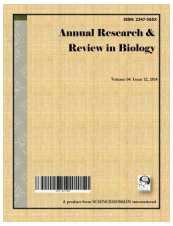

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Scientific Studies on the Variability of Phytochemical, Antioxidant and Antimicrobial Activities of Essential Oils of *Thymus hirtus sp. algeriensis*

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Authors' contributions

This work was carried out in collaboration between all authors. Author FG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FG, SI, HN and AL managed the analyses of the study. Author FG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: To delineate the mechanism of action of bioactive compounds extracted from the aerial parts of *Thymus algeriensis* collected from four mounts in Tunisia.
Materials and Methods: Essential oil phenolic content was measured, and its effect on the free radicals was investigated, along with their antibacterial potential following exposure to various doses.
Results: Our finding suggested that the essential oil of four populations contain a high amount of phenolic compounds. The antioxidant effect was detected with a low dose of *Thymus* samples.

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Whereas, *T. algeriensis* collected in the Orbata location exhibited the moderate antiradical effect. The exposure to thyme essential oils suppressed the bacteria strains growth. TJO and TJB exhibited the best antibacterial activities amongst all essential oil.
Conclusion: The volatile compounds and its antiradical and antibacterial effects support the effectiveness of Thyme towards several diseases.

Keywords: Thymus Eos; phenolic content; DPPH; FRAP; antibacterial effect.

1. INTRODUCTION

Plant extracts and natural compounds purified from plants have been used by humans for many centuries for the treatment and alleviation of a variety of inflammation-related diseases, including cancer [1]. In many countries, including Tunisia, traditional medicines widely resort to medicinal plants as an important drug to treat many diseases. *Thymus algeriensis*, locally known as "Mougecha" or "Mazoukcha", a member of the Lamiaceae family, is the most widespread North African species (Guesmi et al., 2014). Populations of this species are distributed from the sub-humid to the lower arid bioclimates [2]. According to Guesmi et al. [3], the aerial parts of *Thymus algeriensis*, widely used in Tunisian folk medicine, have an aromatic taste and are used as a flavouring and anti-inflammatory agent. Its leaves are rich with essential oils contained, such as linalool (17.62%), camphor (13.82%), terpinen-4-ol (6.80%), α-terpineol (6.41%), together with small amount of thymol [4]. Such variation in the chemical composition of the essential oil obtained from different populations was described by Guesmi et al. [5].

The problem of increased antimicrobial resistance becomes even more menacing when the delay in the discovery and development of new antibiotics is taken into account [6]. Natural products from mother nature would replace at least a part of the antibiotics. Essential oils (EOs) are aromatic and volatile liquids, mixtures of organic compounds extracted from plant materials and characterised by a strong and generally pleasant flavour [7]. It has already been documented that *the* essential oils of *Thymus* plant origin are shown to have antioxidant and antimicrobial properties and could be used as a therapeutic agent to treat several diseases. Thyme EOs as a mixture of terpenes, terpenoids, and phenylpropene, is extensively studied in literature because it possessed effective antimicrobial activities against several pathogenic and spoilage bacteria and fungi, like *S. aureus* and *E. coli*, with low MICs (≤1100 µL/mL) [8].

Dietary components derived from some species collected in Orbata mount in Tunisia have been indicated as an antitumor and cytotoxic suppressor in animals [9,10].

In this context, the objective of the present report is to study the antioxidant and antibacterial potential of four thyme populations growing in different mounts in Tunisia.

2. MATERIALS AND METHODS

2.1 Reagents

Stock solutions of essential oil fractions of the *Thymus* upper parts (500 µg/mL) were dissolved in 100% dimethyl sulfoxide (DMSO) (purchased from Sigma-Aldrich, St. Louis, MO, USA) and then diluted as needed *in vitro* studies. Folin-Ciocalteu reagent, gallic acid, catechin, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) diluted in methanol (99.95% purity), ferric reducing antioxidant power (FRAP), Potassium ferricyanide ($K_3Fe(CN)_6$) (1%), Trichloro acetic acide (TCA) (10%), Ferric Chloride ($FeCl_3$) (0.1%), Butyl-hydroxytoluene (BHT), Ascorbic acid (1%) were obtained from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA), Merck (Nottingham, UK), and Fluka Chemie (Buchs, Switzerland).

