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Bio-chemical study of essential oils of *Thymus vulgaris* on pathogenic strains responsible for urinary tract infections

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ABSTRACT

The present work investigates the chemical composition; the essential oils antioxidant and antibacterial activities of Thymus vulgaris on the pathogenic bacteria strains responsible of the urinary tract infections. The essential oils obtained by the hydrodistilation of studied plants areal parts; were analyzed by GC/MS. The essential oil'antioxidant activity capacity was measured using: the DPPHand the FRAP methods, while the antibacterial activity was determined by the agar well diffusion method against twelve pathogenic strains, responsible of the urinary tract infections. For the DPPH and the FRAP tests, The Thymus vulgaris from the two Algerian regions presented a very interested activity. Moreover, it was found that the studied plants essential oils exhibited an important resistance against the most of the pathogenic bacteria tested. The relation study between the essential oils chemical composition, the antibacterial and the antioxidant activities, reveal the presence of different strong correlation with some major identified compounds. As a conclusion, the tested essential oils in this study showed an interesting strong antioxidant, and antibacterial activities. These effective activities are due to the presence of several chemical compounds.

***Keywords*:** Antibacterial activity, antioxidant activity, essentials oils, *Thymus vulgaris,* urinary tract infections.

INTRODUCTION

Urinary tract infections are a common reason for consultation and prescribing in general medicine. They are considered as the second site of bacterial infection after lung infections (Barrier Letertre, 2014). They are also, one of the most common infections in both outside and in hospital. Several studies showed that these infections affect about 40% to 50% of women (Dauquanand Abdullah, 2017; Giordani *et al.,* 2008). A portion of the world's population uses traditional medicine to heal. This widespread use is explained by, its accessibility and availability of this medicine in developing countries on the one side, and the harmful effects of synthetic drugs on the other side (Kanoun *et al.,*2020).The natural resources uses, particularly medicinal plants becomes very important and interesting for the new antibacterial products search; more effective in view of the resistant strains emergence to antibiotics (Lavigne, 2007; Prasanth *et al.,* 2014). Medicinal plants becomes important for pharmalogical research and drug development or production, not only when plant components are directly used as therapy agent, but also as materials for drug synthesis or as models for pharmacologically compounds (Burt, 2004).

Algeria, by its climatic diversity and its geographical situation, possesses a considerable set of natural species that represents a great importance phylogenetic patrimony, considering their spatial distribution and their role in ecological equilibrium (Ruberto and Barrata, 2000). The studies indicated that the essential oils of *Thymus vulgaris* have an antioxidant proprieties by protecting cells against the free radicals damaging impact (Hussain *et al.,* 2011). The aim of this study was to investigate the chemical composition of essential oils of *Thymus*

vulgaris and its antimicrobial activity collected from two regions (Chlef and Sidi-Bel-Abbes), against a pathogenic strains responsible of urinary tract infections. The antioxidant capacity tests such as FRAP and DPPH scavenging activity are also demonstrated. Finally, the correlation between the *Thymus vulgaris* essential oils chemical composition and its antibacterial and antioxidant activities was explored by a statistical analysis, which was carried out, in order to determine the natural chemical compounds responsible for the different essential oils activities.

MATERIALS AND METHODS

Plant material

This study was performed on the *Thymus vulgaris* leaves harvested from Medajaja region (Chlef) and Tessala region (Sidi-Bel-Abbes) (Algerian west), during the flowering stage (late May and early June 2019). Two samples from two different regions were collected in order to compare their essential oils composition differences, as well as their antioxidant and antibacterial activities. The plant samples were identified by the regional flora; as well as by floristic and taxonomic references. Once harvested, The *Thymus vulgaris* fresh leaves were air-dried for 20 days. After drying, the leaves are ground with an electric grinder until a fine powder was obtained, which was kept in bottles and stored, away from light and humidity until use.

