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Phytochemistry, antioxidant and antimicrobial activities of the essential oils of *Mentha rotundifolia* L. in Tunisia

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ABSTRACT

The present study is the first investigation of the chemical composition, antioxidant and antimicrobial activities of *Mentha rotundifolia* L. essential oils in Tunisia. Results show that essential oils from Beja locality were most complex and present 45 compounds representing 96.83% of the total oil composition. The major components of the studied oils in this site are β-Caryophyllene (26.67%), Germacrene D (12.31%) and Carveol (7.38%). 40 components representing 95.81% of the total oil were identified in Bizerte site. Those essential oils are dominated by Pulegone (32.09%), Piperitenone oxide (17.28%) and 5-Acetyl Thiazole (11.26%). Considerable levels of antioxidant activities of the investigated essential oils were highlighted. Variations in antioxidant activities may be attributed to the concentrations of major components and the presence of some phenolic compounds like Diosphenol and 2-Allyl-4-methylphenol. Our results showed strong activities of the investigated oils against all tested microorganisms. The highest antimicrobial activities were observed against Gram+ bacteria followed by Gram− ones then fungal species.

1. Introduction

Lamiaceae species are considered of high importance because of their use in folk medicine, culinary, cosmetics, flavoring and production of essential oils throughout the world. The genus *Mentha* which comprises 20 species distributed all over the world is among the major genera belonging to Lamiaceae family (McKay and Blumberg, 2006). It comprises herbaceous, perennial plants, common in temperate climates in Mediterranean region, Australia and South Africa (Lange and Croteau, 1999).

The industrial mint crops are cultivated in several countries for their essential oils commonly used in the cosmetics, pharmaceuticals, food, confectionery and liquor industries (Khanuja et al., 2000). Aerial parts from *Mentha* species have been widely used for the treatment of cold, cholera, bronchitis, tuberculosis, sinusitis and for their diuretic, carminative, antiflatulent, expectorant, antitussive and antioxidant properties (McKay and Blumberg, 2006; Kamkar et al., 2010).

Among plant extracts, essential oils and their components are gaining increasing interest in the food, cosmetic and pharmaceutical industries because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use. The essential oils from *Mentha* species have been used since ancient times for the treatment of many digestive tract diseases and in culinary (Iscan et al., 2002). Moreover, *Mentha* species have been used widely for their antimicrobial properties (Naigre et al., 1996; Flamini et al., 1999; Al-Bayati, 2009). Antimicrobial activities of mint essential oils were revealed in several species like *Mentha suaveolens* (Oumzil et al., 2002), *Mentha rotundifolia* (Ladjel et al., 2011), *Mentha pulegium* (Mahboubi and Haghi, 2008), *Mentha aquatica* and *Mentha longifolia* (Mimica-Dukic et al., 2003; Gulluce et al., 2007).

Nowadays, scientific research reveals that the antioxidant property of the plant extracts gives beneficial effect to human health (Puangpronpitag and Sittiwet, 2009). The *Mentha* species are cited as favorable free radical scavengers as well as primary antioxidants that may react with free radicals and limit ROS attack on biological and food systems (Nickavar et al., 2008). Antioxidant capacity is widely used as a parameter to characterize nutritional health food or plants and their bioactive components. Recently, interest

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has considerably increased in finding naturally occurring antioxidant to replace synthetic antioxidants, which were restricted due to their side effects such as carcinogenesis (Zhou et al., 2000).

Recently, investigation of natural products for the discovery of active compounds with antimicrobial and antioxidant properties from plant origin that can be applied to the food industry has gained interest. Natural active compounds could be an alternative to the employ of synthetic chemicals. Such compounds can be used to prolong the storage stability of food, by inhibiting the growth of foodborne spoilage or pathogenic microorganisms and protecting food from oxidative stress damage (Teixeira et al., 2012).

Located in the North African region with Mediterranean climate, Tunisia has a breeding ground for the development of a large number of medicinal and aromatic species currently experiencing a renewed interest in their use in the pharmaceutical, food and cosmetics. Thus, over than 500 species out of 2103 (approximately 25% of the total flora) are considered of therapeutic use (Le Floc'h, 1983). The species *M. rotundifolia* L. considered as a hybrid of *M. longifolia* and *M. suaveolens* (Lorenzo et al., 2002; Lawrence, 2007) is cited among medicinal and aromatic floral species present in Tunisia (Chemli, 1997).

Despite its importance as a medicinal and aromatic crop with industrial potentials, investigations regarding *M. rotundifolia* L. are still limited. Until now, there are no published reports concerning phytochemical characterization and biological activities of the essential oils of *M. rotundifolia* L. in Tunisia. Thus, chemotypes of this species in Tunisia are still unknown. Based on those considerations, the chemical composition, antioxidant and antimicrobial activities of their essential oils were described in this work based on two study sites. In this report, we aim to define for the first time the Tunisian chemotype of the investigated species and valorize its essential oil as antioxidant and antimicrobial agents.

