

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 Biototoxicology, 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 Biototoxicology 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

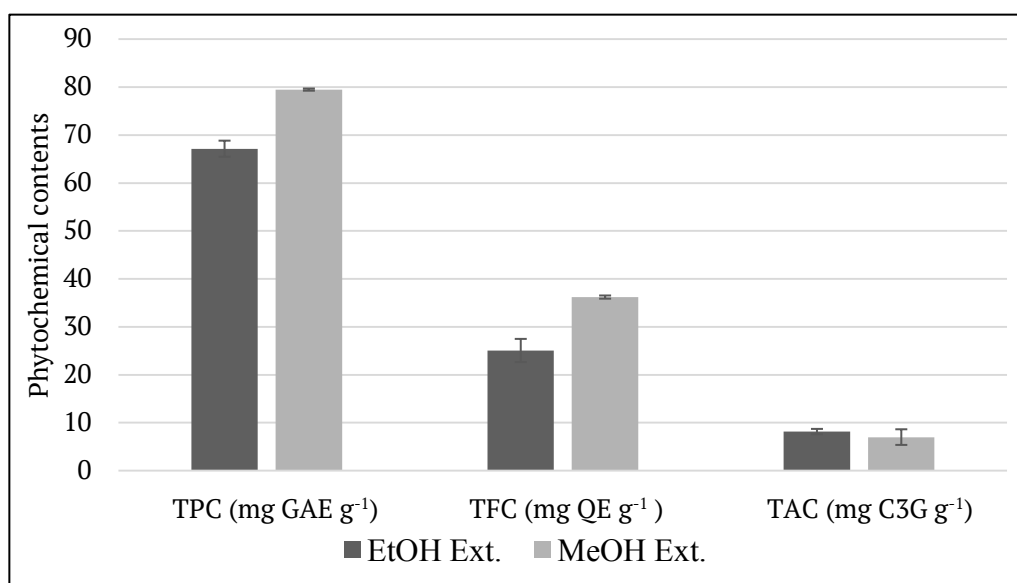


Figure 2. Results of phytochemical screening of *Thymus algeriensis* methanolic and ethanolic extracts. TPC (mg GAE g⁻¹), TFC (mg QE g⁻¹) and TAC (mg C3G g⁻¹)

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 µg 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 µg 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 µg mL⁻¹ followed by *E. coli* (62.5 µg mL⁻¹), *S. typhimurium* (125.0 µg mL⁻¹) and *K. pneumoniae* (500.0 µg mL⁻¹).

Inhibition zone and MICs obtained using *Thymus spathulifolius* methanolic fraction were: *E. faecalis* (10 mm, CMI= 250 µg mL⁻¹), *E. coli* (27 mm, CMI 31.25 = µg mL⁻¹), *P. aeruginosa* (12 mm, CMI= 500 µg mL⁻¹), *S. aureus* (14 mm, CMI= 250 µg 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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References

