Cushnie & Lamb, 2005), which can be exerted in three ways: directly kill the bacteria, attenuate the bacterial pathogenicity and synergistically activate the antibiotics (Xie, Yang, Tang, Chen, & Ren, 2015).

On the other hand, the absence of activity against Gram-negative bacteria could be related to the outer membrane and its permeability properties that perform the crucial role of providing an extra layer of protection against potentially harmful compounds. (Delcour, 2009), (Zgurskaya, López, & Gnanakaran, 2015), (Wiener & Horanyi, 2011).

The results are in agreement with those of Martins et al. (2015a) using methanol water⁻¹ extract of thyme that related higher antibacterial capacity with higher contents in phenolic.

The most pronounced effect was observed for Gram-negative bacteria, zones inhibition were: *E. coli* (> 11 mm), *P. vulgaris* (8-10 mm), *P. aeruginosa* = *E. aerogenes* = *E. sakazakii* = *S. epidermidis* (< 7 mm). No activity was observed against *S. aureus* and *Klebsiella* spp.

Ethanol extract (1:1) of *Thymus vulgaris* presented an important antibacterial activity, It was able to inhibit (50 %) types of susceptible microorganisms tested, among them: *P. aeruginosa*; and *Proteus* spp.

However, it had no activity neither against the susceptible strains: *Salmonella choleraesuis*; *S. aureus*; *B. subtilis*; nor against the resistant ones: *S. aureus*; *K. pneumoniae*; *E. coli*; *Shigella* spp. MIC against both antibiotic-resistant bacteria *P. aeruginosa* and *Enterobacter aerogenes* was 70 $\mu\text{g mL}^{-1}$. (Nascimento, Locatelli, Freitas, & Silva, 2000).

The plant *Caryophyllus aromaticus* extracted and reextracted again for 3 times with 70% methanol presented the highest anti-*S. aureus* activity (MIC₉₀ = 460 $\mu\text{g mL}^{-1}$) and was effective against all bacterial strains tested: *E. coli*, *Salmonella*, *S. aureus* and *Enterococcus* sp. (Ushimaru, Silva, Di Stasi, Barbosa, & Fernandes Junior, 2007).

According to Al-Bayati (2008), *Thymus vulgaris* essential oil was found to be active against all the pathogenic bacteria tested except *P. aeruginosa*. The strongest antibacterial activity was seen against *S. aureus* with a MIC value of 31.2 $\mu\text{g mL}^{-1}$ followed by *E. coli* (62.5 $\mu\text{g mL}^{-1}$), *S. typhimurium* (125.0 $\mu\text{g mL}^{-1}$) and *K. pneumoniae* (500.0 $\mu\text{g mL}^{-1}$).

Inhibition zone and MICs obtained using *Thymus spathulifolius* methanolic fraction were: *E. faecalis* (10 mm, CMI= 250 $\mu\text{g mL}^{-1}$), *E. coli* (27 mm, CMI 31.25 = $\mu\text{g mL}^{-1}$), *P. aeruginosa* (12 mm, CMI= 500 $\mu\text{g mL}^{-1}$), *S. aureus* (14 mm, CMI= 250 $\mu\text{g mL}^{-1}$). No antibacterial effects were recorded against *E. cloacae* and *K. pneumoniae*. (Sökmen et al., 2004).

Methanolic extracts of *T. algeriensis* and *Thymus capitatus* demonstrated the same inhibition zone diameter in opposition to *E. coli* 7 mm, *P. aeruginosa* 7.33 mm, *S. aureus* = *E. faecalis* 10 mm. In contrast, *T. algeriensis* inhibited *Klebsiella* sp. 10.33 and *S. typhi* 7 mm, while inhibited *Thymus capitatus* by 8.33 and 10.33, respectively. (Megdiche-Ksouri et al., 2015).

Except *P. aeruginosa* at 10 mm, methanolic extract of thyme had no inhibitory action against the microorganisms tested: *K. pneumoniae*, *S. aureus*, and *E. coli*. (Albayrak et al., 2013).

The inhibitory effects of *Thymus pubescens* methanolic extract indicated significant bacterial growth inhibition zone diameters ranging from 8 to 16 mm against Gram-positive bacteria including *S. aureus*, methicillin-resistant *S. aureus* and *E. faecalis*. However, it showed no activity against Gram-negative bacteria (Mehrgan, Mojab, Pakdaman, & Poursaeed, 2008).