Used bacteria

All clinical strains were obtained from the Chlef Hospital Microbiology Laboratory, which are involved in urinary tract infections. These microorganisms were identified using conventional, morphological and biochemical tests. The stored bacterial cultures were maintained in PBS (Phosphate Buffered Saline), with 20% glycerol at -70°C. The essential oils used in this research were extracted by hydrodistillation using a Clevenger apparatus. For this, a 50g sample of each plant powder was mixed with 500 ml of distilled water, and then placed in the hydrodistillation instrument for 3 hours. The extracted essential oils were then dried on anhydrous sodium sulfate and then stored in dark glass bottles at +4°C until use (Mansour *et al.,* 2021).

Essential oils analysis by GC–MS

The essential oils chemical composition was determined, by a gas chromatography coupled with Mass Spectrometry GC/MS type Perkin Elmer 500, equipped with a capillary column Elite Series 5 % Phenyl Dimethyl polysiloxane (30 m x 0, 25 mm), with a film thickness of 0.25 mm, and the a split injector was calibrated at 250°C. The injection mode is split (leak ratio: 1/50, flow rate: 66 ml / min). Samples are diluted in methanol (1/20 v/ v), 2 ml constituting a manual injection (Hussain *et al.,* 2011). The gas used is the helium with 1 ml/ min of flow rate/min. The column temperature was programmed from 60 to 275°C (Burits and Bucar, 2000), the fragments are carried out by electronic impact under 70 EV field, with abalayage of 80 to 600 Uma, a quadruple analyzer and a solvent's delay: 5.90 mn. The component identification was based on Kovats indices (KI) and the equipment was connected to a computer system that manages a library of NIST 98 mass spectra.

Antimicrobial activity determination

The antibacterial activity of essential oils was identified by the agar well diffusion, approach as reported by Rather *et al*. (2012). Firstly, the clinical strains were cultured in Brain Heart Infusion (BHI) broth at 37°C for 24h. After incubation, each bacterial suspensions was adjusted to *turbidity* standard of *0.5 McFarland units* in the same medium .0.1ml from the bacterial suspension was uniformly spread on the agar Mueller Hinton surface, the Petridishes were then dried. Moreover, 5 mm diameter wells were formed on the petridishes surfaces under aseptic conditions, 50 µl of each essential oils were applied in the well formed on the inoculated Petridishes; which were incubated at 37°C for 24h. After incubation, The inhibition zones diameters were measured in mm, including the well diameter (5mm) (Burits and Bucar, 2000; Oyaizu,1986). All tests carried out in triplicate, and the results are expressed as the zone diameter mean values.

The Minimum Inhibitory and Bactericidal Concentrations were determined using a dilution test. The Minimum Inhibitory Concentrations (MICs) are defined as the lowest concentration of an antibacterial agent, that inhibits the visible growth of a microorganism after an overnight incubation, for the Minimum Bactericidal Concentrations (MBCs), are defined as the lowest concentration of an antibacterial, that kill the

microorganism after sub-culturing on the antibacterial agent-free medium. These methods are based on those described by Ericsonand Sherris (1971). In the dilution test, the microorganisms are tested for their capacity to generate a visible growth on a series of agar plate (agar dilution) or in a broth (broth dilution) containing the antibacterial agent dilutions (in mg/l) which, under defined *in vitro* conditions, inhibits the appearance of the microorganism visible growth, within a defined time period, is known as the Minimum Inhibitory Concentration (MIC).

A volume of bacterial suspension equal to the diluted antibacterial solution volume is added to each antibacterial agent tube or well. The periodic viability counts should be performed on the inoculum suspensions to ensure that the inoculums contain approximately 5x10u CFU/ml (colony forming units per milliliter). This can be performed by removing 10µl from the growth control well of the tube immediately after inoculation and diluting in 10ml of broth. 100µl of this dilution is spread on the agar plate surface, which is then incubated overnight. Fifty colonies are expected from an original inoculum of 5x10u CFU/ml. It is recommended that a purity be performed on the inocula; by placing a sample on a non-selective agar plate and incubating it overnight. The results should be read when the test organism growth is sufficient. The *Minimum Bactericidal Concentration* (MBC) is determined by calculating the relative proportion of live and dead bacteria. The tests were performed in microtiter plates. The bacterial viability determination was performed after exposure to the antibacterial agent (essential oil) for 4 h. The *Minimum Bactericidal Concentrations*(MBCs) calculation was carried out by graphical extrapolation and by the mathematical approximation method (Ericson and Sherris, 1971).

