Volume 23(4). Published novembre, 01, 2015
www.jnsciences.org
ISSN 2286-5314

Potential use of wild *Thymus algeriensis* and *Thymus capitatus* as source of antioxidant and antimicrobial agents

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Abstract - Thyme species are aromatic plants widely used in Tunisia as spices and for its antispasmodic, antimicrobial, expectorant and antioxidant activities. This work aimed to assess the richness of *Thymus capitatus* and *T. algeriensis* leaves on phenolics and essential oils (EOs) and to evaluate the antioxidant and antibacterial potential of these compounds. According to the chemical EO composition, *T. algeriensis* was 1,8-cineole/α-pinene/camphor chemotype. While, *T. capitatus* EO was noteworthy dominated by carvacrol (76.5%). As compared to EOs, antioxidant capacity of polar fractions were higher with a strong antiradical capacity ranged between IC50 = 6 and 7 µg/ ml. These high capacities positively correlated with high phenolic contents. However, EOs showed a best and broader antimicrobial spectrum activity than polar fractions. These results confirmed the possibility of using thyme essential oils and phenolic components as a natural preservative ingredient in food and/or pharmaceutical industries.

Key words: antiradical activity; antibacterial activity; essential oils; phenolic; polar fraction; *Thymus*.

1. Introduction

Among the various medicinal and culinary herbs, some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing molecules with significant antioxidant capacities and health benefits (Ksouri et al. 2011). Many herb spices rich in phenolic compounds, especially those belonging to the Lamiaceae family, such as sage, oregano and thyme are increasingly of interest in the food industry because of their strong antioxidant capacity (Hirasa and Takemasa 1998) retarding oxidative degradation of lipids and thereby ameliorate the quality and nutritional value of food.

The genus *Thymus* L. is a member of the Lamiaceae family and contains about 215 species particularly prevalent in the Mediterranean area (Bounatirou et al. 2007). They are commonly used as spices and as remedies in traditional medicine. They are also reported to possess some biological effects such as antispasmodic, antibacterial, antiviral, expectorant and antioxidant activities (Ismaili et al. 2004). In Tunisia, *Thymus* genus is represented by two species *Thymus algeriensis* Boiss. et Reut. and *Thymus capitatus* Hoff. et Link.

Tunisian *T. algeriensis* populations grow wildly on poor fertile calcareous soils and in different bioclimatic zones extending from the sub-humid to the lower arid (Ben El Hadj Ali et al., 2012). This species is widely used in local medicine against illnesses of the digestive tube and antiabortion (Ben El Hadj Ali et al. 2012). *T. algeriensis* has high content of oxygenated monoterpenes (79.5%) and also possess major compounds, such as linalool (47.3%), thymol (29.2%) and p-cymene (6.8%) that were the most abundant reported compounds (Dob et al. 2006). In other reports, it was found the *T. algeriensis* essential oil possess an interesting inhibitory activity against angiotensin I-converting enzyme suggesting the potential of this plant as an antihypertensive agent (Zouari et al. 2011).

T. capitatus, locally known under the common name ‘‘zaâtar” is endemic to Algeria and Tunisia and also the most widespread North African species. In Tunisia, *T. capitatus* is widely used in folk

medicine as stomachic, diaphoretic, antispasmodic specifically for whooping cough, stimulant for the blood circulation, and aphrodisiac (Bounatirou et al. 2007; Hazzit et al. 2009).

Thyme oil is among the world's top 10 essential oils used as a preservative for food (Ehivet et al. 2011). The demand for essential oils from these species is increasing for perfumery, cosmetic and medicinal applications (Hazzit et al. 2009). Chemical classification of *Thymus* species was based on the main essential oil components and their chemical polymorphism. Numerous chemotypes have been defined, such as carvacrol and thymol, geraniol, γ-terpineol, thujone and linalool (Thompson et al. 2003).

Regarding *Thymus* EOs, variations in the chemical composition and biological activity of plants growing in different countries have been reported (Cosentino et al. 1999; Rota et al. 2008). The aim of this study was to determined the richness of *Thymus capitatus* and *T. algeriensis* leaves on phenolics and essential oils (EOs) and to evaluate the antioxidant and antibacterial potential of these metabolites.