2.2 Plant Material

The aerial parts of *Thymus hirtus sp. algeriensis* were collected from Februray to March 2016 from four different wild populations in Tunisia during the flowering phase. Voucher specimens have been deposited at the Herbarium of the National Institute of Agronomic of Tunisia (INAT) under registration number 1188. Different thyme populations collected in Tunisia Mounts were: TJO: *Thymus algeriensis* collected in the Orbata mount; TJB: *Thymus algeriensis* collected in the Berda mount; TJA: *Thymus algeriensis* collected

in the Asker mount; TJS: *Thymus algeriensis* collected in the Swinia mount. Chemical compounds were analysed by GC/MS (Fig. 1A).

2.3 Total Phenolic Content

Phenolic, condensed tannin and flavonoid content were assessed by the method of Singleton and Rossi [11], Makkar and Becker [12] and Dewanto et al. [13], respectively.

2.4 Antiradical Scavenging Activity

To evaluate the antioxidant potential of *Thymus* Eos against DPPH free radicals ($DPPH^{\cdot}$), we used the modified DPPH method as described by Abdel Rahman et al. [14] with slight modifications, using ascorbic acid as a standard solution.

The evaluation of the ferric reducing antioxidant power (FRAP) of *Thymus* EOs was determined by the method of potassium ferricyanide-ferric chloride as described by Oyaizu [15] with slight modifications, using BHT as a standard solution.

2.5 Antibacterial activity

2.5.1 Bacteria strains

The microorganism strains used in this study including *Escherichia coli* (*E.coli*), *Staphylococcus aureus* (*S.aureus*), *Bacillus subtilis* (*B.subtilis*) and *Klebsiella pneumoniae* (*K.pneumoniae*) were purchased from ATCC.

2.5.2 Antibiotics

Ertapenem (ETP) (10 µg/µl) ; Aztreonam (ATM) (5 µg/µl) ; Chloramphenicol (CFM) (10 µg/µl) ; Cefotaxim (CTX) (10 µg/µl) ; Gentamicin (CN) (10 µg/µl) ; Cefixim (cystites) (CFM) (10 µg/µl) ; Ciprofloxacin (CIP) (5 µg/µl) ; Ceftazidim (CAZ) (10 µg/µl) ; Cefoxitin (FOX) (10 µg/µl) ; Ceftazidime (CAZ) (10 µg/µl); Tobramycin (TOB) (10 µg/µl) ; Cefoxitime (FOX) (30 µg/µl) ; Cefotaxime (CTX) (30 µg/µl); Fosfomycin (FF) (200 µg/µl); Amoxicilline-acid clavulanic (AMC) (30 µg/µl); Ticarcillin (TIC) (75 µg/µl) and Imipenem (IPM) (10 µg/µl) were obtained from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA).

2.5.3 Disc diffusion assay

The *in vitro* bactericidal and bacteriostatic effects of *Thymus* EOs was evaluated by disc_diffusion assay as described by Vlietinck et al. [16] and Abdel-Rahman [17,14]. Whatman discs (5 mm) were impregnated with 1 µl of different samples (TJO, TJB, TJA, TJS) and placed in plates and microorganism was suspended in Mueller - Hinton agar (10^6 colony forming units/mL). DMSO saturated discs were used as negative controls. After incubation of plates for 24 h at 37°C, we measured inhibition zones (in mm) around the disks [18].

2.6 Statistical Analysis

In vitro Experiments were repeated a minimum of three times. The results were expressed as mean ± SD. Differences between assays were compared by a one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Study

As reported in Fig. 1Bi, the total phenolic level of thyme samples, obtained from hydrodistillation extraction was found to be between 42 µg/mg DW - 125 µg/mg DW, respectively, as gallic acid equivalents. Amongst all essential oil of *Thymus* used in this report, TJO showed three times higher amount of phenols and the lowest level was detected in the TJA. An earlier study [18] has shown that acetonitril/water extract of *T.algeriensis* contain a high total phenolic content (12,384 µg GAE/g DW).

