Research Article

Chemical Composition and Antibacterial Activity of the Essential oil of *Thymus citriodorus L.* Growing Wild in Morocco: Preventive Approach against Nosocomial Infections

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ABSTRACT

In order to elucidate the chemical character of lemon thyme that grows wild in Morocco (*Thymus citriodorus L.*), chromatography (GC) and (GC–MS) was used to analyze the Hydro-distilled essential oil from this Moroccan Thymus specie. Forty three components representing 95.4% of the total oil composition were identified. The yield of essential oil was 1% and the predominant components were as follows: terpinyl formate (10.4%), geraniol (8.7%), isogeraniol (7.2%), cubenol (7%), citronellyl tiglate (6.4%), Thymol (5.3%), pulegone (4.8%) and Caryophyllene oxide (4%). Antibacterial activity of the oil was tested against five bacterial strains responsible of Nosocomial Infection: *Escherichia coli*; *Pseudomonas aeruginosa*; *Klebsiella pneumonia; Staphylococcus aureus* and *Citrobacter sp* through using disc diffusion method. Results showed that the essential oil from *Thymus citriodorus* exhibited the higher activity against all bacterial strains tested. Therefore, the essential oil extracted from lemon thyme can be used to clean the environment of polyvalent reanimation and Anesthesia service.

Keywords: *Thymus citriodorus*, Essential oils, Chemical composition, Antibacterial activity, Nosocomial infection.

INTRODUCTION

The genus Thymus (family Lamiaceae) is widely distributed in the Mediterranean region.[1] There are twenty one species of this genus in Morocco, twelve are endemic.[2] Some of these species have been used as infusions, powders and decoctions for their preservative and medicinal properties [3] and have been added to foods [4]; their oil is applied in cosmetic such as deodorants and toothpastes.[5]

The previous studies reported that Thymus species have strong antibacterial [6, 7], antifungal [8], antioxidant [9, 10], anticancer [11], antispasmodic and antiviral activities. [3]

Until recently, essential oils have been studied most from the viewpoint of their flavour and fragrance chemistry only for flavouring foods, drinks and other goods. Actually, however, the essential oils of plants have been of great interest as sources of natural products and biologically active compounds because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use.[12]

However, a number of Essential Oil (EO) components have been identified as effective antibacterial against multiple microorganisms[13,14,15] and numerous publications have presented data on the composition of the various EOs.[16] The essential oils from different Thymus species are characterised by their high content of monoterpenes especially phenolic compounds (thymol and carvacrol) and other more or less biologically active compounds (eugenol, p-cymene, ϒ-terpinene, linalool, geraniol and bornéol).[17,18,19,3]

Furthermore, the Thymus essential oils are being regarded as the most potent antimicrobial plant agents because of their highest activity, especially pathogens resistant to antibiotic.[17, 13, 20]

The objectives of the present study was to determine, for the first time, the chemical composition of the leaf essential oil of *Thymus citriodorus* from Morocco, as well as to evaluate its antibacterial activity against five bacterial strains responsible of Nosocomial Infections encountered in the University Centre Hospital of Fez (Morocco).

MATERIALS AND METHODS

Plant material

Fresh leaves of *Thymus citriodorus* were collected in Morocco at flowering stage (Mars 2014) from the region of Berkin. They were identified by Professor Amina Bari, botanist (Department of Biological Sciences, Faculty of Science, Sidi Mohammed Ben Abdellah University, Fez (Morocco).

Preparation and analysis of essential oil

Air-dried leaves were subjected to hydrodistillation for 3 h with 500 ml distilled water using a Clevenger-type apparatus, according to the European Pharmacopoeia.[21] Essential oil yields of *T. citriodorus* were 1% (v/w) respectively. The oils obtained was collected and dried over anhydrous sodium sulfate and stored in a

refrigerator at 4-5°C prior to analysis. Oil yield was based on dry weight of the simple.