2. Matériels et méthodes

2.1 Plant sampling

The samples from wild growing *T. algeriensis* and *T. capitatus* plants were collected during the vegetative stage in January 2013, respectively from Ras-Jdir (latitude: 33°8'N, longitude: 11°33'E; mean annual rainfall < 200mm) and Zaghouan (latitude 36°16'N, longitude 9°59'E; sub humid bioclimatic stage).

2.2. Isolation of the essential oil

Leaves collected were air dried in the shadow at room temperature then slightly ground before extraction. Essential oils were extracted using the traditional water distillation method. Triplicate samples of 200 g were subjected to hydrodistillation in 1 L of deionized water using a Clevenger apparatus at 90°C for up to 5 h, time was necessary for a complete extraction. The obtained EOs were dried over Na2SO4 and stored in sealed dark vials, at 4°C (Adams, 2001).

2.3. Gas chromatography/mass spectrometry (GC–MS) analysis

The identification of the EOs was performed using a Hewlett Packard HP5890 series II GC–MS equipped with a HP5MS column (30 m x 0.25 mm). The carrier gas was helium at 1.2 ml min-1. Each sample (1 µl) was injected in the split mode (1:20), the program used was isothermal at 70 °C, followed by 50–240 °C at a rate of 5 °C min-1, then held at 240 °C for 10 min. The mass spectrometer was an HP 5972 and the total electronic impact mode at 70 eV was used. The components were identified by comparing their relative retention times and mass spectra with the data from the library of EOs constituents, Wiley, Mass-Finder and Adams GC-MS libraries (Adams, 2001).

2.4. Extraction of phenolic compounds

Dried and powered leaves (2.5 g) were extracted with 25 ml of solvents mixture (chloroform/methanol) (12/5). Extraction was repeated two times. Both polar (aqueous) and non-polar (chloroform) phases were separated by addition 3.5 ml of water and after decantation. The non-polar compounds were removed from the plant material during extraction into the chloroform extract, which contained most essential oils components, besides non-volatile components. Methanolic / water phase contained non-volatile compounds (flavonoids and phenolic compounds) was used for our experimentation. This later fraction was evaporated under reduced pressure. Dry residue was weighed and removed in pure methanol. Aqueous phase was then stored at 4°C until analysis.

2.5. Total phenolic contents

Total phenolic compounds were assayed by the Folin-Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. (2002). An aliquot (0.125 ml) of suitable diluted phase was added to 0.5 ml of distilled water and 0.125 ml of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 ml of 7% Na2CO3 solution. The solution was then diluted with deionised water to a final volume of 3 ml and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 760 nm. Total phenolic content of leaves (three replicates) was expressed as mg gallic acid equivalents (GAE)/g DW through the calibration curve with gallic acid.

2.6. Total flavonoid contents

Total flavonoids were measured according to Dewanto et al. (2002). An aliquot (0.25 ml) of diluted sample was added to 0.075 ml of NaNO2 solution (5%), mixed and left for 6 min, before adding 0.15 ml of a freshly prepared AlCl3 solution (10%). After 5 min, 0.5 ml of 1 M NaOH solution was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoids were expressed as mg (+)-catechin/g DW (mg CE/g DW), through the calibration curve of (+)-catechin.

2.7. Condensed tannin contents

Proanthocyanidins were measured using the modified vanillin assay described by Sun et al. (1998). To 50 µl of suitably diluted sample, 3 ml of methanol vanillin solution (4%) and 1.5 ml of HCl was added. The mixture was maintained at ambient temperature for 15 min. The amount of total condensed tannins was expressed as mg (+)-catechin/g DW (mg CE/g DW). The calibration curve range was 0-400 µg/ ml. All samples were measured in three replicates at 500 nm against methanol as a blank.

2.8. Antioxidant activities

2.8.1. Evaluation of total antioxidant capacity

The assay was based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH (Prieto et al. 1999). An aliquot (0.1 ml) of leaves fraction was combined to 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature; the absorbance of each solution was measured at 695 nm (Anthelie Advanced 2, SECOMAN) against a blank. The antioxidant capacity was expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW).