The flavonoid level of *Thymus* EOs was found to be between 20 µg/mg DW – 60 µg/mg DW, respectively, as quercetin equivalents and shown in Fig. 1Bii. Moreover, the condensed tannins detected in plant samples vary between 4.2 µg/mg DW - 12 µg/mg DW, respectively, as catechin equivalents (Fig. 1Biii). The level of tannins in TJO was also high even compared with other EOs.

The phytochemical study indicated a moderate amount of phenolic contents in *Thymus* EOs, including total phenols, condensed tannins and Flavonoids. Flavonoids and phenolic compounds are the most important groups of secondary metabolites and bioactive compounds in plants [19] and were responsible for biological activities.

3.2 Antiradical Scavenging Activity

Natural products, as substitutes of synthetic chemical preservatives, are increasingly accepted because they are innately better tolerated in human body and have inherent superiorities for food industry [20]. Thyme was used in some foods to extend the shelf-life [8].

After the evaluation of phenolic content of the aerial parts of *T. algeriensis*, we investigated the antioxidant effect of EOs. Our findings, validated through DPPH and FRAP assays, showed that *Thymus* EOs has a potential application as an antioxidant agent. As indicated in Fig. 1Ci, *Thymus* EOs act as potent free radical scavengers of the DPPH. DPPH radical scavenging activity was in the range of 52-91.96%. In addition, this essence is able to reduce Fe^{3+}/ferricyanide complex to the ferrous form (Fig. 1Cii). TJS sample was proved to be the most effective at all concentrations (10 µg/ml, 100 µg/ml, and 500 µg/ml). The antioxidant effect of TJS is close to that of ascorbic acid and BHT. Our finding indicates that EOs contain thymol, as a minor compound in TJO. Plants belonging to *Thymus* genus contain thymol as the active and interested molecules. Previous work done by Gavaric et al. [21] has shown that thyme essential oil and thymol were able to reduce DPPH radical into DPPH-H, with IC50 values of 0.24 and 70.06 µg/ml. Another study reported that thymol showing antioxidant activity with DNA-protective effects on cells [22]. The antioxidant properties of thymol may be related to its phenolic structure, which may adsorb and neutralise free radicals and exhibit redox properties [23].

3.3 Antibacterial Activity

The therapeutic potential of various natural plants and vegetables has already been described by practitioners of traditional medicine for many disorders. The antimicrobial effects of pure EOs *Thymus* specie were evaluated *in vitro* by agar diffusion assay. As shown in Fig. 2, EOs extracted from *Thymus algeriensis* collected from four sites showed effective antibacterial activity (P<0.05) against all tested bacteria with inhibition zone (IZ) ranging from 8 to 47 mm. The high content of eucalyptol and camphor supports the folk medicinal use of thyme as an antibacterial