GC and GC-MS analysis

The isolated oil was diluted with hexane (dilution ratio 10:100), and 1 mL was sampled for the gas chromatographic analysis. Trace gas chromatograph (GC) (ULTRA S/N 20062969, Thermo Fischer), gas chromatograph equipped with HP-5MS non polar fused silica capillary column (60 m × 0.32 mm, film thickness 0.25 mm) was used. Operating conditions: oven temperature program from 50 _C (2 min) to 280 _C at 5 _C/min and the final temperature kept for 10 min; 2"split mode" ratio 1:20; carrier gas Azoth (N), flow rate 1 mL/ min; temperature of injector and detector (flame ionization detector) were fixed at 250 _C and 280 _C, respectively.

Gas chromatography–mass spectrometry (GC–MS)

The volatile constituents were analysed on a Thermo Fischer capillary gas chromatograph directly coupled to a mass spectrometer system (model GC ULTRA S/N 20062969; Polaris QS/N 210729), HP-5MS non polar fused silica capillary column (60 m x 0.32 mm, 0.25 µm film thickness). The oven temperature was maintained at 40°C for 2 min, then increased at a programmed rate of 2°C/min to a final temperature of 260°C, which was maintained for 10 min; injector temperature 250°C. The carrier gas was helium at a flow rate of 1ml/min. Samples were run in *n*-hexane with a dilution ratio of 10:100. Compounds were identified by matching their MS and retention index with those reported in the literature.[22] The volume of injected specimen was 1µl of diluted oil; split injection technique; ionization energy 70eV in the electronic ionization mode; ion source temperature 200°C, scan mass range m/z 40-650, and interface line temperature 300°C. The essential oil components were identified by comparing their retention times and mass fragmentations with the NIST-MS and by literature comparison.[23]

Antimicrobial activity assessment

The microorganisms tested were *the Escherichia coli; Pseudomonas aeruginosa; Klebsiella pneumonia; Staphylococcus aureus and Citrobacter sp*. These were isolated in a hospital environment from clinical patients in reanimation service (CHU, Morocco). For the susceptibility screening test, an agar-disc-diffusion procedure was adapted from a method used earlier.[24] Each microorganism stock was suspended in Mueller-Hinton (MH) broth and incubated at 37°C for 18–24 h. The overnight cultures were diluted and adjusted in order to get a density of 10^8 CFU/ml (0.5 McFarland turbidity standards). They were flood-inoculated onto the surface of MH agar plates and 6 mm diameter, sterile filter discs of Whatman paper N_3, impregnated with 15 µg/disc of the essential oil were delivered onto the inoculated agar MH. The plates were incubated for 18 h at 35°C. Antimicrobial activity was evaluated by measuring the zones of inhibition. The antibody standards used were Imipenem, Ampicillin, Kanamycin and Ceftriaxone.[25, 26] The tests were carried out in triplicates.

RESULTS AND DISCUSSION

Essential oils composition

Table 1: Chemical composition of the essential oil from leaves of *Thymus citriodorus* (%).

Compounds	%	RI
3-Carene	0.4	917
m-Mentha-4,8-diene	2.7	923
Terpinyl propionate	2.2	929
2,3-bornanediol	0.6	932
1R-à-Pinene	0.4	937
Isopulegyl acetate	1.9	941
p-mentha-1,4-diene	0.3	954
Squalene	1.6	960
Terpinene	0.3	970
Phorbol	0.9	974
terpinyl formate	10.4	984
Camphidine	1.3	987
p-menth-4-en-3-one	2.8	1004
isogeraniol	7.2	1012
Pulegone	1.0	1014
Humulene	0.8	1016
á-Guaiene	0.3	1024
citronellyl tiglate	6.4	1027
Pulegone	4.8	1033
Geraniol	8.7	1036
Caryophyllene	3.0	1063
A-terpenyl ester	0.3	1073
Eudesma-4,11-diene	0.4	1075
Longifolene	3.9	1086
Himachala-2,4-diene	1.6	1089
Himachala-3(12),4-diene	1.4	1093
Isoledene	0.6	1094
Di-epi-à-cedrene	0.5	1096
Patchoulene	1.7	1199
Muuralene	0.92	1100
A-santol acetate	0.2	1102
A-cedrene oxide	5.3	1102
Thymol	7.0	1108
Cubenol	0.5	1111
Ç-Himachalene	0.3	1113
Neoisolongifolene,8-bromo	3.2	1115
Calarene epoxide	4.0	1118
Caryophyllene oxide	0.5	1121