2.8.2. DPPH radical–scavenging activity

DPPH quenching ability of leaves extracts and EOs was measured according to Hanato et al. (1988). One milliliter of the extract at known concentrations (2.5, 5, 7.7 and 10 µg/ ml for polar fractions; 1.25, 2.5, 5 and 10 mg/ ml for *T. algeriensis* EO and 0.1, 0.5, 1 and 2 mg/ ml for *T. capitatus* EO) was added to 0.25 ml of a DPPH. methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance was measured at 517 nm and corresponded to the ability of extract to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The antiradical activity was expressed as IC50 (l g/ ml), the extract dose required to induce a 50 % inhibition. A lower IC50 value corresponds to a higher antioxidant activity of plant extract. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH. scavenging effect = [(A0 - A1)/A0] x 100 Eq (1)

Where A0 is the absorbance of the control at 30 min, and A1 is the absorbance of the sample at 30 min. Each phase was analyzed in triplicate.

2.8.3 Iron reducing power

The method of Oyaizu (1986) was used to assess the reducing power of *Thymus* leaves. Methanolic phases (1 ml) at different concentrations (20 – 500 µg/ ml) were mixed with 2.5 ml of a 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 ml of 1% potassium ferricyanide K3Fe(CN)6, then incubated in a water bath at 50°C for 20 min. After that, the mixture was centrifuged at 650xg for 10 min and 2.5 ml of 10% trichloroacetic acid were added. The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution. The intensity of the blue–green appearing colour was measured at 700 nm. Ascorbic acid was used as a positive control. Results are expressed as Effective Concentration at which the absorbance was 0.5 (EC50 in mg/ ml) obtained from linear regression analysis.

2.9. Screening for antimicrobial activity

The antibacterial activity was assessed by using agar disk diffusion against three human pathogenic Gram-positive bacteria including Staphylococcus aureus (ATCC) 25923, Micrococcus luteus (NCIMB) 8166 and Enterococcus faeccalis ATCC 19436 and five Gram-negative bacteria including

Escherichia coli DH5α, Salmonella typhi ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Klebsiella sp. From the collection of the Institut Pasteur (CIP), Tunis and Shigella flexneri ATCC 12022.

All bacteria were grown overnight on Mueller–Hinton broth at 37 °C prior to inoculation onto the nutriment agar. Suspension of the tested microorganisms (100 µl) containing 5 x 105 CFU ml-1 was spread with a sterile cotton swab into Petri plates containing 10 ml of API suspension medium. The filter paper discs with 6 mm of diameter were individually impregnated with 10 µl of each sample of polar extracts (30µg/ disc) and EOs (10 µl/ disc) and then placed onto the agar plates seeded with bacteria. The treated Petri dishes were kept at 4 °C for 1 h, and incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. Standard discs of gentamycin (10 µg/ ml) or chloramphenicol (30 µg/ ml) served as positive antibiotic controls. Discs with 10 µl of pure methanol were used as negative controls. For the antifungal activity of the same *Thymus* extracts, the agar-disc diffusion method was used as previously described by Cox et al. (2000). Four Candida strain (C. albicans ATCC 10281, C. glabrata ATCC 90030, C. tropicalis ATCC 13803 and C. krusei ATCC 6258) was first grown on Sabouraud chloramphenicol agar plate at 30 °C for 18–24 h. The colony was transferred into Api suspension medium and adjusted to two McFarland turbidity standard. The inocula of the yeast was streaked onto Sabouraud chloramphenicol agar plates at 30 °C using a sterile swab and then dried. A sterile filter disc, diameter 6 mm was placed in the plate. Ten microlitres of each polar extracts (30 µg/ disc) and EOs (10 µl/ disc) were impregnated on each paper disc. The treated Petri dishes were placed at 4 °C for 1–2 h and then incubated at 37 °C for 18–24 h. The antifungal activity was assessed by measuring the zone of growth inhibition surrounding the disc. The susceptibility of the standard was determined using a disc paper containing Amphotericin B (10 µg/ ml). The antimicrobial potentials were estimated according to indices reported by Rodriguez Vaquero et al. (2007). All tests were performed in triplicate.