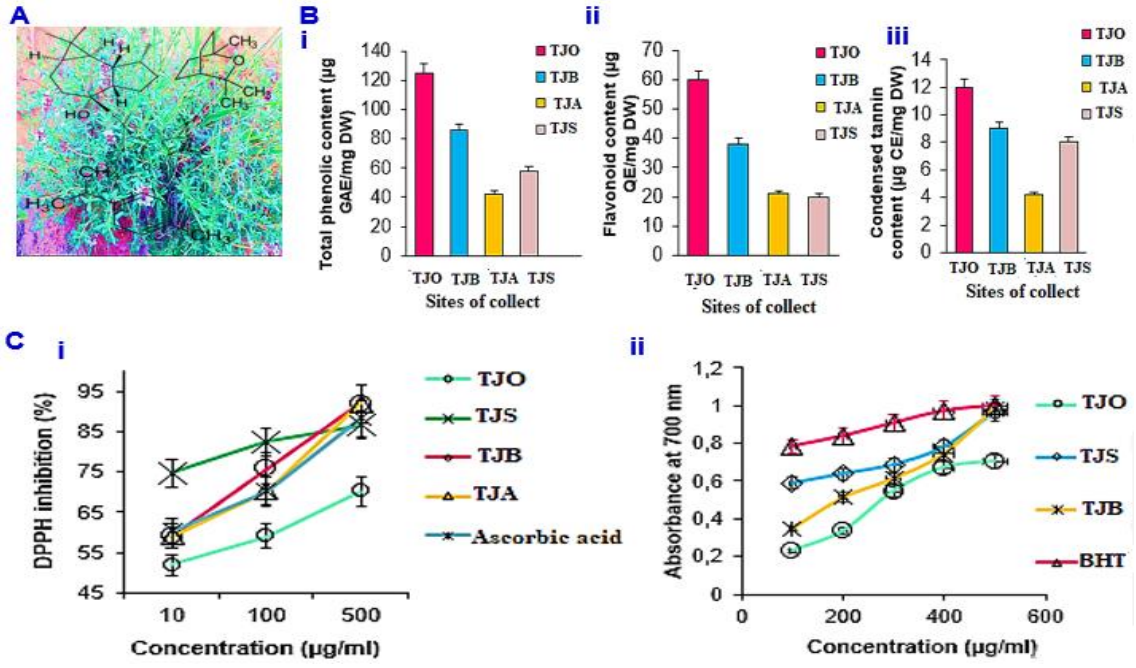

Fig. 1. Structure of major terpenic compounds isolated from the *Thymus* specie (A), Phenolic content (B) and antioxidant activity of thyme essential oil determined using DPPH (ii) and FRAP (iii) assay.

TJO : Thymus algeriensis collected in the Orbata mount; TJB : Thymus algeriensis collected in the Berda mount; TJA : Thymus algeriensis collected in the Asker mount; TJS: Thymus algeriensis collected in the Swinia mount.

agent. Furthermore, our data revealed that EOs isolated from *Thymus algeriensis* collected from the Orbata and Asker mounts were more effective (P<0.05) in the inhibition of all microorganism strains, than those of Ertapenem (10 µg/ml) and Chloramphenicol (10 µg/µl). The TJO have shown a highly potent antibacterial effect towards strains growth because it contains the highest total phenolics, flavonoids and tannins. The profiles obtained by chromatography, as well as the activity of the microorganisms tested showed very distinct aspects, indicating that the variation of results was due to the geographical region [24].

This finding is in harmony with the findings of Nikolić et al. [25], who found that *Thymus algeriensis* EO act as an inhibitor of *Streptococcus salivarius* growth, with a minimal inhibitory concentration (MIC) between 20 and 80 µg/ml. In addition Girova et al. [26], reported that thyme EO exhibited the highest antimicrobial activities against psychrotrophic microorganisms (*P. fluorescens*, *Pseudomonas putida* (*P. putida*), *P. fragi*, *B. thermosphacta*, and *C. albicans*) isolated from spoiled chilled meat products and some reference strains (*P. fluorescens* ATCC 17397, *P. putida* NBIMCC, *P. aeruginosa* ATCC 9027, and C. albicans ATCC 10231) using the method of disc diffusion and serial broth dilution, among five plant EOs with the MICs ranging from 0.05% to 0.8% w/v. Additionally, thyme EO was shown to be the most efficient against many *Vibrio alginolyticus* strains [27]. EO of *T. serpyllum* (8 mg/disc) was shown to inhibit the germination of spores from 80% to 100% [28]. As reported by Guesmi et al. [29], essential oil isolated from *Teucrium alopecurus* collected from Orbata mount (Gafsa, Tunisia) was effective (P < 0.05) in inhibiting various tested bacteria, than those of antibiotics. Similarly, other reports indicated that species cultivated from the same location (Orbata Mount, Tunisia) are considered as antibacterial agent [18].