Antioxidant capacity determination

The essential oil antioxidant capacity was established using two different methods: the ferric reducing antioxidant power (FRAP) method as described by (Oyaizu, 1986), and the 2,2-diphenyl-1-picrylhydrazyl DPPH method as reported by Burits and Bucar (2000), with some modifications.

For the FRAP test, six various concentrations of methanolic-aqueous solutions (0.66-16.66 mg/ml) and the essential oils were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of potassium ferricyanide solution $K_3Fe(CN)_6$, 1% (w/v). The mixtures were incubated for 20 minutes in a water bath at 50°C. The incubated mixtures were then cooled at room temperature. Once cooled; 2.5 ml of 10 % (w/v) of TCA solution (*Trichloroacetic acid*) was added. The absorbances were measured at 700 nm. The positive control was represented by a standard antioxidant solution; the ascorbic acid was measured under the same conditions as the sample. An increase in the absorbances corresponds to an increase in the essential oils reducing power tested (Oyaizu, 1986).

For the DPPH test; the analysis was realized with 300 µl of six different methanolic aqueous solutions concentrations (0.66-16.66 mg/ml) with the essential oils, then they were mixed with 2.7ml of radical DPPH ($6x10^{É5}$mol/l in methanol). A control solution was also prepared with 300 µl of Milli-Q water and 2.7ml of DPPH solution. The mixture was vigorously vortexed and left to stand for 60 minutes in the dark at room temperature. A colometric evaluation was then performed using a spectrophotometer at 517 nm. The DPPH free radical inhibition by ascorbic acid was also tested for comparison with the same concentration. The reaction kinetics and the parameters determination for the ascorbic acid antioxidant activity and the essential oils inhibition percentage determination (%), and IC_{50} were performed in triplicate (Imelouane *et al.,*2009).

Inhibition percentage determination

According to Sharififar *et al.* (2007), The free radical DPPH reduction as percentage (I %) was calculated as follows: I % =(A control - A sample) / A control) x 100.

A control: the control absorbance, and **A Sample:** the test absorbance. The essential oils and the ascorbic acid kinetics reactions with the DPPH are registered at each studied concentration. The essential oils and ascorbic acid rates, as a function of the inhibition percentage, were noted at the reaction end to obtain the index. The essential oils concentration providing 50% of inhibition was calculated. A lower IC_{50} value signified a higher antioxidant activity (Rather *et al*., 2012).

Statistical analysis

Data were submitted to the correlation analysis; it was realized using the SPSS statistical package program (SPSS 17.0 for windows, SPSS Inc. Chicago, IL., USA).

RESULTS AND DISCUSSION

According to AFNOR (2000), the essential oils (EOs) are generally liquid at room temperature and volatile, which differentiates them from the so-called fixed oils. They are more or less colored; their density is generally lower than that of water (Nostro *et al.,* 2000). The studied essential oils yields were moderate values. The essential oils chemical compositions are presented in Table 1. For the *T. vulgaris* E.O, thirty two different compounds were identified representing 98.26% for the Chlef sample and thirty one components for that of Sidi-Bel-Abbes region, which representing 94.92% with proportion of monoterpene hydrocarbons and oxygenated monoterpenes. The γ-Terpineme was the predominant compound in the Chlef region sample with the percentage of 27.09% followed by Thymol (21.07%), then p-Cymene (21.04%) and Camphor (13.05%).The Sid-Bel-Abbes sample region was also principally composed by γ-Terpineme 24.02%, but with a lower content, than the noted sample from Chlef region. The Thymol presented a high percentage (24.89%) compared to that of Chlef region. In addition, the two samples showed equal percentages of p-Cymene (21.04% and 21.09%). By referring to the literature, a number of important compounds were identified from this essential oils plant; a previous studies confirm the γ-Terpineme predominance (AFNOR, 2000), while others have identified the Thymol as dominant compound (Kulisic *et al.,*2004) . Based on previous works that concentrated on this plant from Algeria, it appeared that there are differences in the composition of the major compounds, which were attributed to a various factors, such as, the climate, the development stage and even the species genetic profiles (Silva *et al.,* 2015).