2.10. Statistical analysis

Means were statistically compared using the STATI-CF program with Student's t test at the $p < 0.05$ significance level. A one-way analysis of variance (ANOVA) and Newman-Keuls multiple range test were carried out to test any significant differences between species used at $p < 0.05$.

3. Results and discussion

3.1. Chemical composition of *T. capitatus* and *T. algeriensis* leaves EOs

Results obtained by the GC–MS chemical analysis of *T. capitatus* and *T. algeriensis* leaves EOs are listed in Table 1. In total, 31 compounds were identified in *T. algeriensis* and 15 in *T. capitatus*, accounting 95.3 and 97.4 % of their total oils. *T. algeriensis* EOs were characterized by domination of monoterpene hydrocarbons (38.3 %), which 1,8 cineol (13.9%) and α-pinene (13.6%) were the main components. The monoterpene ketone formed 16.8% of the oil, represented by camphor (16.7%) as major compound. Monoterpene alcohols and sesquiterpene alcohols were also present in appreciable amounts. Both major predominated compounds from these respective population of compounds were borneol (5.9%) and elemol (8.2%).

Various chemotypes according to the geographical origins of samples have been previously reported in this species. Ben El Hadj Ali et al. (2012) distinguished five chemotypes according their main compounds from eight Tunisian natural populations of *T. algeriensis*. They include caryophyllene oxide/1,8-cineole/α-pinene, 1,8-cineole/α-pinene, 1,8-cineole/α-pinene/camphor, linalool and thymol chemotypes. According to the chemical EO composition, *T. algeriensis* was 1,8-cineole/α-pinene/camphor chemotype.

The chemical composition of EOs of *T. capitatus* was dominated by one monoterpene phenol named carvacrol (76.5%), which is in concordance with the carvacrol chemotype growing in Tunisia previously reported by Hedhili et al. (2002) and Bounatirou et al. (2007). In addition, this oil was characterized by the presence of p-cymene a precusor of carvacrol, γ-terpinene and β-caryophyllene. These three compound have been previously found as the most constituent compound in *Thymus* carvacrol chemotype (Hedhili et al. 2002). However, reports on different region of north Africa have shown that major compound *Thymus* oil species were thymol in Algerian (Kabouche et al. 2005) and Moroccan (Richard et al. 1985) samples.

Results of the scavenging activity of *T. capitatus* and *T. algeriensis* EOs against the radical DPPH are represented in figure 1. Comparison of IC50 values depicted significant variability in the antioxidant activity between the investigated EOs. *T. capitatus* EO was more efficient with an IC_{50} value of 340 µg/ ml, than *T. algeriensis* EO (IC_{50}= 4400 µg/ ml).

Tableau 1 : Compounds identified in leaves essential oils of Tunisian *Thymus capitatus* and *Thymus algeriensis* and their relative percentages at vegetative stage.

Components	*T. algeriensis*	*T. capitatus*
Monoterpene hydrocarbons		
α-thujene	-	0.5
α-pinene	13.6	-
Camphene	3.2	0.1
Sabinene	0.8	0.2
β-pinene	2.9	-
β-myrcene	0.2	0.7
1.8-cineol	13.9	-
α-terpinene	0.1	1.0
α-ocimene	1.1	-
γ-terpinene	0.4	5.7
Pinocarvone	0.6	-
Verbenone	0.9	-
p-cymene	0.6	5.3
Monoterpene phenols		
Carvacrol	-	76.5
Monoterpene alcools		
Thujanol	0.5	0.4
Linalool	2.5	1.2
Terpene-4-ol	1.3	0.3
α-terpineol	1.6	-
Trans-pinocarveol	1.5	-
p-cymen-8-ol	0.1	-
Cis-carveol	0.3	-
Borneol	5.9	0.5
Monoterpene ketone		
Camphor	16.7	-
Carvone	0.1	-
Monoterpene ester		
Carvacryl acetate	-	1.3
Borneol acetate	2.1	-
Monoterpene aldehyde		
Myrtenal	1.2	-
Sesquiterpenes		
β-caryophyllene	2.1	2.8
β-panainsene	3.3	-
Oxygenated sesquiterpenes		
Caryophyllene oxyde	6.8	0.5
Sesquiterpene alcool		
Elemol	8.2	-
β-eudesmol	0.6	-
γ-eudesmol	1.3	-
α-eudesmol	0.6	-
% of identification	95.3	97.4
Oil yield (%)	1.27	0.87

