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Evaluation of biological activities of Thyme extracts and their use as a natural alternative to antibiotics for the reduction of pharmaceutical waste

N. Mebarki[1,2]*, M. Nabiev[2], H. Ziane[3], H. Chader [4], F. Fazouane[1]

[1] Laboratory of Alimentary Technology, Faculty of Engineering Sciences, University of Boumerdes, Boumerdes 35000, Algeria
[2] Department of Chemical and Pharmaceutical Process Engineering, Faculty of Hydrocarbons and Chemistry, University of Boumerdès 35000, Algeria
[3] Microbiology Laboratory, Centre Hospitalo-Universitaire Mustapha Bach, Algiers 16000, Algeria.
[4] National Laboratory of Control of Pharmaceutical Products (LNCPP), Algiers 16000, Algeria.

*Corresponding author: n.mebarki@univ-boumerdes.dz; Tel.: +213 07 77 62 49 15; Fax: +213 023238591

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ABSTRACT/RESUME

Abstract: *Excessive and improper use of chemical antibiotics is dangerous to human health and animals and causes an unhealthy environment. Hence, medicinal plants are expected to be the future alternative source of new antimicrobial products. The present study aims to determine the chemical composition of Thymus fontanesii Boiss. & Reut. essential oil (EO) then to isolate and characterise its main constituent, thymol, and to evaluate their antibacterial and antioxidant activities. The oil was obtained by hydro-distillation from the aerial parts of T. fontanesii at yield of 3.4±0.2% and 06 main compounds were identified using GC/MS technique representing more than 99% of the oil composition. The major constituents were thymol (77.72%) and carvacrol (13.27%). The antimicrobial activity of our extracts was investigated in comparison with four synthetic antibiotics, using the disc diffusion and broth macro-dilution methods against 13 pathogenic bacteria. The antimicrobial evaluation showed that EO and thymol isolate exhibited high antibacterial activity against all the strains tested compared with the synthetic antibiotics, they were capable of inhibiting the growth of bacterial organisms tested with inhibition zones in the range of 10–58.53 mm. The EO was the most active with MIC and MBC values of (0.035 mg/mL to 0.565 mg/mL). The antioxidant activity of our extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH**)** radical scavenging method. The antioxidant activity revealed that the EO and thymol isolate demonstrated a very important anti-radical activity compared to the standards (ascorbic acid and BHT). T. fontanesii essential oil showed the strongest ($p < 0.05$) radical scavenging effect, with 50% inhibitory concentration (IC50) of 15.85 ± 0.23µg/mL. The results showed that the essential oil of T. fontanesii and thymol, its major compound, are an excellent alternative to synthetic antibiotics. They are of great interest because of their availability, non-toxicity and their friendship with the environment, and therefore can be useful for the food and pharmaceutical industries.*

I. Introduction

The therapy of human and animal infections caused by pathogenic bacteria is mainly based on the use of antibiotics. As such, consumption of synthetic antibiotics is increasing day by day, as part of a healthy life- style maintenance by humans and as growth promoters in veterinary medicine **[1]**. However their overuse soon caused problems such as ineffectiveness on pathogenic bacteria due to the accelerated development of antibiotic resistant bacteria **[2]**. In addition, antibiotics have been recently recognized as an emerging class of environmental contaminants since they have been extensively administered in humans and animals and persist in the environment through a complex vicious cycle of transformation and bioaccumulation **[3, 4]**. In fact, antibiotic residues present in the ecosystem provide an ideal setting for the formation of antibiotic resistant bacteria, resulting in serious human health and environmental problems **[5-7]**. Therefore the increase in resistance to conventional antibacterial agents, toxicity and costs involved justified the search for new therapeutic approaches more effective, more ecofriendly and a less toxic alternative for the treatment of bacterial infections **[8]**. Among these novel approaches, essential oils are promising natural compounds for use in the prevention and treatment of microbial infections **[8]**. Numerous studies have shown antimicrobials effects of essential oils **[8-10]**. We chose *Thymus fontanesii* essential oil among other essential oils because this plant is relatively abundant and widely used in traditional medicine in Algeria in the form of powders, infusions and decoctions for the treatment of many ailments including respiratory and digestive tube disorders **[11, 12]**.

The objective of the present study is the characterisation of *Thymus fontanesii* extracts (essential oil and thymol isolated) and the evaluation of their antibacterial and antioxidant activities in comparison with synthetics antibiotics and antioxidants.

II. Materials and methods

II.1 Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH, 95%), ascorbic acid and butylated hydroxytoluene (BHT) were procured from Sigma–Aldrich Chemie (Steinheim, Germany). Analytical grade methanol, ethanol, Tween 80, Sodium hydroxide (NaOH), hydrochloric acid (HCl) and n-Hexane were obtained from Merck (Darmstadt, Germany). Ultra pure water was used for the experiments. Acetic acid and methanol (99,9%, of HPLC grade), were purchased from Sigma–Aldrich (Sigma–Aldrich, St. Louis MO, USA). All the other organic solvents and chemicals used in this study were of analytical grade.