Antioxidant activity

Generally, the unique method is not suggested for the essential oils antioxidant activity determination due to their complex composition (Grigore *et al.,* 2010). Therefore, two different tests were used for the essential oils antioxidant activity determination (the FRAP and DPPH scavenging activity). These tests have various mechanisms. The DPPH method is based on the antioxidants ability operate as radical scavengers; while the FRAP method measures the antioxidants ability to act as reducing agents (Rustaiyan *et al.,* 2000).

DPPH test

The DPPH test measured the sample ability to provide protons; the DPPH radical scavenging activities tests of both samples on the EOs are reported in Table 2, these results are defined as the substrate concentrations that causes 50% loss of the initially introduced DPPH free radical concentration. In comparison to the reference antioxidants (Ascorbic acid and BHA), the EO samples demonstrated a low DPPH radical scavenging activities. These results suggest that; the *T.vulgaris* EO antioxidant activity from Chlef region are twice time more active than that of the Sidi-Bel-Abbes region, the antioxidant activity was higher with lower DPPH value, which could be explained by the different active chemicals in each case. It can be concluded that the EOs samples possessed the powerful antioxidant substances, which that may be responsible for its anti-inflammatory and chemo-protective mechanism, as well as justify the basis of using this plant as popular medicine (Mansour *et al.,* 2021).

FRAP test

As observed, both essential oils exhibited antioxidant capacity. In comparison with the ascorbic acid (Table 2), the EOs exhibited a moderate antioxidant activity. The highest antioxidant capacity was reported for the *T. vulgaris* from Chlef region, whereas the EO with the lowest activity was assigned to *T. vulgaris* the from Sidi-Bel-Abbes region. A high FRAP value indicates a higher antioxidant capacity. It's considered that, the *T. vulgaris* antioxidant capacity is associated to its Thymol and Camphor content, two phenolic compounds with a recognized antioxidant capacity (Marino *et al.,*1999). In a previous study, FRAP values of the EO obtained from six different *T. vulgaris* were found to be in the range 16.23 and 27.84 mmol/l. The results of the present study are

Table 1: ***Thymus vulgaris*** **essential oils samples chemical composition from Chlef and Sidi-Bel-Abbes region**

Compound	*Thymus vulgaris* (Chlef)		*Thymus vulgaris* (Sidi -Bel -Abbes)	
	IK (m/m)	%	IK (m/m)	%
á-Thujen	923	1.01	925	1.03
á-Pineme	929	0.71	926	0.86
Camphene	942	0.04	944	0.08
Sabineme	967	0.03	963	0.01
â-Pineme	980	0.21	978	0.19
Myrcene	987	0.60	982	0.65
á-Phellandere	999	0.17	992	0.14
ä-Carene	1011	0.08	1009	0.07
P-Cymene	**1022**	**21.04**	**1020**	**21.09**
Limoneme	1021	0.015	1019	0.013
Eucalyptol	1038	0.11	1032	0.19
ã-Terpineme	**1057**	**27.09**	**1059**	**24.02**
Terpinoleme	1089	0.17	1084	0.14
Linalool	1097	1.08	1091	1.01
â-Thujone	1117	0.002	1114	0.001
Allo-ocimene	1113	0.08	1117	0.09
Camphor	**1147**	**13.05**	**1143**	**10.07**
Neo-isopilegel	1167	0.013	1161	0.012
Pinocarvone	1160	0.52	1163	0.40
Isopulegol	1168	0.45	1166	0.51
Borneol	1163	1.22	1169	1.02
Terpinene-4-ol	1171	0.58	1172	0.45
á-Terpineol	1174	0.031	1181	0.015
Naphtalene	1177	0.71	1187	0.80
Thymol methyl-l-Ether	**1234**	**7.84**	**1230**	**6.72**
Nerol	1235	0.31	1233	0.39
Pulegone	1239	0.014	1236	0.011
Carvone	237	0.01	1240	0.02
Linaly Acetate	1247	0.003	1249	0.021
Thymol	**1291**	**21.07**	**1293**	**24.89**
Carvenol	1297	0.005	1297	0.01
Total identified		**98.26**		**94.92**
Essential oil yield		**1.69**		**1.56**