The potent antioxidant capacity of EOs seems to be related to the activity of some kinds of compounds (Skotti et al. 2014) especially oxygenated monoterpenes among them alcohols and phenols (Bourgou et al. 2008). In addition, Ruberto and Barrata (2000) found that among 100 pure components of essential oils, phenols were confirmed to possess the highest antioxidant activity. Our findings in radical scavenging activity is in accordance with these reports since the percentage of carvacrol were remarkably high (76.5%) in leaves of *T. capitatus*.

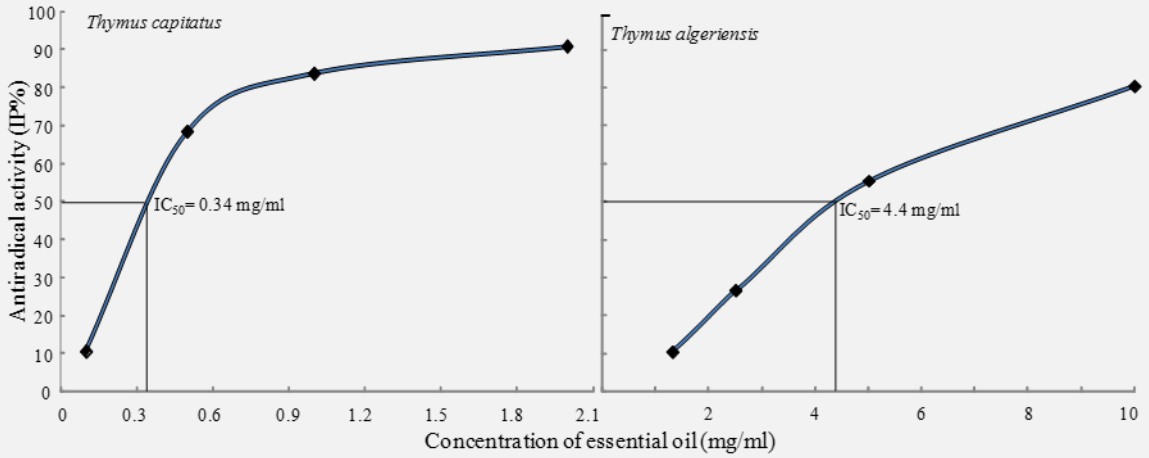

Figure 1. DPPH radical-scavenging of essential oils from *T. capitatus* and *T. algeriensis* leaves.

3.2. Phenolic compounds contents and antioxidant activities of *T. capitatus* and *T. algeriensis* leaves polar fraction

3.2.1. Phenolic compounds contents

Total phenolic (TPCs), flavonoides (TFCs) and condensed tanin (CTCs) contents of both *Thymus* leaves were estimated in aqueous fractions (polar) (Table 2). These extracts contained only non-volatile compounds like flavonoids and phenolics. Non-polar compounds such as essential oils and non volatile components were removed in chloroform. TPCs were determined as gallic acid equivalents in milligrams per gram of dry weight (mg GAE/g DW) while TFCs and CTCs were calculated as catechin equivalents in milligrams per gram of dry weight (mg CE/g DW). The analysis of results showed that both *Thymus* species contained high TPCs ranging from 240.31 and 248.88 mg GAE/g DW. Additionally, total flavonoid contents showed no signicant difference between species and similar trend to that of total polyphenols. Conversely, *T. capitatus* leave extracts contained more condensed tannins (13.95 mg CE /g DW) than *T. algeriensis* extract (6.71 mg CE /g DW).