Egiglobulol	0.9	1122
Spathulenol	0.9	1125
Farnesyl bromide	1.2	1128
Calarene epoxide	0.4	1132
Ledene oxide	0.5	1134
A-terpenyl ester	0.3	1073
Eudesma-4,11-diene	0.4	1075
Longifolene	3.9	1086
Himachala-2,4-diene	1.6	1089
Himachala-3(12),4-diene	1.4	1093
Isoledene	0.6	1094
Di-epi-à-cedrene	0.5	1096
Patchoulene	1.7	1099
Muuralene	0.9	1100
A-santol acetate	2.0	1102

Abbreviations: Retention index (RI); Area (%).

The chemical compositions and yields of essential oils from leaves of *Thymus citriodorus* reported in Table 1. The Essential oils of this genus from Morocco, which has not been investigated before, were yellow in color and were obtained in a yield of 1% (v/w), on dry weight basis. KIZIL et al. reported that the essential oil of *Thymus citriodorus* in turkey was 0.9%.[27] In other studies, Bagdat *et al*. indicated that the yield of essential oil from *T. x citriodorus* was identified between 1.30 and 1.43%.[28]

The GC-MS analyses led to the identification of Forty three compounds constituting 95.4% of the total essential oils of *T.citriodorus*. Table 1 depicts the identified compounds and their percentages as well as their RI values. The major constituents identified were terpinyl formate (10.4%), geraniol (8.7%), isogeraniol (7.2%), cubenol (7%), citronellyl tiglate (6.4%), Thymol (5.3%) pulegone (4.8%) and Caryophyllene oxide (4%). A different composition of essential oil of China *T. citriodorus in which the* main components were borneol (28.82%), thymol (14.43%), 3, 7-dimethyl-1, 6-octadiene-3-ol (8.26%), 1-methyl-4-[alpha-hydroxy-isopropyl] cyclohexene (8.23%) and terpenes camphor (5.1%) [29] and to the essential oils of Turkey *T. citriodorus* in which the major constituents were *trans* geraniol (30.07%), *trans*-citral (15.06%), *cis*-citral (11.71%), and *cis*- geraniol (7.65%).[27] However, the main constituents of the essential oil of *Thymus citriodorus* from Iran were geraniol (54.4%), geranial (13.9%), neral (10.1%), nerol (5.2%), 3-octanone (3.3%) and borneol (3.2%).[30] Stahl-Biskup and Holthuijzen indicated previously for the same species that geraniol was the main compound (more than 60%), geranyl acetate (1.0%), geranyl butyrate (0.8%), nerol (2.8%), and citronellol (0.3%).[31] Bagdat et al. also founded that the main component of *Thymus citriodorus* essential oil was geraniol (from twenty-one constituents).[28]

The differences between the results of our study and those of other studies highlight the impact that geographical and ecological factors can have on the qualitative and quantitative characteristics of the essential oils produced. In addition, other factors, such as the developmental stage of the plant and growing conditions, can influence the essential oil composition[32] and that geraniol can be characterize the Essential Oils of *Thymus citriodorus*.[30, 33]

Antimicrobial activity

Table 2: Antimicrobial activity of *Thymus citriodorus* essential oils using disc diffusion assay.

Inhibition zone (mm)		
Bacterial species	**Essential oils (15µl/disc)**	**Antibiotic standards**
Escherichia coli	32±0.76	40(IMP), 0(AMP), 15(CT),15(K)
Klebsiella pneumoniae	16±0.57	34(IMP), 0(AMP), 12(CT),10(K)
Staphylococcus aureus	10±0.5	16(IMP), 0(AMP)), 10(CT),18(K)
Pseudomonas aeruginosa	12±0.8	44(IMP), 0(AMP), 0(CT),8(K)
Citrobacter sp	28±0.86	35(IMP), 0(AMP) 15(CT),10(K)

Abbreviations Standard antibiotic disks: Imipenem IMP, Ampicillin AMP, Ceftriaxone CT, Kanamycin K.