- Albano, S. M., & Miguel, M. G. (2011). Biological activities of extracts of plants grown in Portugal. *Industrial Crops and Products*, 33(2), 338–343. doi: 10.1016/j.indcrop.2010.11.012
- Al-Bayati, F. A. (2008). Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. *Journal of Ethnopharmacology*, 116(3), 403–406. doi: 10.1016/j.jep.2007.12.003
- Albayrak, S., Aksoy, A., Albayrak, S., & Sagdic, O. (2013). In vitro antioxidant and antimicrobial activity of some Lamiaceae species. *Iranian Journal of Science & Technology*, 37(1), 1–9. doi: 10.22099/IJSTS.2013.1529
- Alonso-Paz, E., Cerdeiras, M.P., Fernandez, J., Ferreira, F., Moyna, P., Soubes, M., ... Zunino, L. (1995). Screening of Uruguayan medicinal plants for antimicrobial activity. *Journal of Ethnopharmacology*, 45(1), 67–70. doi: 10.1016/0378-8741(94)01192-3
- Amarti, F., Satrani, B., Ghanmi, M., Farah, A., Aafi, A., Aarab, L., ... Chaouch, A. (2010). Composition chimique et activité antimicrobienne des huiles essentielles de *Thymus algeriensis* Boiss. & Reut. et *Thymus ciliatus* (Desf.) Benth. du Maroc. *Biotechnology, Agronomy, Society and Environment*, 14(1), 141–148.
- Azwanida, N. N. (2015). A Review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, 4(3). doi: 10.4172/2167-0412.1000196
- Babovic, N., Djilas, S., Jadranin, M., Vajs, V., Ivanovic, J., Petrovic, S., & Zizovic, I. (2010). Supercritical carbon dioxide extraction of antioxidant fractions from selected Lamiaceae herbs and their antioxidant capacity. *Innovative Food Science and Emerging Technologies*, 11(1), 98–107. doi: 10.1016/j.ifset.2009.08.013
- Bekhechi, C., Atik-Bekkara, F., & Abdelouahid, D. E. (2008). Composition and antibacterial activity of the essential oils contained in Algerian *Origanum glandulosum* (Desf.). *Phytothérapie*, 6(3), 153–159. doi: 10.1007/s10298-008-0310-6
- Brahmi, N., Scognamiglio, M., Pacifico, S., Mekhoukhe, A., Madani, K., Fiorentino, A., & Monaco, P. (2015). ¹H NMR based metabolic profiling of eleven Algerian aromatic plants and evaluation of their antioxidant and cytotoxic properties. *Food Research International*, 76(3), 334–341. doi: 10.1016/j.foodres.2015.07.005
- Burnichon, N., & Texier, A. (2003). L'antibiogramme: la détermination des sensibilités aux antibiotiques. *DES Bactériologie*, 1–29.
- Colpaert, P. (2006). *Thymus vulgaris: fiche technique*. Morlanwelz; Mariemont-Chapelle, FR: Ed. Département horticulture de l'Athénée provincial Warocqué Morlanwelz-Mariemont-Chapelle.
- Ćujić, N., Šavikin, K., Jankovic, T., Pljevljakušić, D., Zdunic, G., & Ibric, S. (2016). Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chemistry*, 194(1), 135–142. doi: 10.1016/j.foodchem.2015.08.008
- Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26(5), 343–356. doi: 10.1016/j.ijantimicag.2005.09.002
- Dapkevicius, A., Beek, T. A. V., Lelyveld, G. P., Veldhuizen, A. V., Groot, A. D., Linssen, J. P. H., & Venskutonis, R. (2002). Isolation and structure elucidation of radical scavengers from *Thymus vulgaris* leaves. *Journal of Natural Products*, 65(6), 892–896. doi: 10.1021/np010636j
- Delcour, A. H. (2009). Outer membrane permeability and antibiotic resistance. *Biochimica et Biophysica Acta*, 1794(5), 808–816. doi: 10.1016/j.bbapap.2008.11.005
- El Abed, N., Guesmi, F., Mejri, M., Marzouki, M. N., & Ahmed, S. B. H. (2014). Phytochemical screening and assessment of antioxidant, antibacterial and cytotoxicity activities of five Tunisian medicinal plants. *International Journal of Pharmaceutical Research and Bio-Science*, 3(4), 770–789.