IK: Kovats retention index.

Table 2: ***Thymus vulgaris*****essential oils samples antioxidant activity from Chlef and Sidi-Bel-Abbes regions using the DPPH ,the FRAP methods and** IC_{50}

E.O (Chlef)	IC_{50} (mg/mL)	DPPH%	FRAP(mmol/L)
	10.01±0.7	33	21.21±0.04
E.O (Sidi-Bel-Abbes)	5.3±0.01	67	15.02±0.09
Ascorbic acid	0.080±0.003	83	61.07±0.23
BHA	0.061±0.002	75	46.01±0.63

E.O: Essential Oil BHT: Butyl Hydroxy Anisole.

Table 3: *T. vulgaris*essential oil samples antibacterial activity from Chlef and Sidi-Bel-Abbes regions by the well method

Strains	*T.vulgaris* (Chlef)	*T.vulgaris* (Sidi-Bel-Abbes)
	Inhibition zone diameters (mm)	Inhibition zone diameters (mm)
Enterobacter cloacae	35±0.01	28±0.05
Proteus mirabilis	24±0.001	22±001
Citrobactercoseri	20±0.03	17±0.01
Acinetobacterbaumanii	28±0.07	18±0.05
Seratiaodorifera	23±0.05	21±0.09
Enterobactergergoviae	17±0.02	15±0.06
Staphylococcus .ssp	22±0.08	21±0.02
E.coli	25±0.06	22±0.05
Klebsiellassp	37±0.01	32±0.06
Enterococcus faecium	22±0.05	25±0.09
Pseudomonas.ssp	9±0.002	8.2±0.08
Citrobacterfreundii	30±0.01	27±0.01

Table 4: MIC and MBC for the *T.vulgaris*essential oils samples from Chlef and Sidi-Bel-Abbes regions

Strains	*T.vulgaris* (Chlef)		*T.vulgaris* (Sidi-Bel-Abbes)	
	MICs (mg/mL)	MBCs (mg/mL)	MICs (mg/mL)	MBCs (mg/mL)
Enterobacter cloacae	0.109	1.022	0.111	1.041
Proteus mirabilis	0.101	0.429	0.122	0.508
Citrobacter coseri	0.538	1.057	0.432	1.061
Acinetobacterbaumanii	0.508	1.077	0.490	1.051
Seratiaodorifera	0.112	1.070	0.101	1.063
Enterobactergergoviae	0.125	1.015	0.131	1.032
Staphylococcus.ssp	1.019	1061	1.002	1.021
E.coli	1.001	1.063	0.985	1.052
Klebsiella .ssp	0.099	1.001	0.078	1.095

MICs: Minimum Inhibitory Concentrations; **MBCs**; Minimum Bactericidal Concentrations

similar to those in the above literature with some differences. These differences may be related to the regional conditions, the active substance quantity, the extraction methods and the solvent type (Pina-Vaz *et al.,* 2004).