II.2 Plant materials and essential oil isolation

The aerial parts of *T. fontanesii* were collected at the beginning of the flowering period (March) from the Lakhdaria mountain at 1040 m altitude (Bouira region), 74 km south east of Algiers. A voucher specimen was deposited in the herbarium of the Department of Botany of the National Institute of Agronomy (INA) in Algiers. The essential oil was obtained by the hydrodistillation for 2 hours using a Clevenger-type apparatus and stored in sealed glass vials at 4 °C in the dark until used.

II.3 Isolation of thymol

Thymol is one of the main known phenols in the thyme essential oil of formula $C_{10}H_{14}O$, isolated according to **Mohammed and Al-Bayati (2009)[13]** with some modifications. The treatment of *T. fontanesii* EO, dissolved in hexane, with a solution of caustic soda (25%), the hydrocarbons forming the organic phase are removed and the aqueous phase containing the sodium thymolate ($C_{10}H_{13}ONa$) is neutralized with the hydrochloric acid (5M). The thymol comes to float on the liquor surface; it is collected by decantation followed by an alcoholic re-crystallization.

II.4 Analysis and Characterization of thyme EO and thymol isolate

II.4.1 Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis of the thyme EO and thymol, its main compound isolate, was carried out on an Agilent HP-6890 gas chromatograph with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m - 0.25 mm, 0.25 µm film thickness) coupled to a quadrupole mass spectrometer (model Agilent HP 5973) network mass selective detector in the electron impact mode (Ionization energy: 70 eV). Analytical parameters were the following: the carrier gas was helium at a flow rate of 1 mL/min, the oven temperature was programmed from 60 to 280°C at 2 °C/min and held isothermal for 30 min. Injector and detector temperatures were set at 250 and 280 °C, respectively, and in the GC–MS analyses, temperatures of the ion source and transfer line were 170 and 280 °C, respectively. The Identification of the components was made by visual interpretation, comparing their retention indices and mass spectra with those stored in the mass spectrometry data base and given in the literature by Adams terpene library **[14-16]**.

II.4.2 High-performance liquid chromatography (HPLC)

The HPLC analysis of thymol isolated were performed in the Scientific and technical Research Centre in Physico-chemical Analyses (CRAPC), using a Young Line YL 9100 high-performance liquid chromatography equipped with C18 column

(150x4.6 mm, 5µm, Restek). The mobile phase was composed of ultrapure water/ acetic acid (99:1) (solvent A) and methanol (solvent B) with a flow rate of 1 mL/min. The volume of extract and standards injected was 20 µl. The eluate was detected using an UV detector at 254 nm. Thymol was identified by comparing its UV spectrum and retention time with that of standard.

II.5 Antibacterial activity

II.5.1 Microbial strains

The *in vitro* antibacterial activity of the EO from *T. fontanesii* and thymol isolate were tested against thirteen bacterial strains obtained from the microbiological laboratory of Mustapha Bacha hospital and Research and Development Centre, SAIDAL Algiers, including: six Gram-positive, namely *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 9372), *Bacillus cereus* (ATCC 10876), *Enterococcus faecalis* (ATCC 6569), *Listeria monocytogenes* (ATCC 15313), and seven Gram-negative bacteria: *Escherichia coli* (ATCC 4157), *Escherichia coli* (ATCC 25922), *Salmonella Enteritidis* (ATCC 13076), *Salmonella Typhimurium* (ATCC 13311), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 9027) and *Pseudomonas aeruginosa* (ATCC 27853).

II.5.2 Antibacterial screening

The EO and thymol isolate were screened for antibacterial activity using the disk diffusion method. A suspension (0.2 mL of 10^8 cfu/mL) of each microorganism was spread on Mueller Hinton Agar (MHA). Sterile filter paper disks (6 mm in diameter) were individually impregnated with 10 µL of each extracts (EO and thymol (10mg/mL)) and placed on the inoculated plates. Negative controls were prepared using a disk impregnated with sterile water. The treated Petri dishes were incubated at 37°C for 24 h. Before incubation, all plates were stored in the dark at 4°C for 2h, to allow the diffusion of the oil from disc to medium without microbial growth. Antibacterial activity was evaluated by measuring the diameter of the growth inhibition zones in millimeters (including disc diameter of 6 mm) for the test organisms and comparing to the control. All the tests were performed in triplicate.

II.5.3 Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The broth macro-dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). All tests were performed in Mueller Hinton Broth (MHB) supplemented with 5% Tween 80 at 2%. Tubes of MHB containing various concentrations of EO and thymol were inoculated with 10µl bacterial inoculums adjusted to 10^6cfu/ml of bacteria cells strains. The test tubes were incubated at 37◦C for 24 h. The positive control was performed in the medium containing only the microorganism suspension. For determination of minimum bactericidal concentration (MBC), 0.1 ml of culture medium was a spirited from each micro broth assay tube showing no apparent growth and sub-cultured in fresh Mueller Hinton Agar (MHA). After incubation at 37◦C for 24 h, the least concentration showing no visible growth on sub-culture was taken as the MBC.

