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Phytochemistry of the essential oil of *Melissa officinalis* L. growing wild in Morocco: preventive approach against nosocomial infections

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ABSTRACT

Objective: To determine the phytochemical characterization and antibacterial activity of *Melissa officinalis* essential oil against bacteria responsible for nosocomial infections.
Methods: The phytochemical characterization of essential oil was evaluated using gas chromatography-flame ionization detector and gas chromatography-mass spectrometer analysis. Antibacterial activity of the oil was tested against four bacterial strains responsible for nosocomial infections: *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Citrobacter koseri* using disc diffusion method.
Results: Thirty three components were identified representing 89.30% of the total oil composition. The yield of essential oil was 0.4% and the predominant components were citronellal (14.40%), isogeraniol (6.40%), geraniol acetate (10.20%), nerol acetate (5.10%), caryophyllene (8.10%) and β-caryophyllene oxide (11.00%). Antibacterial activity of the oil showed the higher activity against all bacterial strains tested.
Conclusions: The essential oil extracted from lemon balm can be used to clean the environment of reanimation polyvalent and anesthesia service.

1. Introduction

Lemon balm [*Melissa officinalis* L. (*M. officinalis*)] is a perennial herb in the mint family Lamiaceae, native to Southern Europe and the Mediterranean region[1]. In Morocco, this plant grows wildly in Sefrou region where it's popularized and applied for tea as a tranquilizer due to its health profit. Reports indicated that lemon balm had many beneficial effects such as anti-bacterial, sedative, spasmolytic, mnemonic improvement, and could reduce excitability, anxiety, stress, gastrointestinal disorders and sleep disturbance[1-3]. The essential oil of *M. officinalis* possesses potential anti-inflammatory activities, supporting the traditional application of this plant in treating various diseases associated with inflammation and pain[4]. An aqueous extract of *M. officinalis* demonstrated a high antiviral activity against herpes simplex virus type 1 (HSV-1) *in vitro*[5]. Furthermore essential oil of lemon balm showed an inhibition activity against HSV-1 as well as HSV-2 and might be suitable for topical treatment herpetic infections[6]. Recently, Hasanein and Riahi showed that chronic administration of *M. officinalis* oil displays efficacy in an experimental model of diabetic hyperalgesia and may therefore be a promising treatment for painful diabetic neuropathy[7]. *M. officinalis* leaf showed high acetylcholinesterase inhibitory activity and can be recommended for the treatment of Alzheimer's disease[8].

The essential oil of *M. officinalis* is a well-known antibacterial, antifungal and antioxidant agent[9-11]. A recent review revealed that several essential oils possess strong antimicrobial activity against various microorganisms[12,13], suggesting the possibility of using them as replacements of synthetic drugs to overcome the increasing resistance of some pathogens[12]. These oils might be exploited as natural antibiotic for the treatment of several infectious diseases[14].

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The main goal of the present work was to evaluate for the first time the phytochemicals of the essential oil of *M. officinalis* growing wild in Morocco. We determined, moreover, the antibacterial activity of lemon balm against bacterial responsible for the nosocomial infections contracted at patients in the University Centre Hospital of Fez Morocco. To the best of our knowledge, the antimicrobial activities using lemon balm essential oil belonging to this region has not been carried out before.

2. Materials and methods

2.1. Plant material

Fresh leaves of *M. officinalis* were collected in the winery from the hills of the Sefrou City, Morocco in the April 2013 and were dried for 7 to 10 days in the shade at room temperature then stored in cloth bags at 5 ℃ and transferred later to the laboratory for preparation of the plant extracts.

2.2. Isolation of the essential oil

A total of 200 g air-dried leaves of *M. officinalis* were subjected to hydrodistillation for 3 h with 600 mL distilled water using a Clevenger-type apparatus according to the European Pharmacopoeia[15]. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in a refrigerator at 4-5 ℃ prior to analysis. Yield based on dried weight of the sample was calculated.

2.3. Gas chromatography analysis

The isolated oil was diluted with hexane (dilution ratio 10:100), and 1 μL 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 μm) was used. Operating conditions: oven temperature program from 50 ℃ (2 min) to 280 ℃ at 5 ℃/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 ℃ and 280 ℃, respectively.