Antibacterial activity

The antimicrobial activity essential oils results against 12 microorganisms responsible for urinary tract infections are reported in Table 3, both EOs exhibited an inhibitory effect against all the microorganism with an inhibition zone ranging from 13 to 37 mm (Values are the mean of 3 replicates).The EOs exhibited a high activity (when the inhibition zone diameter was ≥ 20mm) and a moderate activity (when the inhibition zone diameter was <12- 20-mm). Among the EOs investigated, the highest antibacterial activity against *Klebsiella* ssp. was established with *T. vulgaris* from the Chlef region with an inhibition zone diameter of 37mm, followed by *Enterobacter cloacae* with an inhibition zone diameter of 35mm with also *T. vulgaris* of Chlef region; whereas the lowest activity was assigned to *Pseudomonas ssp.* with an inhibition zone diameter of 8.2 mm with *T. vulgaris* of Sidi-Bel-Abbes region. This *Pseudomonas ssp* remarkable resistance was reported by Cosentino *et al.* (1999) and by Hussain

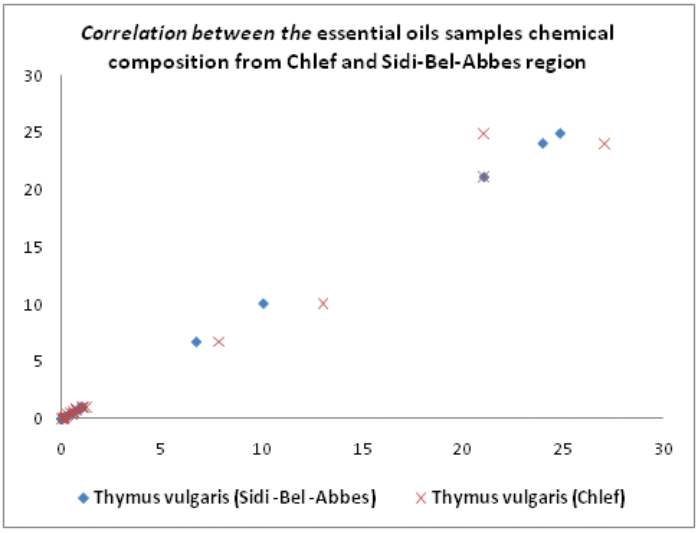

Fig. 1*:* ***Thymus vulgaris*** **essential oils samples chemical composition from Chlef and Sidi-Bel-Abbes region**

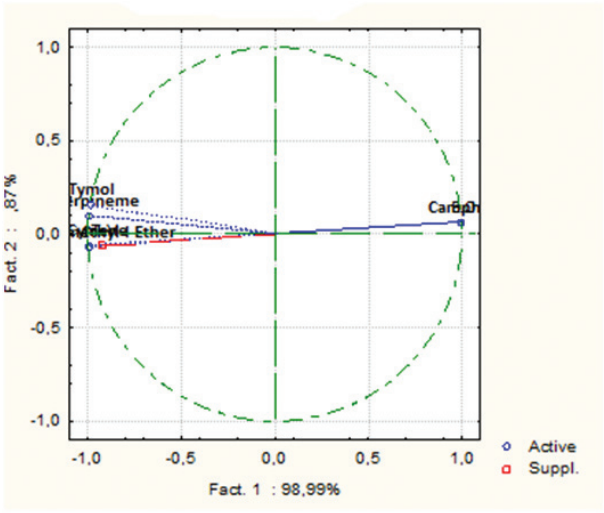

Fig. 2: Two dimensional plot on the F1 and F2 axes of the essential oils samples and their antioxidant activity using principal component analysis.