II.6 Antioxidant Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay was applied according to **Sarikurkcu et al. 2010[17]** with minor modifications. One milliliter of various concentrations of *T.fontanesii* EO and thymol isolate in methanol were added to 1 ml of DPPH radical solution in methanol (0.2 mM). The mixture was shaken vigorously and allowed to stand for 30 min in the dark at room temperature. Absorbance of the resulting solution was measured at 517 nm by a spectrophotometer against methanol as a blank. Ascorbic acid and BHT were used as a control. The percent of DPPH radical inhibition of the samples was calculated according to the following equation: **[18]**

$$\%I = [(A_B - A_A)/ A_B] \times 100$$

Where: A_B: absorbance of DPPH solution without extracts; A_A: absorbance of samples.

The concentration of our extracts or controls that could scavenge 50% of the DPPH radicals (IC50) was calculated by plotting inhibition percentages against concentrations of the samples **[19]**. All tests were carried out in triplicate and IC50 values were reported as means ± SD of triplicates.

II.7 Statistical analysis

Data obtained were analyzed using the student's *t*-test and a *P* value less than 0.05 was considered statistically significant. All experiments were done in triplicate and results were presented as mean ± standard deviation (SD).

III. Results and discussion

III.1 Thyme EO yield and chemical composition

The volatile oil extracted from *T.fontanesii* aerial parts was yellow with agreable perfumery odour. The extraction yield was approximately 3.4 ± 0.2% (v/w) , which is higher than those found by **Haddouchi et al. 2009[20] and Mohammedi et al.**

2010[21] (2%, 3.09% respectively), for the same species of thyme harvested in Mostaganem province and Tlemcen area (Algeria). Furthermore, it was higher than that obtained from other species of thyme from Algeria considered as economically important for essential oils production, such as *Thymus lanceolatus* with 0.9%**[22]**, *Thymus dreatensis* with 2.1% **[23]** and *Thymus pallescens* with 1.7–1.9% yields **[10]**.

The chemical analysis revealed the presence of six main compounds (**Table 1**), with thymol being the major component with 77.72% followed by carvacrol (13.27%). This compound was also shown to be in the majority in other studies with the EO of *T.fontanesii*, in different concentrations, such as 67.80%, 68.20% and 29.30 % **[11, 12, 24]**. Except for results of **Bekhechi et al. 2007[25]**, where carvacrol was reported to be the major oil component (66.7-69.5%) while thymol represented 0.6-0.7%. Our oil consisted mainly in oxygenated monoterpenes (90.99%) and hydrocarbon monoterpenes (6.03%). Sesquiterpenoids represented only 2.96% of the total composition. Our results are in agreement with on other study **[11, 12, 24]**.

It is well known that the yield and the chemical composition of the EO can vary within the same species. Such variability depends on several factors including local climatic and environmental conditions, season, geographical location, geology **[26, 27]**.

Table 1. *Main chemical composition of Thymus fontanesii essential oil*

Compounds	**RI**	**Percentage (%)**
Gamma-terpinene	1060	4.86
Linalool	1106	1.17
Thymol	1290	**77.72**
Carvacrol	1298	13.27
Alpha-gurjunene	1412	0.93
Beta-Caryophyllene	1416	2.03
Monoterpene hydrocarbons		**6.03**
Phenolic monoterpenes		**90.99**
Sesquiterpene		**2.96**
Total		**99.98**

RI : retention index, obtained with reference to an n-alkyne series C8H18–C20H42 using DB-5 column and the Van den Dool and Kratz equation.