Table 1. Antibacterial activity of *Thymus* EOs and antibiotics against four bacterial strains

Essential oils and antibiotics		Inhibition zones (mma) of bacterial strains in presence of Thymus EOs and antibiotics				
		Bacterial strains				
		Gram +		Gram -		
Plant name	Location	E.coli	K.pneumoniae	S.aureus	B.subtilis	K .oxycota
Control: DMSO		*	*	*	*	–
Thymus algeriensis	TJO	10	47	30	16	*
	TJA	12	10	R	25	*
	TJS	12	13	36	15	*
	TJB	13	7	8	12	*
Antibiotics (µg/µl)	ETP	*	30	*	*	24
	CFM10	22	*	28	18	*
	CAZ10	23	*	*	*	24
	AK10	17	*	*	*	21
	CTX30	30	*	*	*	35
	CIP5	40	*	*	*	40
	CT10	12	*	*	*	19
	IPM10	28	*	*	*	34
	TIC75	---	*	*	*	R
	FOX30	29	*	*	*	37
	CN10	18	*	*	*	22
	CFM5	22	*	*	*	33
	FF200	15	*	*	*	18
	AMC30	17	*	*	*	25
	AX10	---	*	*	*	---
	TOB10	17	*	*	*	22
	TJO	10	47	30	16	*

Note : EOs: Essential Oils; S. aureus: Staphylococcus. auerus; E. coli: Eshershia. coli ;K. pneumonie: Klebsiellla pneumoniae; B.s: Bacillus subtilus; a: inhibition zone diameters; : no activity detected ;(-) : not tested ; AMC : Amoxicilline-acid clavulanic, PRL : Piperacillin ; TIC : Ticarcillin ; CFM : Cefixim (cystites) ; FOX : Cefoxitin ; CTX : Cefotaxim, CAZ : Ceftazidim ; ATM : Aztreonam ; ETP : Ertapenem ; IPM : Imipenem, NA : Nalidixic acid ; NOR : Norfloxacin ; CIP : Ciprofloxacin ; AK : Amikacin ; CN : Gentamicin ; TOB : Tobramycin ; TGC : Tigecyclin (E.coli) ; FF : Fosfomycin ; CL : Cefalexin ; TZP : Piperacillin-Tazobactam 1/2*