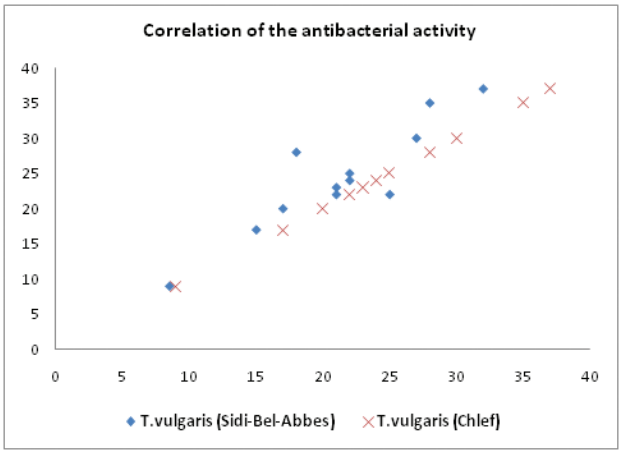

Fig. 3: The correlation between *T.vulgaris* essential oil samples and the antibacterial activity from Chlef and Sidi-Bel- Abbes regions

et al. (2011) for three species of *Thymus* genus, and six plants from the *Lamiaceae* family. These results revealed a significant variation in the antimicrobial properties of EO samples. In general, the EOs contained a phenolic compounds such as Thymol at high level and camphor, which exhibited a strong antimicrobial activity effect against pathogenic microorganisms (Simandi *et al.,*2001).These compounds can inhibit the essential enzymes, interfere with the cell membrane activity or disturb the genetic material's function and perturb the energy production and structural compounds synthesis (Sokoviæ *et al.,*2008).

Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations (MBCs); tests determination

The MIC values obtained ranged from 0.099 to more than 2.004 mg/mL for *Pseudomonas ssp*. The same resistance was observed for *Pseudomonas ssp* towards to both EOs samples (Table 4).The Chlef *T. vulgaris* exhibited a MICs values of 0.099 mg/ml for: *Klebsiella* ssp*, Enterococcus faecium, Enterobacter gergoviae, Seratia odorifera, Acinetobacter baumanii, Citrobacter coseri, Proteus mirabilis, Enterobacter cloacae* and *Citrobacter freundii* and 1.019 mg/ml for *Staphylococcus* ssp*,* and *E.coli,* thus samples of Sidi-Bel-Abbes region, were from 0.101 mg/ml for *Seratia odorifera, Enterobacter cloacae, Proteus mirabilis, Citrobacter coseri, Acinetobacter baumanii, Seratia odorifera, Enterobacter gergoviae, Klebsiella* ssp*, Enterococcus faecium, Citrobacter freundii* and *E. coli* and 1.002 mg/ml for *Staphylococcus* ssp. The *Pseudomonas* resistance found at a concentration of 2.004 mg/ml. Imelouane *et al*. (2009) found that for this same species, the EOs MIC had a value equal to 1.33 mg/ml for *E. coli* and *Staphylococcus ssp*, Kaloustian and Hadji-Minaglou, (2012) found that the MIC_S for *E.coli* and *Staphylococcus ssp* are equal to 1 and 2 mg/ml respectively. For the MBCs for *Proteus mirabilis* was the most sensitive with a MBC equal to 0.429 mg/ml for the Chlef region samples, and 0.508 mg/ml for the Sidi-Bel-Abbes region samples. Numerous previous research confirmed the *Thymus* genus EOs antimicrobial proprieties (Sokoviæ *et al.,*2008).The comparison of the *T.vulgaris* essential oils under different geographic conditions revealed that there are some qualitative and quantitative differences between both Algerian localities (Chlef and Sidi-Bel-Abbes), which may have been influenced by genetics differences and different environmental factors. Others results on other *Lamiaceae* plants have indicated that, their chemical composition variation is assigned to the geographical conditions (Baba-Aissa, 2011).

Determination of the *correlation coefficient*

A correlation coefficient is a number between -1 and 1, that tells us the strength and direction of a relationship between variables: $-1 \leq r \leq +1$ .There is a strong positive correlation between the two species in terms of chemical composition of their essential oils, r=0.99 (Fig.1).