III.2 Identification of thymol extract

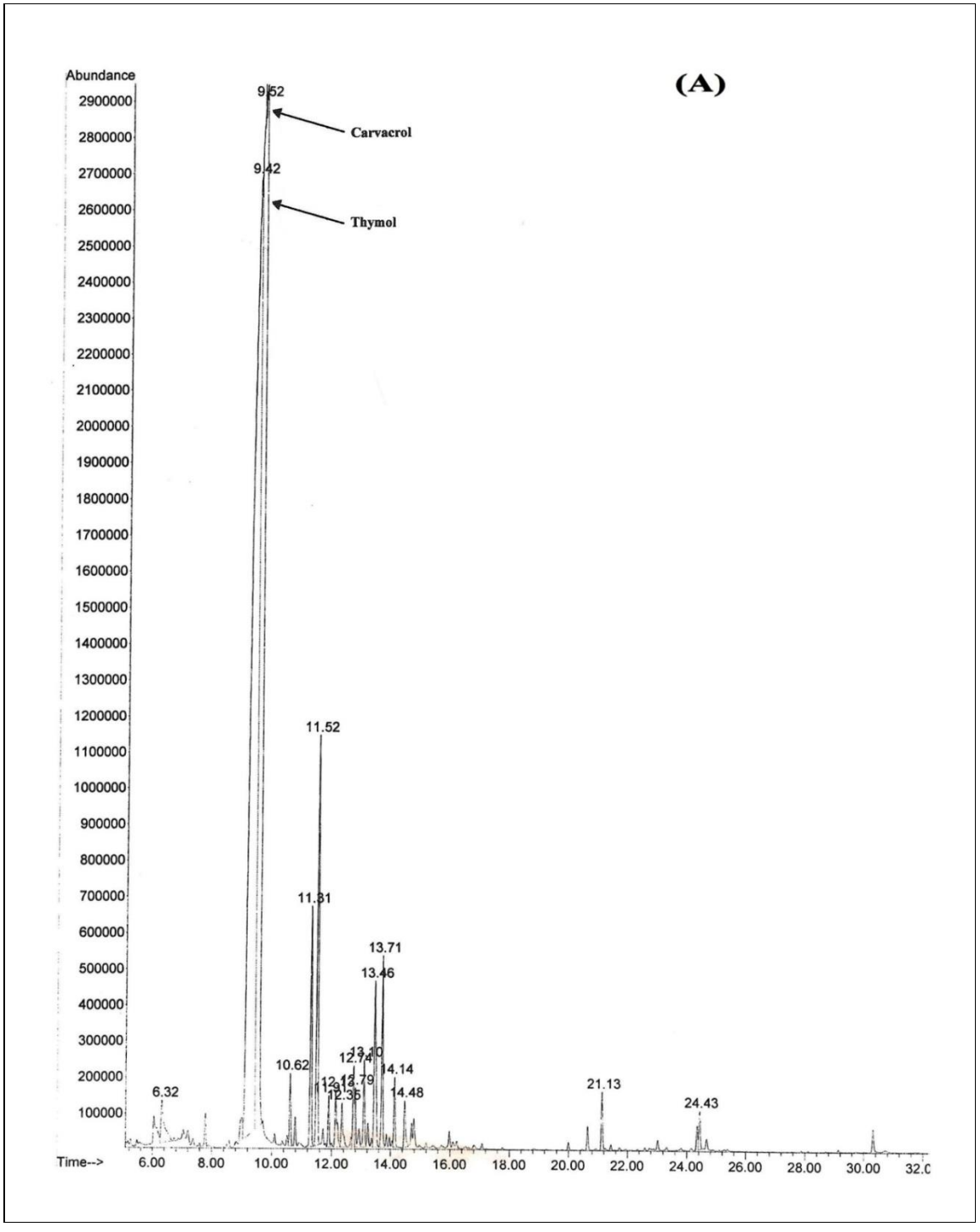
III.2.1 Chemical identification of thymol isolate

The extracted thymol is colourless fusible crystals at 50-51.5 ° C. It has a very strong thyme smell. It is very slightly soluble in water (1.4 g/l) at 40°C. On the other hand, it is very soluble in most organic solvents at 40°C including: ethanol (1000g/L), ether (700g/L). With thymol, $FeCl_3$ does not give a colour reaction. All of our results show that the product extracted from the essential oil of *T. fontanesii* is thymol.

The chemical identification of thymol is a necessary but not sufficient step to characterise our extracted product. It is therefore necessary to supplement it with HPLC and GC/MS chromatographic analyzes. The latter is often used as an analytical means for the structural analysis of volatile substances.

III.2.2 Analysis of thymol isolated by GC/MS and HPLC

A gas chromatographic-mass spectrometry coupling analysis of the EO of *T. fontanesii* before and after extraction of thymol and product extract was considered to confirm that our extracted product is truly thymol **(Figure. 1)**.



