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Chemical composition and antibacterial activity of *Origanum compactum* Benth. essential oils from different areas at northern Morocco

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ABSTRACT

Origanum compactum Benth. is considered a highly threatened species due to its overexploitation by local inhabitants. The selection of the most active species, for later cultivation and domestication, is one of the best solutions to save this Moroccan endemic medicinal plant. The aim of this study was to determine the chemical composition of *O. compactum* essential oils harvested from fourteen sites geographically different within six areas in Northern Morocco and to study their antibacterial activity against four bacterial strains. EOs were isolated using steam distillation and the chemical composition was determined by GC–MS. The antibacterial activity was tested against four bacterial strains (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Listeria innocua*) using the well diffusion assay, then the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by the micro-dilution method. The results showed that the EOs tested presented a wide variation in the chemical composition, noticed mainly by phenols (21.41 to 69.53% in Melloussa and Ben Karrich, respectively), sesquiterpenes (0.41 to 2.49% in Mharech and Fifi, respectively) and monoterpenes (27.58 to 73.98% in Ben Karrich and Melloussa, respectively). The major components were: carvacrol (2.18–63.65%), *p*-cymene (6.69–42.64%), thymol (0.16–34.29%) and γ-terpinene (2.95–22.97%). The EOs of *O. compactum* from the studied sites expressed a significant antibacterial activity against the four strains studied. The diameters of the inhibitory zones varied from 10.33 to 49.00 mm, while the MIC values ranged from 0.06 to 0.25% (v/v) and the MBC values varied from 0.12 to 0.5% (v/v).

1. Introduction

The family of Labiaceae (Lamiaceae) is one of the most homogeneous families of the gamopetal subclass. Its homogeneity is not only morphological, but also physiological because most of the labies are rich in EOs. This family comprises about 3000 species, belonging to 200 genera, distributed throughout the world, with predominance in the Mediterranean and temperate region (Kokkini et al., 1991). In Morocco there are 207 species of this family (Ibn Tattou and Fennane, 1989).

The genus *Origanum* has a local distribution, mainly around the Mediterranean basin, and it is characterized by a great morphological and chemical diversity (Kokkini, 1997). It is a complex taxonomic group of aromatic plants that have been used all over the world for their aromatic and medicinal properties as well as a culinary herb (Aboukhalid et al., 2016). This genus was divided into 38 species, six subspecies and 17 hybrids, grouped into three groups and 10 sections (Ietswaart, 1980). Since this classification, five other species and one hybrid could be identified increasing the number of species to 43 and that of the hybrids to 18 (Carlström, 1984; Danin, 1990; Duman et al., 1995; Danin and Künne, 1996; Duman et al., 1998).

Among these species, *O. compactum* is one of the most important medicinal plants in terms of ethno-botany in Morocco. Called locally "Zaâtre", *O. compactum* grows naturally on dry, limestone and rocky terrain up to 700 m above the sea level. It develops on forest, collective and even cultivated land, sometimes between trees and shrubs and blooms from June to August.

O. compactum has been considered a real drug in Morocco, and it has been applied against several pathologies with a spectrum of use which varies between the regions regarding the pathologies, the parts used and the mode of preparation. The aerial parts of this plant have been used for a long time against lung and gastrointestinal infections as well as spasmolytic and sedative. *O. compactum* leaves and stems have been found to have various applications across Moroccan regions. They have been used against several pathologies such as cardiac diseases, diabetes, inflammation, hypertension, pyelonephritis and cystitis, stomachic, febrifuge, cooling and respiratory diseases. They have been also used against constipation and bile acid pathologies as well as for increased appetite (Bouyahya et al., 2017b). Nowadays *O. compactum* is used in several industrial fields (food, perfumery, pharmacy and aromatherapy) because of its broad spectrum of

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biological activities (Ayvaz et al., 2010; Amorati et al., 2013; Chaouki et al., 2015). *O. compactum* is considered a highly threatened species due to its overexploitation (Aboukhalid et al., 2016).

The chemical composition of EOs was widely studied (Lambert et al., 2001; Roldán et al., 2010; Belkamel et al., 2013). All studies have demonstrated that the chemical composition of EOs vary considerably from a region to another. Indeed, the knowledge of the chemical constituents of EOs is very important to explain their biological activities. In this regard, no study has published before about the comparative data of the chemical composition and antibacterial activity of *O. compactum* EOs from northern Morocco. In northern Morocco, the climatic and the edaphic as well as the topographical conditions are varied significantly from one area to another. The changes of conditions have known to affect the chemical composition and therefore the biological activities of EOs.

The excessive and inappropriate use of antibiotics in human's medication to treat infectious diseases is responsible for the emergence of resistant organisms (Rolain et al., 2016). This antibiotic resistance has become a public health issue of increasing magnitude. EOs from medicinal plants have been known as one of the best sources of antibacterial molecules (Aumeeruddy-Elalfi et al., 2016). Several studies have been conducted to evaluate the antibacterial effect of the EOs (Hammer et al., 1999; Roldán et al., 2010; Faleiro, 2011; Stefanakis et al., 2013; Mith et al., 2014). They have shown that the EOs expressed significant antibacterial activity.

The aim of this study was to evaluate the chemical composition of *O. compactum* EOs harvested from fourteen sites geographically different within six areas in Northern Morocco, and to study their antibacterial activity against four bacterial strains.