- Farhadi, F., Khameneh, B., Iranshahi, M., & Iranshahi, M. (2018). Antibacterial activity of flavonoids and their structure–activity relationship: an update review. *Phytotherapy Research*, 33, 13–40. doi: 10.1002/ptr.6208
- Fernandez-Lopez, J., Zhi, N., Aleson-Carbonell, L., Pérez-Alvarez, J. A., & Kuri, V. (2005). Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Science*, 69(3), 371–380. doi: 10.1016/j.meatsci.2004.08.004
- Gavarić, N., Kladar, N., Misan, A., Nikolic, A., Samojlik, I., Mimica-Dukic, N., & Bozin, B. (2015). Postdistillation waste material of thyme (*Thymus vulgaris* L., Lamiaceae) as a potential source of biologically active compounds. *Industrial Crops and Products*, 74, 457–464. doi: 10.1016/j.indcrop.2015.05.070
- Gedikoğlu, A., Sökmen, M., & Çivit, A. (2019). Evaluation of *Thymus vulgaris* and *Thymbra spicata* essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. *Food Science & Nutrition*, 7(5), 1704–1714. doi: 10.1002/fsn3.1007
- Gharibi, S., Tabatabaei, B. E. S., & Saeidi, G. (2015). Comparison of essential oil composition, flavonoid content and antioxidant activity in eight *Achillea* species. *Journal of Essential Oil Bearing Plants*, 18(6), 1382–1394. doi: 10.1080/0972060x.2014.981600
- Ghedadba, N., Bousselsela, H., Hambaba, L., Benbia, S., & Mouloud, Y. (2014). Evaluation de l'activité antioxydante et antimicrobienne des feuilles et des sommités fleuries de *Marrubium vulgare* L. *Phytothérapie*, 12(1), 15–24. doi: 10.1007/s10298-014-0832-z
- Giordani, R., Regli, P., Kaloustian, J., Mikail, C., Abou, L., & Portugal, H. (2004). Antifungal effect of various essential oils against *Candida albicans* potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris*. *Phytotherapy Research*, 18(12), 990–995. doi: 10.1002/ptr.1594
- Goetz, P., & Ghedira, K. (2012). *Thymus vulgaris* L. (Lamiaceae): thym. *Phytothérapie Anti-Infectieuse: Collection Phytothérapie Pratique*, 357–365. doi: 10.1007/978-2-8178-0058-5_27
- Guesmi, F., Ben Farhat, M., Mejri, M., & Landoulsi, A. (2014). *In vitro* assessment of antioxidant and antimicrobial activities of methanol extracts and essential oil of *Thymus hirtus* sp. *algeriensis*. *Lipids in Health and Disease*, 13(114), 1–12. doi: 10.1186/1476-511x-13-114
- Hazzit, M., & Baaliouamer, A. (2007). Essential oil composition of *Thymus algeriensis* Boiss. ET Reut. and *Thymus numidicus* Poiré from Algeria. *Rivista Italiana EPPOS*, (43), 11–18.
- Hendra, R., Ahmad, S., Sukari, A., Shukor, M. Y., & Oskoueian, E. (2011). Flavonoid analyses and antimicrobial activity of various parts of *Phaleria macrocarpa* (Scheff.) Boerl fruit. *International Journal of Molecular Sciences*, 12(6), 3422–3431. doi: 10.3390/ijms12063422
- Javed, H., Erum, S., Tabassum, S., & Ameen, F. (2013). An overview on medicinal importance of *Thymus vulgaris*. *Journal of Asian Scientific Research*, 3(10), 974–982.
- Jayari, A., El Abed, N., Jouini, A., Wahab, O. M. S. A., Maaroufi, A., Ahmed, S. B. H. (2018). Antibacterial activity of *Thymus capitatus* and *Thymus algeriensis* essential oils against four food-borne pathogens inoculated in minced beef meat. *Journal of Food Safety*, 38(1), 1–10. doi: 10.1111/jfs.12409
- Kabouche, Z., Boutaghane, N., Laggoune, S., Kabouche, A., Ait-Kaki, Z., & Benlabed K. (2005). Comparative antibacterial activity of five Lamiaceae essential oils from Algeria. *International Journal of Aromatherapy*, 15(3), 129–133. doi: 10.1016/j.ijat.2005.03.006
- Kumar, A., & Jnanesha, A. C. (2016). Medicinal and aromatic plants biodiversity in India and their future prospects: a review. *Indian Journal of Unani Medicine*, 9(1), 10–17.
- Lamchouri, F., Benali, T., Bennani, B., Toufik, H., Hassani, L. I. M., Bouachrine, M., & Lyoussi, B. (2012). Preliminary phytochemical and antimicrobial investigations of extracts of *Haloxylon scoparium*. *Journal of Materials and Environmental Science*, 3(4), 754–759.
- Martins, N., Barros, L., Santos-Buelga, C., Silva, S., Henriques, M., & Ferreira, I. C. F. R. (2015a). Decoction, infusion and hydroalcoholic extract of cultivated thyme: antioxidant and antibacterial activities, and phenolic characterisation. *Food Chemistry*, 167, 131–137. doi: 10.1016/j.foodchem.2014.06.094
- Martins, N., Barros, L., Santos-Buelga, C., Henriques, M., Silva, S., & Ferreira, I. C. F. R. (2015b). Evaluation of bioactive properties and phenolic compounds in different extracts prepared from *Salvia officinalis* L. *Food Chemistry*, 170, 378–385. doi: 10.1016/j.foodchem.2014.08.096