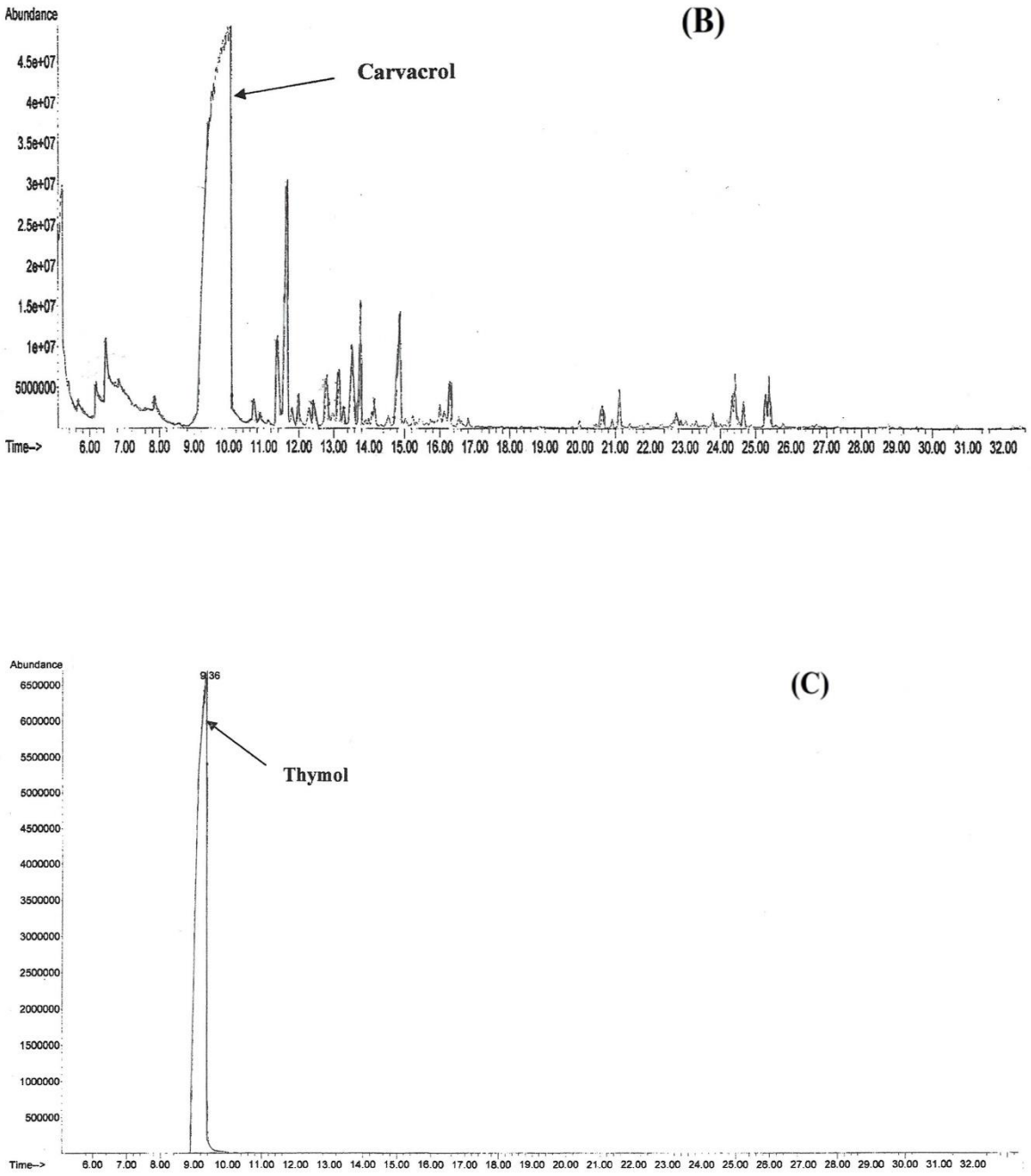


Figure 1. *Chromatogram of Thymus fontanesii essential oil showing the major components: before isolation of thymol. (A), after isolation of thymol (B) and Chromatogram of thymol isolate (C).*

Moreover, the HPLC analysis revealed that the product extract isolate is thymol, the major components of the *T.fontanesii* EO **(Figure. 2)**. The retention time is 33–35min and the spectrum of the isolated compound on HPLC was completely identical to that of standard thymol.

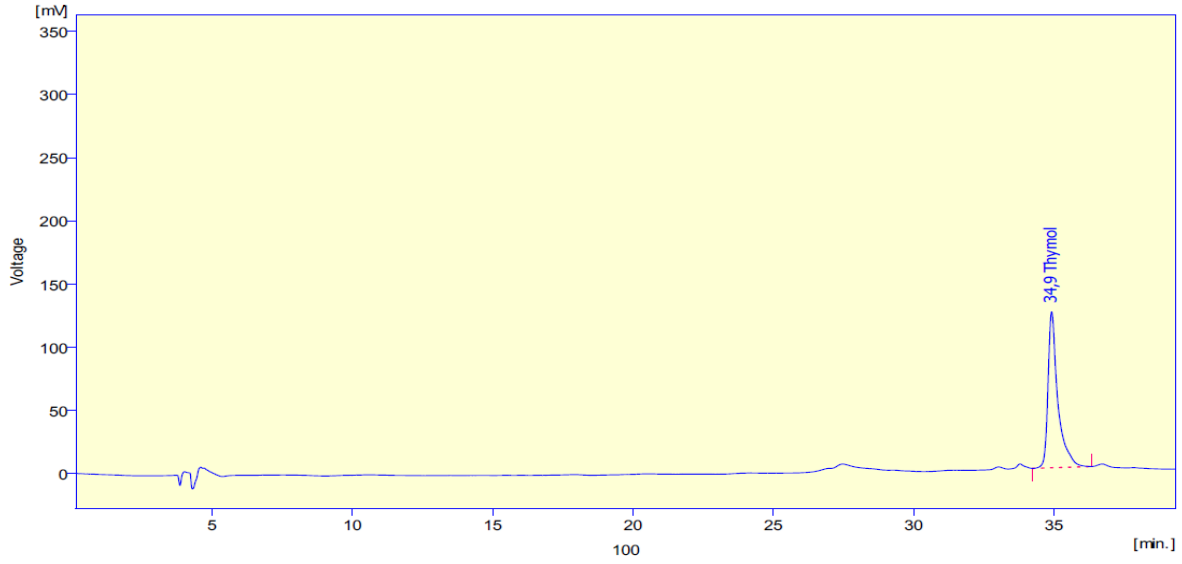


Figure 2. *HPLC Chromatogram of thymol isolate*

III.3 Antibacterial activity

According to the results given in **Table 2**, the *T. fontanesii* essential oil and its isolated compound showed an important *in vitro* antibacterial activity against all bacteria tested. As can be seen from **Table 2**, the EO of *T.fontanesiis* showed the strongest inhibitory activity and this activity was followed by the thymol isolated. The data obtained from the agar disc diffusion method indicated that these extracts exhibited varying degrees of antibacterial activity on the different tested strains. The inhibition zones of bacteria strains were in the range of 13.33–58.53 mm and 12-45 mm for the EO and thymol extract respectively (**Figure. 3 and 4**). The thyme EO oil and thymol isolate showed higher antibacterial activity than Chloramphenicol (30µg/disc), Gentamicin (10µg/disc), Ampicilin (10µg/disc) and Trimethoprim/Sulfamethoxazole (1.25/23.75µg/disc).