The antibacterial activity of the essential oils of *T. citriodorus* was assayed in vitro following the diffusion in agar disc method using five bacteria strains *Escherichia coli; Pseudomonas aeruginosa; Klebsiella pneumonia; Staphylococcus aureus and Citrobacter sp* responsible for nosocomial infections in Centre Hospital University of Fez Morocco. Table 2 shows the microbial growth inhibition achieved by the Eos assayed. As can be seen, strong activities were observed against *Escherichia coli* and *Citrobacter sp* with an inhibition zone of 32 and 28 mm respectively. Antibacterial activities were also observed against *Klebsiella pneumonia* (16 mm), *Pseudomonas aeruginosa* (12 mm) and *Staphylococcus aureus* (10 mm). All strans were resistant to Ampicillin and sensible to Imipenem (Table 2). This later drug is typically used for the treatment of hospitalised patients with Nosocomial Infection in order to avoid bacterial resistance.

CONCLUSION

In this study, we report for the first time antimicrobial activity of essential oil of Moroccan *Thymus citriodorus*. Based on the results obtained, it is possible to conclude that the *Thymus citriodorus* essential oil has a stronger antibacterial activity than standard antibiotics used us controls such as Ampicillin and Ceftriaxone. The performance of the essential oil, on different strains bacterial, can lead to a thorough study and prospects for their application as a phytomedicine and food preservation agent.