Tableau 2 :: Amount of total phenolic (TPCs), total flavonoid (TFCs) and condensed tannin (CTCs) contents in polar phase from leaf of *Thymus capitatus* and *T. algeriensis*.

	TPCs (mg GAE /g DW)	TFCs (mg CE /g DW)	CTCs (mg CE /g DW)
T. capitatus	240.318± 6.23a ns	14.948 ± 7.26a ns	13.956 ± 0.90b ***
T. algeriensis	248.889± 7.18a ns	15.362 ± 2.28a ns	6.712 ± 0.68a***

Values are means ± SD of three determinations. Means followed by the same letter are not significantly different at $p < 0.05$, **$p < 0.001$, ***$p< 0.0001$, ns: not significant.

The amount of total phenolic compounds in two tested *Thymus* species was higher than that found in areal part of *T. vulgaris* (Roby et al. 2013), *T. capitatus* flowers (Jabri-Karoui et al. 2012) and in Jordanian *T. capitatus* shoot methanolic extract (Al-Mustafa and Al-Thunibat 2008). In addition, the amount of phenolics found in both *Thymus* species are largely exceeded those found in other Lamiaceous medicinal plants such as Ocimum basilicum (Javanmardi et al. 2003), sage, marjoram (Roby et al. 2013), Mentha piperita, Melissa officinalis and Rosmarinus officinalis (Zheng and Wang 2001) characterised by this abundance of phenolic compounds. Safaei-Ghomi et al. (2009) reported that the TPCs of Iranian *T. caramanicus* polar subfraction were 124.3 µg GAE/ mg, and were lesser in comparison with our data.

3.3. Antioxidant activities of *T. capitatus* and *T. algeriensis* fractions

Antioxidant capacity of polar phases estimated by three in vitro assays, differed between two species (Table 3). Results analysis depicted that this ability was very high in *T. capitatus* fraction as compared to *T. algeriensis*. Moreover, both *Thymus* displayed high total antioxidant activity and interesting antiradical capacity againt DPPH radical (IC50 = 6 and 7 µg/ ml) that might be attributed to the

presence of compounds that have the ability to interact with the free radicals by acting as an electron donor or hydrogen.

Tableau 3 : Total antioxidant activity (TAA), DPPH radical scavenging activities (IC50 values) and ferric reduced antioxidant power (FRAP) (EC50 values) of *Thymus capitatus* and *T. algeriensis* leaves methanolic phases.

Sample	TAA (mg GAE/g DW)	DPPH test (IC50 µg/ml)	FRAP (EC50 µg/ml)
Thymus capitatus	291.425± 0.23a**	6± 0.01c**	120±1.01b***
Thymus algeriensis	213.061±0.31b**	7± 0.02b**	210±2.11a***
Butylated hydroxytoluene (BHT)	-	11.5± 0.01a**	-
Vitamin C	-	-	37.33± 0.20c***

Values are means ± SD of three determinations. Means followed by the same letter are not significantly different at *p < 0.05, **p < 0.001, ***p< 0.0001, ns: not significant.

Recent studies have shown that polyphenols contribute significantly to the total antioxidant activity of many fruits, vegetables and medicinal plants (Ksouri et al. 2011; Skotti et al. 2014). The antiradical activity of both polar phases of *Thymus* species is very interesting and reflects the high antioxidant potential of these species, since radical scavenging activity is an indicator of the functionality and antioxidant activity of food (Ksouri et al. 2011). In fact, IC50 values of *Thymus* extracts surpass the BHT, a synthtetic antioxidant and many conventional aromatic and medicinal plants such as Origanum vulgare ssp. vulgare (9.9 µl/ ml) (Şahin et al. 2004) and Tunisian Carthamus tinctorius provenances (22-78 µl/ ml) (Ben Abdallah et al. 2013). According to the results, a positive linear correlation was established between the three in vitro assays of antioxidant activity and phenolics (Table 4). The high correlation coefficient (r=0.9, data not shown) estimated between antioxidant activities and phenolics suggests that these compounds may be the major contributors to the antioxidant activities of *Thymus* extracts. Numerous studies correlate the antioxidant activity of the plant extracts in the presence of phenolic compounds (Skotti et al. 2014). These authors indicated that high levels of phenolic content were correlated to significant antioxidant activities (DPPH• and ABTS•+) in five selected Greek medicinal aromatic namely Melissa officinalis L., Origanum vulgare L., Origanum dictamnus L., Salvia officinalis L. and Hyssopus officinalis L.