However, in the present study, *T. fontanesii* EO and thymol extract did not show any selective Gram-positive *vs.* Gram-negative antibacterial activity. Thus, and according to the classification established by **Djabou et al. 2013[28]**, *S. aureus* , *Sal. typhimurium* and *E. coli* were the most sensitive bacterium tested, while the least sensitive micro-organism to our extracts was *P. aeruginosa*. Our results are similar to other researches that have proved that the strong antibacterial activity of essential oils was not specific for Gram-positive or Gram-negative bacteria **[29, 30]**. According to **Ballester-Costa et al. 2013[9]**, the essential oil of *T.fontanesii* has a very strong inhibitory activity against all pathogenic bacteria tested (D>30mm) except *P. aeruginosa* which has moderately inhibitory activity. This bacterium has resistance to many essential oils and antibiotics drugs **[22, 31]**. This could be explained by the structure of cell envelope **[31]**. For the broth macro-dilution method, the essential oil of *T.fontanesii* and its main compound extract showed differences in microbial susceptibility.

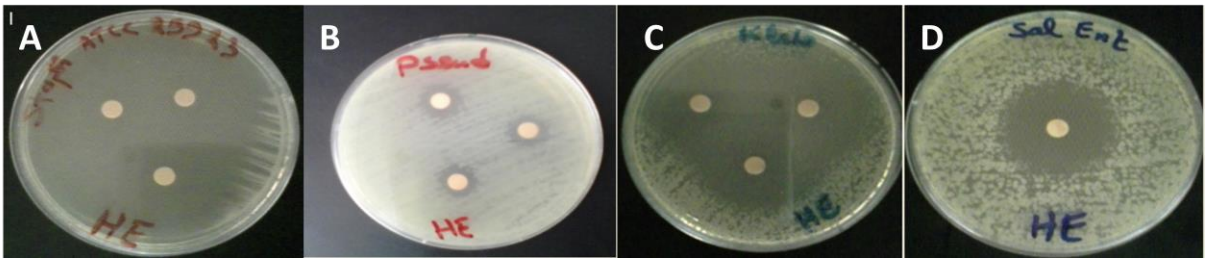


Figure 3. *Inhibition zones of T.fontanesii essential oil on: A. S. aureus, B. P. aeruginosa, C. K. pneumoniae and D. Sal. Enteritidis.*

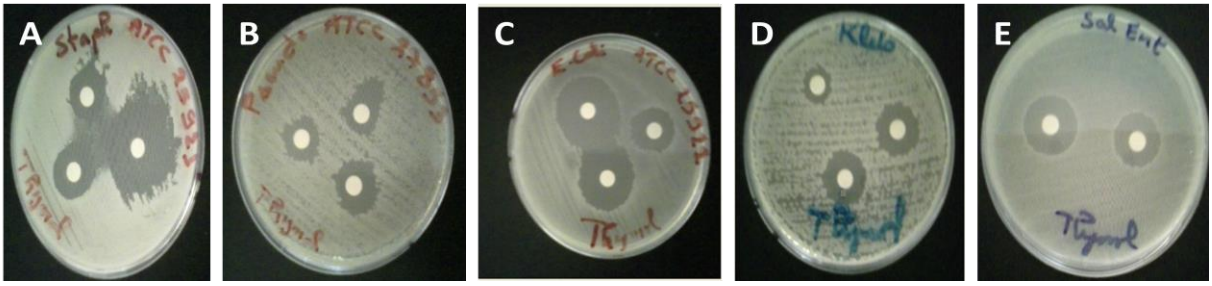


Figure 4. *Inhibition zones of thymol isolate on: A. S. aureus, B. P. aeruginosa, C. E. coli, D. K. pneumoniae and E. Sal. Enteritidis.*

Results, shown in **Table 2**, confirm all investigated samples as having relatively strong antibacterial agents. The EO was the most active with MIC values of (0.035 mg/mL to 0.565 mg/mL) than the thymol with MIC values of (0.093 mg/mL to 3 mg/mL). The antimicrobial activity of plant materials can be classified according to their MIC values **[32]**. The strongest activity of our extracts was observed against *S. aurus* (0.035 mg/mL for EO and 0.093 mg/mL for thymol) and the weak activity was observed against *P. aeruginosa* (0.565 mg/mL for EO and 1.5 mg/mL for thymol), with similar susceptibility of these bacteriums to the other thyme **[29]**. We found that the activity of the essential oil depends on its concentration and the strain of tested bacteria **[33]**. Although the MICs and MBCs results varied between organisms tested, in the most cases the MICs were equivalent to the MBCs, indicating a bactericidal activity of the essential oil and thymol isolated on all strains used in this study **[34-36]**. The *T.fontanesii* essential oil showed better antibacterial activity in comparison with its main component isolate (thymol) that can be attributed to the presence of high percentage of phenolic compounds such as thymol and carvacrol (90.99%) appreciated for their antibacterial potentials **[31, 35, 37]**. Also, many minor constituents of the EO have potential antimicrobial activity as it was reported previously **[32, 33]**. Thus, the antibacterial activity could be caused by the synergistic effect between the major compounds and the minor of the EO. According to the literature, the antimicrobial activity of essential oils could be attributed to their major and minor components **[9, 32, 38]**. Thymol and its isomer carvacrol, the major components of thyme EO, are able to alter the outer bacterial membrane **[9, 39]**. They combine with the charged groups of bacterial membranes thereby increasing their permeability and lead to electrolyte and cellular ATP leakage **[31, 39]**.