2. Materials and methods

2.1. Plant materiel and EOs extraction

The aerial parts of *O. compactum* studied in this work, were collected from its wild habitat in June–July, 2013, from various areas in northern Morocco, including **Tetouan** (Ben Karrich 35°31′21.3″N 5°25′08.3″W and Bni Ider 35°23′04.9″N 5°31′03.1″W), **Tangier** (Melloussa 35°44′ 22.3″N 5°37′48.2″W and Hjar Nhal 35°36′03.3″N 5°36′13.7″W), **Larache** (Haita Graz 35°13′40.3″N 5°59′20.1″W and Twajna 35°11′ 23.6″N 5°59′29.4″W), **Bni Arous** (Mayesra 35°19′10.1″N 5°43′26.2″W and Mharech 35°19′19.0″N 5°34′06.1″W), **Ouezzane** (Nefzi 34°53′ 02.7″N 5°20′19.5″W, El Kalaa 34°49′28.4″N 5°24′45.4″W, Assara 34°45′ 55.3″N 5°24′04.6″W and Dar Elghaba 34°48′34.2″N 5°11′15.6″W) and **Chefchaouen** (Fifi 34°56′39.0″N 5°14′25.7″W and Bab Taza 35°03′ 55.9″N 5°12′39.4″W).

The EOs were extracted from the aerial parts (leaves and flowers) of *O. compactum* by hydro-distillation using Clevenger-type apparatus (Clevenger, 1928). The obtained oils were dried with anhydrous sodium sulphate, weighed then stored at 4 °C until use.

2.2. GC–MS analysis of essentials oils

The EOs were analyzed using a Gas Chromatograph (TRACE GC Ultra) coupled with a mass spectrometer (Polaris Q-Ion Trap MS). Operating in electron-impact EI (70 eV) mode. VB-5 (95% Méthylpolysiloxane; 5% phenyl) and a column (30 m × 0.25 mm × 0.25 μm thickness) were used. The analyses were carried out at National Center for Scientific and Technical Research (NCSTR), Rabat, Morocco. The chromatographic conditions were as follows: Injector and detector temperatures at 220 and 300 °C, respectively; carrier gas, helium at flow rate of 1.4 mL/min; temperature program ramp from 40 to 300 °C with gradient of 4 °C/min (holding the initial and final temperature for 4 min). The identification of EO constituents was made on the basis of their retention time and their mass spectra.

2.3. Antibacterial activity

2.3.1. Bacterial strain and growth conditions

Four bacterial strains were tested: *Escherichia coli* K12 (Laboratory of Food Microbiology, UCL, Belgium: MBLA), *Bacillus subtilis* 6633 (German Microorganism Collection: DCM), *Listeria innocua* 4030 (Spanish Collection of Cultures Types: CECT) and *Staphylococcus aureus* 25,923 (American Type Culture Collection: ATCC). Strains were maintained on an inclined agar medium at 4 °C. Before use, the bacteria were revived by two subcultures in an appropriate culture medium: Lysogeny broth (LB) (Biokar Diagnostics, Beauvais, France) at 37 °C for 18 to 24 h. For the test, final inoculum concentrations of 10^6 CFU/mL of bacteria were used, following the instructions of the National Committee for Clinical Laboratory Standards, USA (NCCLS, 2001).

2.3.2. Agar-well diffusion method

On a basal layer of the Muller-Hinton agar (MHA) medium small glass cylinders of 8 mm in diameter were deposited. A volume of 6 mL of Lauria Bertoni (LB) medium containing 0.8% Agar, maintained supercooled at 45 °C, was seeded with fresh culture of the tested bacterium with a final concentration of about 10^6 CFU/mL. After solidification the cylindrical molds were removed generating at their site wells which were filled with 50 μL of EO. Diffusion of different components of EO in the agar medium was improved by a pre-incubation of Petri dishes at 4 °C for 2 h. The Petri dishes were then incubated at 37 °C for 24 h. The antibacterial activity of EO results in the appearance of an inhibitory zone around the wells (Bouhdid et al., 2009).

2.3.3. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC was determined by the standardized method of microdilution in liquid medium. The study was carried out in 96-well sterile micro-plates. The dilutions of the samples were distributed in the wells starting from the highest to the lowest concentration. 50 μL of the bacterial inoculum (10^6 CFU/mL) was added to each well according to the recommendations of the National Committee for Clinical Laboratory Standard (NCCLS, 2001). Control wells did not contain EO. The microplates were incubated for 18 h at 37 °C. The MIC was determined after verification of the bacterial growth using resazurin as redox indicator. After 2 h of incubation, the MIC (*v*/*v*) corresponded to the lowest concentration of EO which did not produce any change in the resazurin color (Bouyahya et al., 2017a). Chloramphenicol was used as positive control (reference standard). All the tests were performed in triplicate.

To determine the MBC, 10 μL of each well that did not show turbidity or change in resazurin staining was sub-cultivated on the Plate Count Agar (PCA) medium and incubated at 37 °C for 24 h. The MBC (*v*/*v*) corresponds to the lowest concentration of EO for which no overgrowth was observed (Bouhdid et al., 2009). Chloramphenicol was used as positive control (reference standard).

2.4. Statistical analysis

Data were analyzed using SPSS 20. The experiments were carried out in triplicates and the results were expressed as the average of the three measurements ± SD. The comparison of means between groups was performed with one-way analysis of variance (ANOVA) followed by Tukey test. Differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Extraction yield

The yields of EO of *O. compactum* from the various sites are shown in Table 1. The obtained results showed that the yield of extraction varied from 1.22% (Twajna-Larache) as the lowest average yield to 4.24% (Bni Ider-Tetouan) as the highest average yield. Indeed, the extraction