Tableau 4 : Correlation between phenolic compounds and antioxidant activity of plants studied.

	T. algeriensis methanolic phase			*T. capitatus* methanolic phase		
	TPCs	TFCs	CTCs	TPCs	TFCs	CTCs
AAT	0.90	0.934	0.740	0.851	0.994	0.968
DPPH·	0.981	0.96	0.995	0.96	0.931	0.92
FRAP	0.974	0.989	0.869	0.954	0.931	0.999

TPCs: total phenolic compound contents; TFCs: total flavonoid contents; CTCs: condensed tannins contents

3.4. Antimicrobial activity of *T. capitatus* and *T. algeriensis* leaves polar fractions and EOs

Table 5 summarized the mean inhibitory zone of *T. capitatus* and *T. algeriensis* leaves methanolic phases and EOs against 12 microbial species. The antibacterial and antifungal activity of these extracts displayed varying magnitudes of inhibition patterns with standard positive control depending on the susceptibility of the tested microorganism. Results displayed that both *Thymus* aqueous phases have weak to moderate antimicrobial activity against all strains tested. The highest activity of *T. capitatus* was against M. luteus, showing a maximum of 4.66 mm inhibition zone followed by S. thyphi and C. glabrata (4.33 mm). Moreover, the highest inhibitory activity of *T. algeriensis* fraction, was against Klebsiella sp., E. faecalis and C. glabrata with a maximum of 4.33 mm inhibition zone. This possibly means that the compounds responsible for the antibacterial activity in these extracts were at least concentration or totally absent.

In addition, a signifiant difference was recorded between polar fractions and EOs. Bacterial strains were more sensitive to EOs. Antibacterial and antifungal capacities *T. capitatus* EOs exceeded those

of *T. algeriensis. T. capitatus* tested oil exhibited a high to strong activity against all strains tested. Interestingly, the specific antibacterial activity of this oil was against Gram-negative bacterium P. aeruginosa, S. thyphi and S. flexineri and Gram-positive S. aureus that were better and stronger in comparison with the reference drug used as positive control.

T. capitatus and *T. algeriensis* essential oils also efficiently inhibited the growth of Candida sp., which is crucial because all fungal species tested proved to be involved in the diseases, and together with C. albicans followed by C. glabrata represent more than 80% of human cavity clinical isolates (Akpan and Morgan 2002).

Tableau 5 : Antimicrobial activity of *Thymus capitatus* and *T. algeriensis* polar fraction (30 µg/ disc) and essential oils (10 µl/ disc) and antibiotics (GM: Gentamicin, C: Chloramphenicol, AB: Amphotericin B) against different strains of bacteria and Candida species. Inhibition zone was calculated in diameter around the disc (mm ± SD).

	Diameter of inhibition zone (mm ± SD)						
	T. capitatus		*T. algeriensis*		Antibiotics (µg/ml)		
	Polar fraction	Essential oil	Polar fraction	Essential oil	GM 10	C 30	AB 10
Gram-negative							
Escherichia coli	1±0.2	7.66±0.2	1±0.0	4±0.1	15	14	
Pseudomonas aeruginosa	1.33±0.1	34.33±0.3	1.33±0.1	10.33±0.1	14	-	
Klebsiella sp.	2.33±0.2	7.66±0.1	4.33±0.1	4.33±0.1	40	-	
Salmonella thyphi	4.33±0.3	26.33±0.3	1±0.0	6.33±0.2	10	16	
Shigella flexneri	1±0.0	27.66±0.2	4±0.1	5.33±0.2	24	-	
Gram-positive							
Staphylococcus aureus	4±0.1	35.33±0.4	4±0.0	9.66±0.2	16	15	
Micrococcus luteus	4.66±0.2	3±0.1	4±0.1	na	30	-	
Enterococcus faeccalis	4±0.0	27.66±0.2	4.33±0.1	14.66±0.3	-	40	
Candida species							
C. albicans	1±0.1	20±0.2	1±0.0	9.33±0.1			10
C. glabrata	4.33±0.1	25.33±0.3	4.33±0.0	22.33±0.2			9.7
C. tropicalis	2.33±0.2	18±0.1	1.33±0.1	9.33±0.2			7
C. krusei	1±0.0	18±0.1	1±0.0	16±0.3			2.5