Table 2. *Zones of inhibition (IZ mm± SD), minimal inhibition concentration (MIC mg/ml), minimal bactericidal concentration (MBC mg/ml) for T. fontanesii essential oil and thymol isolate against human pathogenic bacteria compared to that of synthetic antibiotics*

Microorganismes	**Essential oil**			**Thymol isolate**			**IZ of Antibiotics**			
	IZ	*MIC*	*MBC*	IZ	MIC	MBC	CHL	AMP	SXT	GEN
Gram +										
Staphylococcus aureus (ATCC 6538)	58.53*** ± 0.83	0.035	0.070	30.16***±0,62	0.187	0.187	23.16±0,62	14.66 1.02	25.33±1.24	18.66±1.24
Staphylococcus aureus (ATCC 25923)	66.5*** ±1.87	0.035	0.035	32.33*** ±1.69	0.093	0.187	26,5±0,40	26.33 1.24	28.00±0.81	21.50±1.22
Bacillus subtilis (ATCC 9372)	43.36*** ± 0.99	0.070	0.070	31.66***±1.24	0.187	0.187	24.16 ± 0,62	20.33± 1,24	00.0±0.0	14.66 ±0.47
Bacillus cereus (ATCC 10876)	45.33***± 2.24	0.070	0.070	30,16*** ±0,62	0.375	0.375	22.33±0.84	10.0± 0.81	00.0±0.00	20.0± 0.81
Enterococcus faecalis (ATCC 6569)	33.19*** ± 1.18	0.140	0.140	26.53***±0.62	0.75	0.75	23.50±1,08	0.00± 0.0	00.0±0.00	10.50± 1.08
Listeria monocytogenes (ATCC 15313)	43.83***±1,01	0.070	0.070	24.16***±0.62	0.375	0.75	28.33±1,24	29.66± 1,24	33.5±1,08	24.66 ±1.24
Gram -										
Escherichia coli (ATCC 4157)	37.33*** ± 0.59	0.070	0.140	28.83*** ±0,47	0.375	0.375	18.16±0.84	7.50± 1.08	19.33±1,24	11.00 ±0.81
Escherichia coli (ATCC 25922)	55.93***±1.17	0.035	0.035	35.16*** ±0,62	0.093	0.187	24,16±1,02	24.75±1.64	20,66±1.69	21.66±1.69
Salmonella Enteritidis (ATCC13076)	38.16*** ±0,62	0.070	0.140	20.33**±0.47	0.75	0.75	33.33±1,24	25.5± 1.08	28.00±0,81	15.83 ±1.02
Salmonella Typhimurium (ATCC 3311)	60.16***±0.62	0.035	0.070	32.16***±0.62	0.187	0.187	18.53±1.04	19.33± 1.24	24.33±1.24	20,0 ±1,63
Klebsiella pneumoniae (ATCC700603)	43.83*** ± 1,31	0.070	0.070	26.16**±0.62	0.375	0.75	20.66±1.69	16.5±1,08	6.66± 0.23	7.5 ±0.40
Pseudomonas aeruginosa (ATCC 27853)	13.93**±0.89	0.281	0.565	10.50** ±0.40	1.5	1.5	6.56±0.30	7.83±1.02	0.0±0.00	12.16±0,62
Pseudomonas aeruginosa (ATCC 9027)	13.33** ± 0.8	0.565	0.565	12.33**±0.62	1.5	3.00	6.30±0.08	6.73± 0.2	0.0±0.00	10.50 ± 0.40

SD: Standard deviation; CHL: Chloramphenicol (30 µg/disc), AMP: Ampicilin (10µg/disc), SXT: Trimethoprim/Sulfamethoxazole (1.25/23.75 µg/disc) and GEN: Gentamicin (10 µg/disc) were used as positive reference standards antibiotic discs. Statistically significant differences compared to control are marked with different letters: extremely significant (***) at $p \leq 0.001$, highly significant (**) at $0.001 \leq p \leq 0.01$ and no significant (*) at $p > 0.01$.