In the aim of searching the correlation between the essential oils chemical compositions of the studied plants and their antioxidant capacity, along with the antimicrobial activity, it was performed to use a statistical methods based on the Principal Components Analysis; PCA, which was used graphically. It described the relationship between the different parameters studied. Both axes represent 90.98% of the total variation. The first axis (98.99%) expressed the largest variation percentage (more than 97%) (Fig. 2).The objective of these both figures is to search the correlation between the essential oils chemical composition and their antioxidant properties, the correlation between the *Thymus vulgaris* essential oils chemical composition and the antioxidant activity, as expressed by the IC_{50} and the antibacterial activity with inhibition zones of the different strains.

The Figure 2 presents the factors projection (F1xF2) of the *T.vulgaris* chemical composition data and the antioxidant activity, which is expressed by the IC_{50} values, which are determined as the substrate concentration that produces the loss of 50% of the initially introduced DPPH free radicals. The antioxidant activity results revealed a great variation in the IC_{50} values and it's related to the plant nature studied. Based on PCA, it was revealed a positive correlation between the EOs chemical composition and its pre-identified main compounds: Cymene, γ-Terpineme, Thymol and Thymol methyl-l-Ether with a significant correlation coefficients higher than 97%.The

results also demonstrate a negative correlation between the EOs compounds (P-Cymene, γ-Terpineme, Thymol and Thymol methyl-l-Ether) and the IC_{50}, with a significant correlation coefficients (-0.97 to-0.99). When the IC_{50} is low, the antioxidant activity is higher and *vice versa*, when the IC_{50} is higher, the antioxidant capacity is lower; we observed that there was a negative correlation between the EOs compounds and the IC_{50}. This provided us the possibility to determine: first, a direct relationship between the antioxidant activity and the EOs compounds; second, each of the antioxidant activity and the EOs compounds have an inverse relationship with the IC_{50}. It considered that the *T. vulgaris* EOs antioxidant capacity associated with its thymol and phenolic compounds content with a recognized antioxidant activity (Sokoviæ *et al.,*2008). Many researchers have also reported that EOs extracted from thyme provide an effective antioxidants (Baba-Aissa , 2011). The results of the present study are also similar to those in the many references .

The Figure 3; illustrates the correlation between *T.vulgaris* essential oil samples and the antibacterial activity from Chlef and Sidi-Bel-Abbes regions**.** From this figure, the EOs sample exhibited a significant resistance against the majority of the tested bacteria; this activity is attributed to the camphor presence. There is a positive correlation between this compounds and the antibacterial activity with a correlation coefficient ranging from 0.70 to 0.99.From these previous results, we concluded that this antibacterial activity is principally based on the Camphor strong contribution. It could be deduced that the Eos *T.vulgaris* were partially effective as antioxidant activity, based on the presence of synergistic relationships that occurred between the EOs constituents such as Cymene-Terpineme, Thymol and Thymol methyl-l-Ether, and partially as antibacterial activity as a result of the Camphor presence. A precedent research (Nikoliæ *et al.,*2014) on the EOs antimicrobial activity of some *Thymus* species revealed that, most of the species which possessed a large quantities of terpenoids compounds, indicated the activities against viruses, bacteria and fungi (Adams, 2007). However, no significant investigations on the relationship between the bacterial inhibition and the total Camphor contents.

CONCLUSION

In the present study, we presented the powerful *T.vulgaris* EOs antibacterial activity from the both Algerian regions against twelve pathogenic *microorganisms*, involved in urinary tract infections, which explains the important use of this plant in the traditional medicine. It is appeared that, modern medicine should give more consideration to the synergistic effect of plant secondary metabolites; as they can help to resolve several problems, especially the microbial resistance to synthetic antibiotics. The tested EOs exhibited also an interesting antioxidant capacity, the relationship study between the EOs chemical composition and the antioxidant or the antibacterial activity indicated the presence of different strong correlation with some identified major compounds in each case of study.

Abbreviations: FRAP: Ferric Reducing Antioxidant Power; DPPH: 2,2-diphenyl-1-picrylhydrazyl ; GC/MS: Gas Chromatography coupled with Mass Spectrometry.

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