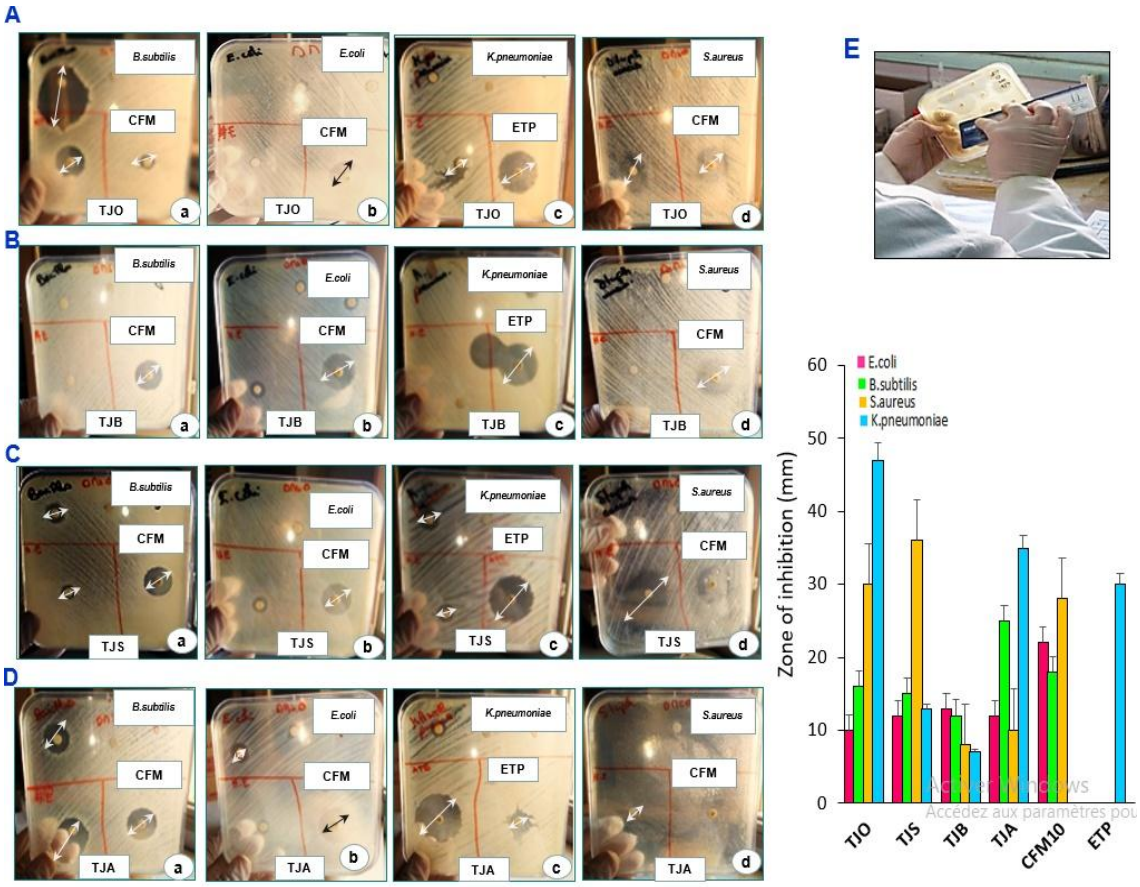

Fig. 2. A. Antibacterial effects of *T. hirtus sp. algeriensis* collected from the Orbata mount (TJO) towards B. subtilis (a), *E. coli* (b), K. pneumoniae (c), *S. aureus* (d). B. Antibacterial effects of *T. algeriensis* collected from the Berda mount (TJB) towards B. subtilis (a), *E. coli* (b), *K. pneumoniae* (c), *S. aureus* (d). C. Antibacterial effects of *T. algeriensis* collected from the Swinia mount (TJS) towards B. subtilis (a), *E. coli* (b), *K. pneumoniae* (c), *S. aureus* (d). D. Antibacterial effects of *T. algeriensis* collected from the Asker mount (TJA) towards B. subtilis (a), *E. coli* (b), *K. pneumoniae* (c), *S. aureus* (d). E. Measure of inhibition zones. Plant essential oil (1 µl) were applied per disc (Whatman No 5 mm) onto the seeded top layer of the agar plates containing tested bacteria (*E. coli*, *S. aureus*, *B. subtilis* and *K. pneumoniae*). The essential oil was tested with a Chloramphenicol, and Ertapenem discs as positive controls. DMSO discs were used as negative controls. The plates were incubated at 37°C for 24 h, and the zone of inhibition was determined. ETP: Ertapenem, CFM10: Chloramphenicol (10 µg/ml).

Thus, *Thymus* EOs has potential to be developed as an antibacterial agent; and this property could be due to their monoterpenes and/or sesquiterpene content (Eucalyptol, camphor, (+)-epi-Bicyclosesquiphellandrene and Thymol). Hence, our study reveals the antibacterial activity of *Thymus* EOs mediated through the inhibition zones and the induction of bacterial death. Thyme essential oil and its principle compound thymol have antimicrobial, antifungal, antioxidant and anticancer activities [30]. Our findings are also consistent with recent publications indicating that thymol acts differentially against a different type of bacteria and exerted its antimicrobial effect through binding to membrane proteins by hydrophobic bonding and hydrogen bonding, and then changing the permeability of the membranes [31]. Another report examining the molecular mechanism of thymol, as a major active compound of thyme, in *E. coli*. It decreased intracellular adenosine triphosphate (ATP) content of this bacteria and increased extracellular ATP, which could disrupt the

function of plasma membranes [32]. Thymol inhibited the *Aspergillus flavus* growth (MICs: 80 µg/ml) [33].

4. CONCLUSION

Thyme EO showed the best antioxidant potential than ascorbic acid. TJS and TJB EOs were the most efficient samples as they scavenge the DPPH free radicals, whereas the significant antibacterial activities, determined by agar diffusion assay, against the bacteria tested was higher in the presence of TJO and TJA EOs. Bioactive compounds are thought to be responsible, at least in part, for this beneficial effect. However, further phytochemical and pharmacological studies of the mechanisms of antibacterial effect of terpenes are warranted for identification of responsible compounds and evaluation of the molecular mechanism of their biological action.