III.4 Antioxidant Activity

The concentrations that led to 50% inhibition (IC50) are given in **Figure 5**. The results of the antioxidant activity study indicated a strong antioxidant potential of the samples tested. The essential oil of *T. fontanesii* has shown an excellent power to scavenge free radicals compared to thymol with IC50 values ($p < 0.05$) of 15.85 ± 0.23 µg / mL and 157.34 ± 1.44 µg / mL respectively. The comparison of these values with those given by reference substances such as ascorbic acid (IC50 = 2.75 ± 0.06 µg / mL) and the synthetic antioxidant BHT (IC50 = 35.90 ± 2.17 µg / mL) proves the excellent antioxidant potential of the essential oil of *T. fontanesii* which is more important than that of BHT.

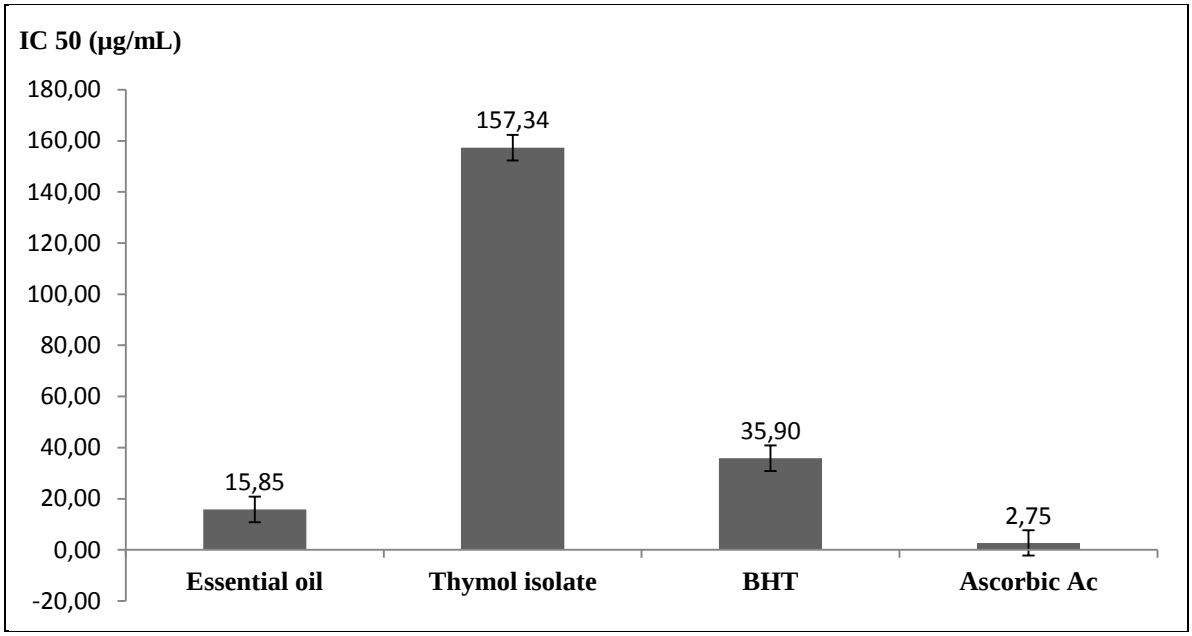


Figure 5. *IC50 values (µg/mL) of T.fontanesii EO, thymol isolate and the reference antioxidants (BHT and ascorbic acid), obtained in DPPH assay. Values are expressed as the means ±SD of three independent experiments.*

The antioxidant activity was in the order: ascorbic acid > EO > BHT > thymol. When compared with other data, the DPPH free radical scavenging activities of *T. fontanesii* EO was significantly higher than that of the EO of *Thymus capitatus* (IC50 = 119 µg /mL)**[40]** and of the EO of *Thymus broussonetii Boiss.* and *Thymus algeriensis Boiss.* from Morocco (IC50 = 90 and 1800 µg /mL respectively) **[16]**. However, the strong antioxidant activity of *T. fontanesii* essential oil may be related to this richness in phenolic monoterpenes (thymol and carvacrol) **[16, 41]**. In general, the oils with a high percentage of phenolic compounds, such as thymol and carvacrol, are potent antioxidants and their use could be beneficial in the antioxidative protection **[40]**. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups **[42]**. The fact that an investigated essential oil exhibited a higher antioxidant potential comparing to the isolated compound (thymol) may be an indication for a synergistic scavenging effect of different compounds present in thyme **[38]**. Therefore, the data obtained in this research are comparable to the results reported on the other members of Thymus family **[19, 40, 43]**.

The action mechanism set in motion by the antioxidant activity of these compounds is still not clearly understood. These compounds exhibit *in vitro* and bactericidal antioxidant activity, thereby inhibiting lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers. In addition, phenols directly scavenge reactive oxygen species (hydroxyl radicals, peroxynitrite and hypochlorous acid) **[44]**.

IV. Conclusion

The results of the present study show that the essential oil of *T. fontanesii* and thymol, its major compound extract, holds significant antibacterial and antioxidant activities. The biological activities of the oil reported here could be justified mainly by the synergistic effects and the diversity of major and minor constituents present in this oil. Therefore, *T. fontanesii* essential oil and this isolated constituent (thymol) are an excellent alternative to synthetic antibiotics as a means to reduce negative impacts to human health and the

environment. They are safe and ecofriendly and more compatible with the environmental components than synthetic antibiotics. They can also be used as natural preservatives in the food or pharmaceutical industries.

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