2.4. Gas chromatography-mass spectrometry (GC-MS)

The analysis of the volatile constituents was run on a Thermo Fischer capillary gas chromatograph directly coupled to the mass spectrometer system (model GC ULTRA S/N 20062969; Polaris QS/N 210729), using an HP-5MS non polar fused silica capillary column (60 m × 0.32 mm, 0.25 μm film thickness). The operating condition of GC oven temperature was maintained as: initial temperature 40 ℃ for 2 min, programmed rate 2 ℃/min up to final temperature 260 ℃ with isotherm for 10 min; injector temperature 250 ℃. The carrier gas was helium, flow rate 1 mL/min. Samples were run in hexane with a dilution ratio of 10:100. The volume of injected specimen was 1 μL of diluted oil, splitless injection technique; ionization energy 70 eV, in the electronic ionization mode; ion source temperature 200 ℃, scan mass range of m/z 40-650 and interface line temperature 300 ℃. Components identification was made by determination of their retention indices (KI) relative to those of a homologous series of *n*-alkanes (C8-C20) (Fluka, Buchs/sg, Switzerland) and by matching their recorded mass spectra with those stored in the spectrometer database (NIST MS Library v. 2.0) and the bibliography[16].

2.5. Antimicrobial activity assessment

Microorganisms included *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*), *Staphylococcus aureus* (*S. aureus*) and *Citrobacter koseri* (*C. koseri*). These bacteria were isolated in hospital environment from clinical patients in reanimation service (CHU, Morocco).

For the experiments of susceptibility screening test of the bacterial we used the agar-disc-diffusion method as mentioned earlier[17,18]. Each microorganism stock was suspended in Mueller-Hinton (MH) broth and then incubated at 37 ℃ 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 standard). They were flood-inoculated onto the surface of MH agar and 6 mm diameter, and sterile filter discs of Whatman paper No. 3 were impregnated with 15 μg/disc of the essential oil and were delivered into the inoculated agar (MH). The plates were incubated for 18 h at 35 ℃. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested microorganisms. The discs antibiogram of imipenem (IMP), cefaclor (CEF), oxacilin (OXA), vancomycin (VAC) are the standard drugs for comparison. The tests were carried out in duplicates. Results were interpreted in terms of a diameter of inhibition zone: resistant ($D < 6$ mm), intermediaries (6 mm $< D <$ 13 mm) and sensible ($D > 13$ mm). An average zone of inhibition was calculated for three replicates. ANOVA test was used to determine whether there are any significant differences between all inhibition tests.

3. Results

3.1. Essential oil composition

The essential oils obtained from the leaves of *M. officinalis* from Sefrou region, Morocco were yellow in colour with a yield of 0.4% v / m and were subjected to gas chromatography-

flame ionization detector and GC-MS. Thirty three components were identified representing 89.30% of the total oil in leaves composition (Table 1). Six predominant components followed in the essential oils from Sefrou lemon balm were citronellal (14.40%), isogeraniol (6.40%), geraniol acetate (10.20%), nerol acetate (5.10%), caryophyllene (8.10%) and caryophyllene oxide (11.00%), representing 55.20% of the total oil (Table 1).

Table 1

Chemical composition of the essential oil from leaves of *M. officinalis*.

Compounds	Area (%)	Retention index (RI)
Camphene	2.10	915
α-pinene	0.60	936
cis-p-Meth-2 en-7-ol	3.80	956
2-pinen-4-one	1.75	967
Nerol acetate	5.10	980
Citronellal	14.40	1021
Nerol	3.50	1036
Patchoulene	1.60	1062
1R-à-Pinene	0.70	1077
Isogeraniol	6.40	1089
Geraniol	1.00	1137
Verbenol	0.90	1136
Carane	2.32	1149
Geraniol acetate	10.20	1151
Menthol	2.20	1172
Cinerone	0.70	1206
cis-Z-Bisabolene oxide	0.60	1235
Verbenone	0.60	1259
Aromadendrene oxide	1.60	1287
β-Caryophyllene	8.20	1309
Aromadendrene oxide	1.80	1333
Andropholide	0.60	1365
Caryophyllene oxide	11.00	1411
cis-Myrtanol	0.90	1446
Germanicol	1.20	1489
Longifolene	0.70	1499
Himachalene	0.70	1515
Himachala-2,4-diene	0.60	1531
Cubenole	0.60	1565
Pimara-7,15-dien-3-one	1.80	1586
Cycloisolengifolene	1.70	1605
Cholest-5-en-7-ol	0.90	1632
Lupan-3-ol acetate	0.50	1665
Total	89.30	

3.2. Antibacterial activity

The antibacterial activities of essential oil from *M. officinalis* were tested by agar disc diffusion method against four bacteria strains (*P. aeruginosa*, *K. pneumonia*, *S. aureus* and *C. koseri*) responsible for nosocomial infections in Centre Hospital University of Fez Morocco. The results in Table 2 revealed that the essential oil of *M. officinalis* inhibited the growth of bacteria such as *P. aeruginosa* (16 mm), *K. pneumonia* (13 mm), *S. aureus* (20 mm) and *C. koseri* (14 mm) and showed stronger antibacterial activity when compared to standard antibiotics used as controls (CEF, OXA and VAN). However, the antibacterial effect of *M. officinalis* essential oil was lower when compared to IMP standard antibiotic.