- Megdiche-Ksouri, W., Saada, M., Soumaya, B., Snoussi, M., Zaouali, Y., & Ksouri, R. (2015). Potential use of wild *Thymus algeriensis* and *Thymus capitatus* as source of antioxidant and antimicrobial agents. *Journal of New Sciences, Agriculture and Biotechnology*, 23(4), 1046–1056.
- Mojab, F., Poursaeed, M., Mehrgan, H., & Pakdaman, S. (2008). Antibacterial activity of *Thymus daenensis* methanolic extract. *Pakistan Journal of Pharmaceutical Sciences*, 21(3), 210–213.
- Mehrgan, H., Mojab, F., Pakdaman, S., & Poursaeed, M. (2008). Antibacterial activity of *Thymus pubescens* methanolic extract. *Iranian Journal of Pharmaceutical Research*, 7(4), 291–295. doi: 10.22037/IJPR.2010.778
- Msaada, K., Tammar, S., Salem, N., Bachrouch, O., Sriti, J., Hammami, M., ... Marzouk, B. (2016). Chemical composition and antioxidant activities of Tunisian *Thymus capitatus* L. methanolic extract. *International Journal of Food Properties*, 19(6), 1381–1390. doi: 10.1080/10942912.2015.1082138
- Murray, P., Rosenthal, K., & Pfaller, M. (2016). *Medical Microbiology* (8th ed.). Philadelphia, PA: Elsevier.
- Nascimento, G. G. F., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31(4), 247–256. doi: 10.1590/S1517-83822000000400003
- Nezhadali, A., Nabavi, M., Rajabian, M., Akbarpour, M., Pourali, P., & Amini, F. (2014). Chemical variation of leaf essential oil at different stages of plant growth and in vitro antibacterial activity of *Thymus vulgaris* Lamiaceae, from Iran. *Beni-Suef University Journal of Basic and Applied Sciences*, 3(2), 87–92. doi: 10.1016/j.bjbas.2014.05.001
- Nikolić, M., Glamoclija, J., Ferreira, I. C. F. R., Calhelha, R. C., Fernandes, Â., Markovic, T., ... Sokovic, M. (2014). Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils. *Industrial Crops and Products*, 52, 183–190. doi: 10.1016/j.indcrop.2013.10.006
- Regnier, T., Combrinck, S., Veldman, W., & Du Plooy W. (2014). Application of essential oils as multi-target fungicides for the control of *Geotrichum citri-aurantii* and other postharvest pathogens of *Citrus*. *Industrial Crops and Products*, 61, 151–159. doi: 10.1016/j.indcrop.2014.05.052
- Roby, M. H. H., Sarhan, M. A., Selim, K. A. H., & Khalel, K. I. (2013). Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Industrial Crops and Products*, 43, 827–831. doi: 10.1016/j.indcrop.2012.08.029
- Sagdic, O., Ozkan, G., Aksoy, A., & Yetim, H. (2009). Bioactivities of essential oil and extract of *Thymus argaeus*, Turkish endemic wild thyme. *Journal of the Science of Food and Agriculture*, 89(5), 791–795. doi: 10.1002/jsfa.3513
- Şahin, F., Gulluce, M., Daferera, D., Sökmen, A., Sökmen, M., Polissiou, M., ... Özer, H. (2004). Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. *Food Control*, 15(7), 549–557. doi: 10.1016/j.foodcont.2003.08.009
- Santos, F. S. M., Bezerra, J. W. A., Kamdem, J. P., Boligon, A. A., Anraku, M. M., Silva, A. R. P., ... Santos, J. E. G. (2019). Polyphenolic composition, antibacterial, modulator and neuroprotective activity of *Tarenaya spinosa* (Jacq.) Raf. (Cleomaceae). *Asian Pacific Journal of Tropical Biomedicine*, 9(1), 12–17. doi: 10.4103/2221-1691.250264
- Sarker, S.D. (2005). *Natural products isolation* (2nd ed.). Totowa, NJ: Humana Press (p. 1–25).
- Saxena, G., McCutcheon, A. R., Farmer, S., Towers, G. H. N., & Hancock, R. E. W. (1994). Antimicrobial constituents of *Rhus glabra*. *Journal of Ethnopharmacology*, 42(2), 95–99. doi: 10.1016/0378-8741(94)90102-3
- Siri, M., Villanueva, P., Pianzzola, M. J., Fraguas, L. F., Galván, G., Acosta, M., & Ferreira, F. (2004). In vitro antimicrobial activity of different accessions of *Solanum commersonii* Dun. from Uruguay. *Potato Research*, 47(3–4), 127–138. doi: 10.1007/BF02735979
- Skendi, A., Irakli, M., & Chatzopoulou, P. (2017). Analysis of phenolic compounds in Greek plants of Lamiaceae family by HPLC. *Journal of Applied Research on Medicinal and Aromatic Plants*, 6, 62–69. doi: 10.1016/j.jarmap.2017.02.001