2. Materials and methods

2.1. Plant material

The leaves of *M. rotundifolia* L. were collected from the regions of Bizerte (N: 36.7271°, E: 9.1880°, Sub-humid) and Beja (N: 36.7271°, E: 9.1880°, Sub-humid) during the vegetative stage of the plant. The samples were dried in the shade away from light at room temperature. After drying, the samples were ground to a fine powder, used for the extraction of essential oils. The botanical identity of collected samples (Family, Genus, and Species) was carried out by Professor M. Sadok Bouzid, a botanist in the Faculty of Sciences of Tunis.

2.2. Essential oil distillation and analysis

For each site, three lots of 100 g of *M. rotundifolia* leaves were separately hydrodistilled for 3 h using a Clevenger-type apparatus. The essential oils were dried using anhydrous sodium sulphate and then stored in sterile tubes at 4 °C until analyses.

The essential oil composition was determined by GC-FID and GC–MS analyses. GC analysis was performed in triplicate by an Agilent 6980 gas chromatograph equipped with a flame ionization detector and split-splitless injector attached to HP-INNOWAX polyethylene glycol capillary column (30 m × 0.25 mm). One microliter of the sample (dissolved in hexane as 1/50 (v/v)) was injected into the system.

The identification of the essential oil was performed using a Hewlett Packard HP5890 series II GC–MS equipped with a HP5MS column (30 m × 0.25 mm). The carrier gas was helium at 1.2 ml/min. 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, 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. Oil components were identified by comparison of their retention indices determined with reference to a homologous series of C_9–C_{24} n-alkanes and with those of authentic standards available in our laboratory. Identification was confirmed by comparison of their mass spectra with those recorded in NIST08 and W8N08 libraries. Component relative percentages were obtained from GC-FID peak areas without correction factors.

2.3. Antioxidant activities

2.3.1. Free radical-scavenging activity

The DPPH radical scavenging capacity was measured according to Hanato et al. (1988). 1 ml of each essential oil extract (at different concentrations) was mixed with 0.5 ml of 0.2 mM DPPH methanolic solution. The reaction was allowed to stand at room temperature in the dark for 30 min and the absorbance was recorded at 517 nm. The scavenging activity was estimated using the following equation: Scavenging effect (%) = [100 × (Ac − AS/Ac)], where Ac is the absorbance of the control reaction (containing all reagents except the test sample) and AS is the absorbance of the tested sample. The concentration of extract that could scavenge 50% of the DPPH radicals (IC_{50}) was calculated.

2.3.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM TPTZ in 40 mM HCl) and $FeCl_3 \cdot 6H_2O$ (20 mM) in a ratio of 10:1:1 (Benzie and Strain, 1996). To perform the assay, 900 μl of FRAP reagent, 90 μl distilled water and 30 μl of diluted essential oil were mixed and incubated at 37 °C for 15 min. The absorbance was measured at 595 nm, using FRAP working solution as a blank. The antioxidant potential of samples was determined from a standard curve plotted using the $FeSO_4 \cdot 7H_2O$ linear regression. The results were expressed as μmol Fe^{2+} equivalents/g essential oil. All assays were determined in triplicate.

2.4. Antimicrobial activity

2.4.1. Microbial species

Antimicrobial activities of *M. rotundifolia* L. essential oils were investigated against four bacteria strains (*Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus cereus*) representing Gram-negative and Gram-positive bacteria species. Additionally, the antimicrobial activity of the studied essential oils was tested against fungus (*Aspergillus niger*) and yeast (*Candida albicans*) species.

2.4.2. Disk diffusion method

The antibacterial and antifungal activities of *M. rotundifolia* essential oils were determined by disk diffusion assay according to Sacchetti et al. (2005) with modifications. Briefly, 100 μl of suspension of the tested microorganisms, containing 10^8 CFU/ml of bacteria cells, 10^6 CFU/ml of yeast, and 10^4 spore/ml of fungi were spread on Petri plates containing TSA, SDA, and PDA medium, respectively. The filter paper discs (6 mm in diameter) were individually impregnated with 10 μl of essential oil and then placed onto the agar plates. Discs without samples were used as a negative control. Before incubation, all Petri dishes were kept at 4 °C for 1 h and then incubated for 24 h for bacterial strains, 48 h for yeast and 72 h for fungi isolates at 37 °C, 30 °C and 27 °C, respectively. After incubation, the diameters of the inhibition zones were measured in mm including the diameter of discs. Measurements were realized in triplicates.