Further results recorded for *Thymus daenensis* methanol extract by Mojab, Poursaeed, Mehrgan, and Pakdaman (2008), indicated a significant antibacterial activity against Gram-positive bacteria including *S.*

aureus, MRSA and *E. faecalis* but it has shown no activity against Gram-negative bacteria. The produced zone of inhibition ranged from 8 to 29 mm.

Ethanollic flower extract of some studied Uruguayan medicinal plants has presented high antibacterial activity against: *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*. (Alonso-Paz et al., 1995). Methanol extract of *Rhus glabra*, a species used in folk medicine by North American native people, prevents the growth of *E. coli*, *P. aeruginosa*, *S. aureus*; for which MICs were 400, 100 and 100 $\mu\text{g mL}^{-1}$, respectively (Saxena, McCutcheon, Farmer, Towers, & Hancock, 1994).

Some researchers noticed that the major activity of polar extracts is mainly due to their richness with active compounds, essentially terpenes and phenols (Bekhechi, Atik-Bekkara, & Abdelouahid, 2008). However, the others have explained the absence of antibacterial activity in polar extracts by the fact that the compounds constituting the apolar fractions are at the origin of the antibacterial action.

Those compounds, probably be the phenolic diterpenoids, because of their highly lipophilic character, which allows them to be extracted with low polarity solvents such as chloroform (Fernandez-Lopez, Zhi, Aleson-Carbonell, Pérez-Alvares, & Kuri, 2005, Albano & Miguel, 2011).

Another study concluded that medicinal plants essential oil contained more antimicrobial compounds than other types of plant extracts like methanol, ethanol, water, and hexane (Şahin et al., 2004).

In the study done on the antibacterial effects of *T. algeriensis* essential oil, *Bacillus subtilis* was more resistant with a concentration of inhibition of $1/250 \text{ v v}^{-1}$, while other bacteria were inhibited from $1/500 \text{ v v}^{-1}$ the case of *Escherichia coli*, *Micrococcus luteus* and *Staphylococcus aureus*.

The inhibition zones observed in another study of antibacterial activity of the *T. algeriensis* essential oil were 13 mm against *P. aeruginosa*, 12 mm against *S. aureus*, 28 mm against *E. coli* and 20 mm against *S. typhimurium*. (Jayari et al., 2018).

However, *Thymus ciliatus* oil exerted a strong antibacterial activity where the concentration of $1/2000 \text{ v v}^{-1}$ was sufficient to inhibit the growth of *Escherichia coli*, while *S. aureus* was more sensitive with an inhibition concentration of $1/3000 \text{ v v}^{-1}$ (Amarti et al., 2010).

For many research groups, the inhibitory effect of *T. algeriensis* essential oil could be resulted from its composition of linalool and camphor, known to have excellent antibacterial properties or could be related to monoterpene hydrocarbons and oxygenated monoterpenes which are able to affect cell integrity, conducting to both the inhibition of the respiration and an alteration of the permeability. (Jayari et al., 2018).

Kabouche et al. (2005), reported that very high antibacterial potential (inhibition zones) of the essential oils of *Thymus numidicus* from 34 to 66 mm and 26-54 mm with *Thymus fontanesii*, antagonistic toward *E. aerogenes*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, but *S. typhimurium* was not affected.

Plant extracts have high potential as antibacterial agents, thus, they can be used in the treatment of infectious diseases. *T. algeriensis* methanolic and ethanolic extracts showed an exceptional richness in phenolic compounds, approved by the appreciable contents measured by the various tests but also by the well-known positive impact of such molecules on human health.

The results, as well as literature data, revealed the great potential of medicinal plants for therapeutic purposes, although they have not been completely investigated. So, more studies need to be conducted to search for new active compounds.

Conclusion

The results indicated that both methanolic and ethanolic extracts of *T. algeriensis* displayed strong antibacterial ability against the most pathogenic strains tested. It can, therefore, be inferred that those extracts could be useful as a natural antibacterial agents.

Additionally, the findings of this study could be important for further studies to identify, purify and elucidate the exact role of bioactive molecules responsible for such activity and determine possible applications for both food preservation and pharmaceutical purposes.

This study showed that the Algerian flora can constitute an important reserve of interesting plant species, including active compounds that can be used in several fields such as pharmaceutical industries.

More studies about the use of plants for therapeutic issues should be emphasized, especially those relevant to prevent the proliferation of antibiotic-resistant microorganisms.

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