REFERENCES

1. Ghasemi PA, Barani M, Hamedi B, Ataei KM, Karimi A. Environment effect on diversity in quality and quantity of essential oil of different wild populations of Kerman thyme. *Genetika*, 45, 2013, 441–450.
2. Benabid A. Flore et écosystèmes du Maroc. Evaluation et préservation de la biodiversité, Paris, 2015.
3. Stahl-Biskup E, Saez F. Thyme. London: Taylor & Francis 2002.
4. Zhang DY, Yao XH, Duan MH, Wei FY, Wu GH, Li L. China. *Industrial Crops and Products*, 77, 2015, 772–779.
5. Costa P, Medronho B, Gonçalves S, Romano A. *Industrial Crops and Products*, 70, 2015, 341–346.
6. Rota MC, Herrera A, Martinez RM, Sotomayor JA, Jordan MJ. *Food control* 19, 2008 681-687.
7. Gianni S, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M and Bruni R, Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods, *Food Chemistry*, 91 (4), 2005, 621–632.
8. Pinto E, PinaVaz C, Salgueiro L, Gonçalves MJ, Costa-de-Oliveira S, Cavaleiro C, Palmeira A, Rodrigues A, Martinez-de-Oliveira J. *Journal of medical microbiology*, 55, 2006, 1367-1373.
9. SariKurkcu C, Sabih Ozer M, Eskici M, Tepe B, Can S., Mete E. *Food and chemical toxicology,* 48, 2010, 1801- 1805.
10. Pereiraa OR, Macias RIR, Perez MJ, Marin JJG, Cardoso SM . Protective effects of phenolic constituents from Cytisus multiflorus, Lamium album L. and Thymus citriodorus on liver cells. *Journal of functional foods,* 5, 2013, 1170-1179.
11. S. Wu, FX. Wei, HZ. Li, XG. Liu, JH. Zhang, JX. Liu, Chemical composition of essential oil from Thymus citriodorus and its toxic effect on liver cancer cells, Zhong Yao Cai. 36(5), 2013,756-9
12. Ormancey X, Sisalli S, Coutiere P. Formulation of essential oils in functional perfumery. Parfums, Cosmetiques, Actualites, 157, 2001, 30–40.
13. Burt S. Essential oils: their antibacterial properties and potential applications in foods. *International Journal of Food Microbiology*, 94, 2004, 223– 253.
14. Lang G, Buchbauer G. A review on recent research results (2008-2010) on essential oils as antimicrobials and antifungals. A review. *Flav Frag J*, 27, 2012, 13-39.
15. Pirbalouti AG, Mirbagheri H, Hamedi B, Rahimi R. Antibacterial activity of the essential oils of myrtle leaves against Erysipelothrix rhusiopathiae. *Asian Pacific Journal of Tropical Biomedicine*, 4, 2014, S505-509.
16. Bauer K, Garbe D, Surburg H. Common Fragrance and Flavor Materials: Preparation, Properties and Uses. Wiley-VCH, Weinheim, 2001, 293.
17. El Bouzidi L, Jamali Alaoui C, Bekkouche K, Hassani L, Wohlmuth H, Leach D, Abbad A. Chemical composition, antioxidant and antimicrobial activitiesof essential oils obtained from wild and cultivated Moroccan Thymus species. *Ind. Crops Prod*, 43, 2013, 450–456.
18. Fadli M, Saad A, Sayadi S, Chevalier J, Mezrioui NE, Pagès JM, Hassani L. Antibacterial activity of Thymus maroccanus and Thymus broussonetii essential oils against nosocomial infection-bacteria and their synergistic potential with antibiotics. *Phytomedicine* 19, 2012, 464–471.
19. Saad A, Fadli M, Bouaziz M, Benharref A, Mezrioui NE, Hassani L. Anticandidal activity of the essential oils of Thymus maroccanus and Thymus broussonetii and their synergism with amphotericin B and fluconazol. *Phytomedicine* 17, 2010, 1057–1060.
20. Hudaib M, Aburjai T. Volatile components of Thymus vulgaris L. from wild growing and cultivated plants in Jordan. *Flavour Frag. J*. 22, 2007, 322–327.
21. Maisonneuve SA. *European pharmacopoeia*. Sainte-Ruffine: Council of Europe; 3, 1975, 68.
22. Joulain D, Konig WA. The atlas of spectral data of sesquiterpene hydrocarbons. E. B. Verlag Hamburg, Hamburg. 1998.
23. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream, 2001, 455.
24. Vuddhakul V, Bhooponga P, Hayeebilana F. Inhibitory activity of Thai condiments on pandemic strain of *Vibrio parahaemolyticus. Food Microbiology*, *24*, 2007, 413–418.
25. Mharti FZ, Lyoussi B, Abdellaoui A. Antibacterial Activity of the Essential Oils of *Pistacia lentiscus* Used in Moroccan Folkloric Medicine. *Natural product communications*, 6, 2011, 1505-1506.
26. Jallal Z, ELAtki Y,Lyoussi B, Abdellaoui A. Phytochemestry of the essential oil of Melissa officinalis L. Growing wild in Morocco: Preventive approach against nosocomial infections. *Asian Pacific Journal of Tropical biomedicine*, 5(6), 2015, 458–461.
27. Kizil S, Toncer O. Determination of essential oil and microelement composition *Thymus citriodorus* and *Lippia citriodora*. *1st Mediterranean Symposium on Medicinal and Aromatic Plants-MESMAP, Cyprus (Abstract),* 2013.
28. Bagdat RB, İpek A, Arslan N. Yield and quality parameters of *Thymus × citriodorus* (Pers.) Schreb. (synonym *T. fragrantissimus*, *T. serpyllum citratus* and *T. serpyllum citriodorum*) cultivated under Ankara ecological conditions. *Planta Medica Proceeding Book*, 2011, 1298, Antalya, Turkey.
29. Wu S, Wei FX, Li HZ, Liu XG, Zhang JH, Liu JX. Chemical composition of essential oil from Thymus citriodorus and its toxic effect on liver cancer cells, *Journal of Chinese Medicinal Materials*. 36 (5), 2013, 756-9.
30. Omidbaigi R, Sefidkon F, Hejazi M. Essential oil composition *of Thymus citriodorus* L. cultivated in Iran. *Flavour and fragrance journal,* 20(2), 2005, 237-238.
31. Stahl BE, Holthuijzen J. Essential oil and glycosidically bound volatiles of lemon scented thyme, Thymus × citriodorus (Pers.) Schreb. *Flavour and Fragrance Journal*, 10 (3), 1995, 225–229.
32. Argyropoulou C, Daferera D, Tarantilis PA, Fasseas C, Polissiou M. Chemical composition of the essential oil from leaves of Lippia citriodora H.B.K. (Verbenaceae) at two developmental stages. *Biochem Syst Ecol*, 35(12), 2007, 831-837.
33. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry,* 91, (2005), 621–632.

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