No antimicrobial activity (na), inhibition zone < 1 mm. Weak antimicrobial activity (w), inhibition zone = 1 mm. Slight antimicrobial activity, inhibition zone 2–3 mm. Moderate antimicrobial activity, inhibition zone 4–5 mm. High antimicrobial activity, inhibition zone 6–9 mm. Strong antimicrobial activity, inhibition zone > 9 mm (Rodríguez Vaquero et al., 2007).

The chemotype carvacrol of *T. capitatus* seemed to present a great effect on all studied bacteria. Several authors reported that this terpene phenol join to the amine and hydroxylamine groups of the proteins of the bacterial membrane altering their permeability and resulting in the death of the bacteria (Lambert et al. 2001). Carvacrol was also found to disintegrate the outer membrane of E. coli and S. typhimurium bacteria at levels close to the MIC (Helander et al. 1998). Cosentino et al. (1999) reported that the antimicrobial properties of thyme essential oils are mainly related to their high phenolic content. The same authors noted also that among the single compounds tested carvacrol and thymol turned out to be the most efficient against several strains and food-derived bacteria. This finding corroborate with *T. capitatus* results that contained higher phenolics and good antimicrobial activities. However, other compounds in EOs than phenols can present good antibacterial activity. The antimicrobial activity of the EO of *T. algeriensis* studied in this work may also be attributed to the dominant presence of 1,8-cineole, α-pinene, and camphor which has been found to have relatively good antimicrobial properties against many important pathogens (Ait-Ouazzou et al. 2011). However, some contradictory reports on the role of these compounds. Dorman and Deans (2000) reported that α-pinene exhibit a low antibacterial activity. Ait-Ouazzou et al. (2012) noted that Rosmarinus officinalis

EO rich in 1,8-cineole, showed less antibacterial activity. It is difficult to attribute the activity of a complex mixture to a single or particular constituent. Thus, a high level of some component does not necessarily mean the best antimicrobial effects against the strains assayed and possible synergistic and/or antagonistic effects of compounds in the oil should also be given consideration. Thus, the effective antimicrobial effect of *T. algeriensis* against bacterial and fungal strains was attributed to the presence of a mixture of major compounds like 1,8-cineole, α-pinene, and camphor and other minor compounds such as sesquiterpenes. Several studies reported a good antimicrobial activity of sesquiterpene-rich EOs (Maxia et al. 2009).

Based on these results, it is possible to conclude that both *Thymus* essential oils have good and broader spectrum of antimicrobial activity as compared to the polar fractions tested. This observation confirmed the evidence in a previous study reported that the essential oil include more antimicrobial substances from medicinal plants than other extracts such as water, methanol, ethanol and hexane (Şahin et al. 2004).

4. Conclusion

Hence, the present results suggest that polar fraction in *T. capitatus* and at lower extent *T. algeriensis* possess phenolic compounds with high antioxidant property which can be used in place of synthetic antioxidant (e.g., BHA, BHT) to prevent quality deterioration of food during storage, as well as for pharmaceuticals and natural therapies uses. In addition, essential oils of *T. capitatus* and *T. algeriensis* can be used in microbial food control against the well known causal agents of food borne diseases and food spoilage such as Enterococcus faecalis, Staphylococcus aureus, Salmonella thyhi, Shigella flexneri and Candida spp., isolates.

Acknowledgements

This work was supported by the Tunisian Ministry of Higher Education and Scientific Research (LR10CBBC02). The authors would like to thank Professor Abderrazak Smaoui for his help and plant identification.

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