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Research Paper

Comparative study of the chemical composition and bioactivities of essential oils of fresh and dry seeds from *Myoporum insulare* R. Br.

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ABSTRACT

Essential oils of fresh and dry fruits from *Myoporum insulare* R. Br. were analyzed by GC and GC–MS. A total of twenty eight components were identified in essential oil of fresh fruits with γ-irone (14.4%), bicyclogermacrene (13.9%) and α-cadinol (13.2%) as main constituents. Twenty seven compounds were characterized in the oil of dry seeds with elemicin (20.1%), spathulenol (16.8%), α-cadinol (16.8%) and T-cadinol (14.2%) as main compounds. The present study was undertaken to evaluate the antioxidant, antibacterial, anti-tyrosinase and cytotoxic activities of essential oils of fresh and dry fruits from *M. insulare*. Dry seeds oil exhibited the highest activity of DPPH (IC_{50} = 54.0 ± 1.3 µg/mL), ABTS (IC_{50} = 68.0 ± 2.0 µg/mL), catalase (504.0.28 ± 0.655 u/mg protein) and paraoxonase (77.51 ± 0.47 µM/min/L). It's also exerted the best cytotoxic effect against A549 cell line and an interesting anti-tyrosinase activity with 81% of inhibition at 100 µg/mL. Essential oil of fresh fruits exhibited the highest antibacterial and antifungal activities against all tested organisms and fungi with IZ values 15.5–25.0 mm and 16–24.5 mm, respectively.

1. Introduction

The Myoporacea is a relatively small family consists of three genera, with the genus *Myoporum* comprising some 31 species which are distributed along the coastal areas from Eastern Asia to the Pacific Islands and Australia (Richmond and Ghisalberti, 1995). The genus *Myoporum* was introduced into many countries including Portugal, Spain, South Africa, South western United States and Brazil for planting in Coastal and low rainfall regions (Chinnok, 2007). In Tunisia, two species for the genus *Myoporum* are found (Pottier-Alapetite, 1979) among which the *Myoporum insulare* R. Br. has not been studied before. It is known by a variety of common names including Boobialla, Water Bush, Native Mangrove and Blueberry Tree. In temperate climates, the plant is used for ornamental hedges and forms a good barrier against onshore winds so it is frequently planted around homes, caravan parks and along paths as shelter belts (Chinnok, 2007). Various species of this genus are also employed in traditional medicine in several countries and it is beneficial in the treatment against many human diseases. In particular, *M. montanum* have a tonic, laxative and headache cure effects (Richmond and Ghisalberti, 1995). The decoction of *M. bontioides* A. Gray has an antidermatosis, antipyretic and antipsychotic effect (Li et al., 2014). Juice from leaves of *M. laetum* is used for curing ulcers, skin eruption and for toothache and decoction of leaves of *M. tenuifoluim* is used for curing toothache (Chinnok, 2007). The biological investigation resulted in finding of some new biological activities of the plants of this genus. These include antiviral and antibacterial activities (Ibrahim et al., 2006). The essential oils of a number of *Myoporum* species are characterized by furanoid sesquiterpene ketones, which are essentially oxygenated farnesols (Sutherland and Rodwell, 1989). Other sesquiterpenes such as myodesmone, isodesmone with their presumed precursor, (−) myoporone, 10,11-dehydromoyoporone were obtained from *Myoporum* species (Blackburne et al., 1971). A sesquiterpene alcohol was characterized from the wood essential oil of *M. crassifolium* (O'Donnell and Sutherland, 1989). Elemicin and nagaione were also describes as constituents of essential oil from leaves of Egypt *M. laetum* (Mohamed and Omer, 2009). Most previous studies concerned with the chemical composition of *Myoporum* species essential oil were focused on leaves and little is known about fruits oil constituents and biological

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activities. Therefore, the present study was intended at identifying for the first time the chemical composition of essential oils from fresh and dry fruits of *M. insulare* R. Br and the evaluation of its antioxidant, antibacterial, antifungal, cytotoxic and tyrosinase activities.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were of analytical grade. All reagents were purchased from Sigma-Aldrich-Fluka (Saint-Quentin France).

2.2. Plant material

Myoporum insulare R. Br. was collected in Monastir (Tunisia) in April 2016 and identified by Professor Fethia Harzallah-Skhiri at the Laboratory of Genetics, Biodiversity and Valorization of Bioresources (LR11ES41), Higher Institute of Biotechnology of Monastir, University of Monastir, Tunisia. A voucher specimen of *Myoporum insulare* has been deposited at the Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity, Faculty of Science of Monastir, Tunisia (MI-Mo/16). Fresh and dry seeds were weighed before the extraction of the volatile oils.

2.3. Essential oils extraction

Fresh and dry fruits of *Myoporum insulare* R. Br. were submitted separately to hydrodistillation for 4 h, using a Clevenger-type apparatus. Essential oils were collected by decantation, dried over sodium sulphate, filtered and stored at 4 °C until analyzed.