- Sökmen, A., Gulluce, M., Askin Akpulat, H., Daferera, D., Tepe, B., Polissiou, M., ... Sahin, F. (2004). The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. *Food Control*, 15(8), 627–634. doi: 10.1016/j.foodcont.2003.10.005
- Soni, N. R. (2012). To study the herbalism of thyme leaves. *International Journal of Pharmacy and Industrial Research*, 2(3), 252–259.
- Tohidi, B., Rahimmalek, M., & Arzani, A. (2017). Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran. *Food Chemistry*, 220, 153–161. doi: 10.1016/j.foodchem.2016.09.203
- Ushimaru, P. I., Silva, M. T. N., Di Stasi, L. C., Barbosa, L., Fernandes Junior, A. (2007). Antibacterial activity of medicinal plant extracts. *Brazilian Journal of Microbiology*, 38(4), 717–719. doi: 10.1590/S1517-83822007000400024
- Wiener, M. C., & Horanyi, P. S., (2011). How hydrophobic molecules traverse the outer membranes of Gram-negative bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 108(27), 10929–10930. doi: 10.1073/pnas.1106927108
- Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Current Medicinal Chemistry*, 22(1), 132–149. doi: 10.2174/0929867321666140916113443
- Yihune, E., & Yemata, G. (2019). Antibacterial activity of medicinal plant extracts against *Ralstonia solanacearum* (Smith) that causes bacterial wilt in hot pepper (*Capsicum annuum* L.). *Acta Scientiarum. Biological Sciences*, 41(e45402), 11. doi: 10.4025/actascibiolsoci.v41i1.45402
- Zgurskaya, H. I., López, C. A., & Gnanakaran, S. (2015). Permeability barrier of gram-negative cell envelopes and approaches to bypass it. *American Chemical Society Infectious Diseases*, 1(11), 512–522. doi: 10.1021/acsinfecdis.5b00097
- Festy, D. (2014). *Huiles essentielles le guide visuel*. France: Éditions Quotidien Malin, Leduc. S.