COMPETING INTERESTS

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

REFERENCES

1. Ishikawa F, Yasukawa M, Lyons B, Yoshida S, Miyamoto T, Yoshimoto G, Watanabe T, Akashi K, Shultz LD, Harada M. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor {gamma} chain(null) mice. Blood. 2005;106(5):1565-73.
2. Pottier-Alapetite G. Flore de la Tunisie. Angiospermes. Dicotylédones. Gamopétales, Vol II. Tunis (Tunisia). Tunisia: Imprimerie officielle de la République Tunisienne. 1981;809-811.
3. Guesmi F, Ben Ali M, Barkaoui T, Tahri W, Mejri M, Ben-Attia M, Bellamine H, Landoulsi A. Effects of *Thymus hirtus sp. algeriensis* Boiss. et Reut. (Lamiaceae) essential oil on healing gastric ulcers according to sex. Lipids in Health and Disease. 2014a;13:138.
4. Ben Hadj Ahmed S, Sghaier RM, Guesmi F, Kaabi B, Mejri M, Attia H, Laouini D, Smaali I. Evaluation of anti-leishmanial, cytotoxic and antioxidant activities of essential oils extracted from plants issued from the leishmaniasis-endemic region of Sned (Tunisia). Natural Product Research. 2011;25:1195-1201.
5. Guesmi F, Ben Farhat M, Mejri M, et al. *In vitro* assessment of antioxidant and antimicrobial activities of methanol extracts and essential oil of *Thymus hirtus sp. algeriensis*. Lipids in Health and Disease. 2014b;13:114.
6. Chaves TP, Fernandes FHA, Santana CP, Santos JS, Medeiros FD, Felismino DC, et al. Evaluation of the interaction between the *Poincianella pyramidalis* (Tul.) LP queiroz extract and antimicrobials using biological and analytical models. PLoS ONE. 2016;11(5):e0155532. DOI:10.1371/ journal.pone.0155532.
7. Mejri J, Aydi A, Abderrabba M, Mejri M. Emerging extraction processes of essential oils: A review. Asian Journal og Green Chemistry ; 2018. DOI:10.22631/ajgc.2018.119980.1053.
8. Liu Q, Meng X, Li Y, Zhao C-N, Tang G-Y and Li H-B. Antibacterial and antifungal activities of spices. International Journal of Molecular Sciences. 2017;18:1283. DOI:10.3390/ijms18061283.
9. Guesmi F, Prasad S, Tyagi AK, et al. Antinflammatory and anticancer effects of terpenes from oily fractions of *Teucruim alopecurus*, blocker of IκBα kinase, through downregulation of NFκB activation, potentiation of apoptosis and suppression of NF-κB-regulated gene expression. Biomedicine & Pharmacotherapy. 2017a; 95:1876-1885.
10. Guesmi F, Beghalem H, Tyagi AK, Ben Ali M, Mouhoub RB, Bellamine H, Landoulsi A. Prevention of H2O2 induced oxidative damages of rat testis by *Thymus algeriensis*. Biomedical and Environmental Sciences. 2016;29:275-85.
11. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965;16:144-58.
12. Makkar HP, Becker K. Vanillin-HCl method for condensed tannins: Effect of organic solvents used for extraction of tannins. Journal of Chemical Ecology. 1993;19: 613-21.
13. Dewanto V, Wu X, Adom KK, et al. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agriculture and Food Chemistry. 2002;50: 3010-3014.
14. Abdel-Rahman LH, Abu-Dief AM, El-Khatib RM, Abdel-Fatah S M. Sonochemical

synthesis, DNA binding, antimicrobial evaluation and *in vitro* anticancer activity of three new nano-sized Cu(II), Co(II) and Ni(II) chelates based on tri-dentate NOO imine ligands as precursors for metal oxides. Journal of Photochemistry and Photobiology, B : Biology. 2016;162:298–308.

15. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japan Journal of Nutrition. 1986;44:307.
16. Vlietinck AJ, van Hoof L, Totte J, Lasure A, Vanden Berghe D, Rwangabo PC, Mvukiyumwani J. Screening of a hundred Rwandese medicinal plants for antimicrobial and antiviral properties. Journal of Ethnopharmacology. 1995;46: 31-47.
17. Abdel-Rahman LH, El-Khatib RM, Nassr LAE , Abu-Dief AM, Ismael M , Abdou Seleem A. Metal based pharmacologically active agents: Synthesis, structural characterization, molecular modeling, CT-DNA binding studies and in vitro antimicrobial screening of iron(II) bromosalicylidene amino acid chelates, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;117: 366–378.
18. Guesmi F, Ben Hadj Ahmed S, and Landoulsi A. Investigation of extracts from tunisian ethnomedicinal plants as antioxidants, cytotoxins, and antimicrobials. Biomedical and Environmental Sciences. 2017b;30(10): 323-332.
19. Boutaoui N, Zaiter L, Benayache F, Benayache S, Carradori S, Cesa S, Giusti AM, Campestre C, Menghini L, Innosa D and Locatelli M. Qualitative and quantitative phytochemical analysis of different extracts from *Thymus algeriensis* Aerial Parts. Molecules. 2018;23:463. DOI:10.3390/molecules23020463.
20. Silva F, Domingues FC. Antimicrobial activity of coriander oil and its effectiveness as food preservative. Crit. Rev. Food Sciences and Nutrition. 2017;57:35–47.
21. Gavaric N, Smole Mozina S, Kladar N, & Bozin B. Chemical profile, antioxidant and antibacterial activity of thyme and oregano essential oils, thymol and carvacrol and their possible synergism. Journal of Essential Oil Bearing Plants. 2015;18(4): 1013-1021.
22. Slamenova D, Horvathova E, Sramkova M and Marsalkova L. DNA-protective effects of two components of essential plant oils carvacrol and thymol on mammalian cells cultured *in vitro*. Neoplasma. 2007;54:108-112.
23. Salehi B, Mishra AP, Shukla I, Sharifi-Rad M, Contreras MM, Segura-Carretero A, Fathi H, Nasrabadi NN, Kobarfard F, Sharifi-Rad J. Thymol, thyme, and other plant sources: Health and potential uses. Phytotherapy Research. 2018;1-19.
24. Machado BAS, Silva RPD, Barreto GdA, Costa SS, Silva DFd, Brandão HN, et al. Chemical composition and biological activity of extracts obtained by supercritical extraction and ethanolic extraction of brown, green and red propolis derived from different geographic Regions in Brazil. PLoS ONE. 2016;11(1):e0145954.
25. Nikolić M, Glamočlija J, Ferreira ICFR, Calhelha RC, Fernandes Â, Markovic T, Soković M. 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. 2014;52:183-190.
26. Girova T, Gochev V, Jirovetz L, Buchbauer G, Schmidt E, Stoyanova A. Antimicrobial activity of essential oils from spices against psychrotrophic food spoilage microorganisms. Biotechnology and Biotechnological Equipment. 2010;24:547–552.
27. Hajlaoui H, Snousi M, Noumi E, Zaneti S, Ksouri R, Bakhrouf A. Chemical composition, antioxidant and antibacterial activities of the essential oils of five Tunisian aromatic plants. Italian Journal of Food Sciences. 2010;22:320–329.
28. Georgescu C, Mironescu M. Obtaining, characterisation and screening of the antifungal activity of the volatile oil extracted from *Thymus serpyllum*. Journal of Environmental Protection and Ecology. 2011;12:2294–2302.
29. Guesmi F, Tyagi AK, Prasad S, Landoulsi A. Terpenes from essential oils and hydrolate of Teucrium alopecurus triggered apoptotic events dependent on caspases activation and PARP cleavage in human colon cancer cells through decreased protein expressions. Oncotarget. 2018;9: 32305-3232.
30. Sertel S, Eichhorn T, Plinkert PK, Efferth T. Cytotoxicity of *Thymus vulgaris* essential

oil towards human oral cavity squamous cell carcinoma. Anticancer Research. 2011;31:81-88.
31. Burt S. Essential oils: Their antibacterial properties and potential applications in foods—A review. International Journal of Food Microbiology. 2004;94:223–253.
32. Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen PJ. Application of natural antimicrobials for food preservation. Journal of Agriculture and Food Chemistry. 2009;57:5987–6000.
33. Shen Q, Zhou W, Li H, Hu L, Mo H. ROS involves the fungicidal actions of thymol against spores of *Aspergillus flavus* via the induction of nitric oxide. PLoS one. 2016;11(5):e0155647.

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