2.4. Chromatographic analysis

GC analyses were carried out with an HP-5890 Series II instruments equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with this temperature program: 60 °C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures 250 °C; carrier gas was helium (2 mL/min); detector dual FID; split ratio 1:30; injection of 0.5 μL (10% hexane solution). Components identification was carried out, for both columns, by comparing their retention times with those of pure authentic samples and by means of their linear retention index (LRI), relative to the series of *n*-hydrocarbons. Gas chromatography-electron impact mass spectroscopy (GC-EIMS) analyses were performed with a Varian CP-3800 gas-chromatograph, equipped with a HP-5 capillary column (30 m × 0.25 mm; coating thickness, 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were set as follows: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 μL (10% hexane solution); split ratio of 1:30. Constituents identification was based on comparison of retention times with those of authentic samples; this implied comparing their LRIs with the series of *n*-hydrocarbons and using computer matching against commercial (NIST 14 and ADAMS) and home-made library mass spectra (built up from pure substances and components of known oils and mass spectra literature data (Stenhagen et al., 1974; Massada 1976; Jennings and Shibamoto 1980; Swigar and Silverstein 1981; Davies 1990; Adams 1995; Benelli et al., 2013).

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity was measured from the bleaching of purple colored ethanol solution of DPPH· according to the method described by Hatano et al. (1988). 0.5 mL of each sample was mixed with the same volume of DPPH· ethanol solution. After 30 min incubation in the darkness at 25 °C, the absorbance of the sample at 520 nm was read. A mixture of 0.5 mL of DPPH· solution and 0.5 mL of ethanol was used as a blank. The decrease in absorption induced by the samples was compared to that of the positive control, BHT (butylatedhydroxytoluene). The calculated IC_{50} values denoted the concentration required to scavenge 50% of DPPH radicals. The results were expressed in inhibition percentage versus samples concentrations (mg/mL) at 30 min. All the measurements were performed in triplicate.

2.5.2. ABTS radical scavenging activity assay

The radical scavenging capacity of antioxidants for the ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical was determined as described by Re et al. (1999) $ABTS^{\cdot+}$ was generated by mixing 7 mM of ABTS at pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4 and 154 mM NaCl) with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using an UV–vis spectrophotometer (Helios, Unicam, Cambridge UK). For each sample, the diluted methanol solution of the essential oil (100 μL) was allowed to react with the fresh $ABTS^{\cdot+}$ solution (900 μL), and then the absorbance was measured 6 min after the initial mixing. BHT (butylatedhydroxytoluene) was used as a positive standard. The capacity of free radical scavenging was expressed as IC_{50} (mg/mL) value, which represents the concentration required to scavenge 50% of ABTS radicals. The capacity of free radical scavenging IC_{50} was determined using the same equation previously used for the DPPH method. All measurements were performed in triplicate.

2.5.3. Reducing power assay

The ferric-reducing power of essential oils and references was tested using the assay of Oyaizu (1986) 1 mL of different concentrations of the essential oils as well as chlorogenic acid as reference for comparative purposes were added to 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide. Later, the mixture was incubated at 50 °C for 20 min and then 10% trichloroacetic acid was added. The mixture was shaken vigorously and this solution was mixed with distilled water and $FeCl_3$ (0.1%, w/v). After 30 min incubation, absorbance was read at 700 nm. Increased absorbance of the reaction meant increased reducing power. BHT was used as control positive. All the measurements were performed in triplicate.

2.5.4. Catalase activity

Catalase activity was measured according to Aebi's (1984) method Hydrogen peroxide (H_2O_2) disappearance was monitored kinetically at 240 nm for 1 min at 25 °C. The enzyme activity was calculated using an extinction coefficient of 0.043 $mM^{-1}cm^{-1}$. One unit of activity is equal to one μmol of H_2O_2 destroyed/min/mg protein. Vitamin C was used as control positive. The assay was performed in triplicate.

2.5.5. Paraoxonase activity (PON1)

Paraoxonase 1 activity was determined using paraoxon (1.2 mmom/L) as substrate in 0.1 M tris-HCl buffer at pH 8.0 containing 2 mM $CaCl_2$. The sample tested was added (5 μL) to start the reaction, and the increase in absorbance at 405 nm was recorded (Araoud et al., 2011). PON1 activity was measured by a simple and rapid automated method adapted on Konelab 30™ (Thermo Electron Corporation, Ruukintie, Finland). Paraoxonase 1 activity is defined as 1 μmol *p*-nitrophenol formed per minute per L (μM/min/L). Vitamine C was used as control positive and the assay was performed in triplicate.

2.6. Anti-tyrosinase activity

The assay was performed according to the protocol described by Gouzi and Benmansour (2007) with some modifications. The effect of