Table 2

Antibacterial activity of *M. officinalis* essential oils.

Bacterial species	Inhibition zone (mm)	
	Essential oil (15 µL/disc)	Antibiotics
K. pneumoniae	13.0 ± 0.6	22 (IMP), 0 (CEF), 0 (OXA), 0 (VAN)
P. aeruginosa	16.0 ± 1.2	27 (IMP), 7 (CEF), 0 (OXA), 12 (VAC)
S. aureus	20.0 ± 1.6	42 (IMP), 0 (CEF), 18 (OXA), 15 (VAN)
C. koseri	14.0 ± 1.0	12 (IMP), 0 (CEF), 7 (OXA), 11 (VAC)

Data are expressed as mean±SD.

4. Discussion

The essential oils obtained from the leaves of *M. officinalis* from Sefrou region, Morocco were yellow in colour with a yield of 0.4% v / m and were subjected to GC-MS.

The obtained yields are higher than the leaves yielded and studied as usually between 0.06% and 0.39% v / m[15]. The total content in the herb is relatively low increasing its production cost and consequently its commercial price.

Thirty three components were identified representing 89.30% of the total oil in leaves composition. Six predominant components followed in the essential oils from Sefrou lemon balm were citronellal (14.40%), isogeraniol (6.40%), geraniol acetate (10.20%), nerol acetate (5.10%), caryophyllene (8.10%) and caryophyllene oxide (11.00%), representing 55.20% of the total oil. This composition is slightly different to the essential oil of Germany *M. officinalis* in which the major components were α-citral (20.13%) β-caryophyllene (17.31%), β-citral (13.58%), citronellal (3.86%)[19] and to the essential oil of Turkey *M. officinalis* in which the major components were citronellal (39%) and citral (33%)[20]. In India, the major constituents found in the *M. officinalis* essential oil were geranial (24.53%), neral (18.8%) and trans-caryophyllene (7.7%)[21]. In Iran, the main components were (E)-citral (37.2%), neral (23.9%) and citronellal (20.3%)[22]. However, the age of lemon balm plants affected the concentration of other constituents and the proportions of the following compounds were subject to especially high fluctuations: citronellal (8.7% and 0.4%), geraniol (trace amounts and 0.6%), and geranyl acetate (0.5% and 3.0%), as well as, among others, isogeranial, E-caryophyllene, caryophyllene oxide, germacrene D, and carvacrol[23].

Antibacterial activity of *M. officinalis* essential oil was studied against four bacteria strains (*P. aeruginosa*, *K. pneumonia*, *S. aureus* and *C. koseri*) responsible for nosocomial infections in Centre Hospital University of Fez Morocco. The results of antimicrobial activity revealed that the essential oil of *M. officinalis* inhibited the growth of all bacteria tested and, except for IMP, showed stronger antibacterial activity when compared to standard antibiotics used as controls (CEF, OXA and VAN). This result may be explained by the high content of citronellyl tiglate, geraniol acetate, caryophyllene, caryophyllene oxide,

isogeraniol and nerol acetate found in the essential oil. However, it is possible that other minor molecules modulate the activity of the main components.

The biological activity and medicinal value of plants are usually due to their phytochemical profiles, whose composition is totally dependent on geographical and environmental factors. *M. officinalis* from Morocco is rich in these compounds and shows an interesting antibacterial activity. Lemon balm is used in the Sefrou region in folk medicine for the treatment of headaches, indigestion, colic, nausea, nervousness, anemia, vertigo, syncope, malaise, insomnia, epilepsy, depression, psychosis and hysteria.

The oil was found to have significant antibacterial activity and therefore can be used as a natural antimicrobial agent for the treatment of several infectious diseases caused by these germs, which have developed resistance to antibiotics in Centre Hospital University of Fez Morocco.

Conflict of interest statement

We declare that we have no conflict of interest.

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