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

Afifa Zardi-Bergaoui[a], Salma Jelizi[a], Guido Flamini[c,d], Roberta Ascrizzi[c], Hichem Ben Jannet[a,*]

[a] Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity (LR11ES39), Team: Medicinal Chemistry and Natural Products, Faculty of Science of Monastir, University of Monastir, Avenue of Environment, 5019 Monastir, Tunisia

[b] Laboratory of Bioressources: Integrative Biology and Valorization, High Institute of Biotechnology of Monastir, University of Monastir, Avenue of Tahar Haddad, 5000 Monastir, Tunisia

[c] Dipartimento di Farmacia, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy

[d] Centro Interdipartimentale di Ricerca "Nutraceutica e Alimentazione per la Salute" Nutrafood, University of Pisa, Italy

**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 Myoporaceae 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 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 appa- ratus. 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 $DPPH^{\cdot}$ according to the method described by Hatano et al. (1988). 0.5 mL of each sample was mixed with the same volume of $DPPH^{\cdot}$ 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 $DPPH^{\cdot}$ 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 $ABTS\%^{+}$ solution (900 μL), and then the absorbance was measured 6min 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 per- formed 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 30TM (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 inhibitor on mushroom tyrosinase was determined by using L-tyrosine (1 mM) as substrate. Hydroquinone (1 mM) was used as tyrosinase inhibitor. Both substrate and inhibitor were prepared in 0.1 M phosphate buffer pH 6,5. Inhibition of tyrosinase activity was tested in a reaction mixture (4 mL) consisting on 1.960 mL phosphate buffer, 2 mL L-tyrosine (1 mM), 20 µL mushroom tyrosinase and 20 µL hydroquinone (1 mM). The reaction was initiated by addition of enzyme to the solution of substrate and inhibitor. The reaction cell and all solutions were both thermostated at 25 °C. Inhibition effect was determined by the diminution of the maximum quantity of dopachrome formed and the absorbance was measured spectrophotometrically at 475 nm. The inhibition percentage of tyrosinase activity was calculated as: Inhibition (%) = (A – B)/A × 100, Where A represents the optical density of the tyrosinase enzyme and B represents the optical density of the tested essential oil during 30 min. The assay was carried out in triplicate.

2.7. Cytotoxic activity

2.7.1. Cell cultures

The human A549 lung epithelial carcinoma and HeLa cervix cells lines were obtained from the American Type Culture Collection (ATCC, Rockville,MD, USA) and cultured in DMEM medium (Dulbecco's Modified Eagle, Gibco) containing 10% (v/v) fetal calf serum, 2 mM glutamine and antibiotics (200 U/L of penicillin and 50 mg/L of streptomycin). Cells were maintained at 37 °C in a humidified 5% $CO_2$ atmosphere.

2.7.2. Cell viability assay

Cytotoxicity was measured using MTT (3-[4,5-dimethylthiazol-2- yl]-2,5-diphenyl tetrazolium bromide) test with slight modifications (Yan et al., 2009). Cells were seeded at $5 \times 10^3$cells/well in 100 µL of growth medium and incubated at 37 °C for 24 h to adhere. The cells were treated by essential oils and incubated for 48 h; then 10 µL of MTT (5 mg/mL) was added to each well and the incubation lasted 2 h. After this, 100 µL of DMSO were added to each well. The absorbance (A) was measured at 540 nm by a Multiskan Ascent (Ascent Software version 2.6) microplate reader. This assay was conducted in triplicate and the percentages of cell growth were calculated as follow:

Cell growth (%) = [A (sample)/A (control)] × 100.

Cytotoxicity was expressed as the concentration of sample inhibiting cell growth by 50%.

2.8. Antibacterial activity

The isolated oils were screened for their antibacterial activity by the agar disc diffusion method (Denis et al., 2011). NA medium cooled at 45 °C was supplemented with a bacterial suspension ($10^6$CF/mL)

and poured into Petri plates. After solidification, sterile Whatman paper discs (diameter 6 mm) were placed at the surface of the culture medium and 20 μL (1000 μg/mL) of the essential oils dissolved in hexane was dropped on to each disc. The negative and positive control plates added to the filter paper, discs were impregnated with the same volume of hexane and ampicillin solution (5 mg/mL). The treated Petri dishes were incubated at 25 °C for 48 h. The antibacterial activity was eval- uated by measuring the diameter of the inhibitory zones formed around the discs. The experiment was replicated twice.

### 2.9. Antifungal activity

*Aspergillus niger, Penicillium digitatum* and *Penicillium italicum* were used for screening of antifungal activity of the products tested by using the disc diffusion method (Barry and Thornberry, 1991). A conidial suspension of the tested fungi was prepared ($10^4$-$10^5$ CFU/mL) and added to PDA medium cooled at 45 °C and poured uniformly into Petri plates (diameter 90 mm). Sterilized paper discs (6 mm, Whatman No. 1filter paper) were impregnated with hexane and placed on the culture plates whereas the negative control plates had no product added to the filter paper. In the positive control plates, discs were imbibed with the same volume of fungicide (FONG) suspension (0.5 mg/mL). The diameter of the inhibition zone (mm) around the disc was measured after incubation at 25 °C for 4 days and compared with control. The test was performed in triplicate.

## 3. Results and discussion

### 3.1. Chemical composition

The hydrodistillation of fresh and dry fruits from *M. insulare* R. Br. furnished yellow oils with 0.025 and 0.04% yields, respectively. The chemical compositions of these oils were reported in Table 1 with their linear retention indices (LRIs), the percentages of compounds and the identification methods. A total of 28 compounds were identified in fresh fruits oil, representing 98.9% of the total oil composition, containing mainly γ-irone (18, 14.4%) followed by bicyclogermacrene (20, 13.9%), α-cadinol (39, 13.2%), 7-epi-silphiperfol-5-ene (9, 9.6%), T-cadinol (37, 8.5%) and δ-cadinene (25, 6.8%). All other components were detected at contents below 5.0%. Moreover, 27 constituents were detected in essential oil of dry fruits accounting for 97.2% (Table 1). The major components were elemicin (26, 20.1%), spathulenol (28, 16.8%), α-cadinol (39, 16.8%) and T-cadinol (37, 14.2%). All the other components were present at amounts varying from 0.1 to 3.9%. The chemical classes of the studied oils are reported in Table 1. Some significant differences were noted in the quantitative and qualitative composition of the oils obtained from fresh and dry fruits but we can deduce that both of them may be considered as sesquiterpene rich oils. The main class was formed by the oxygenated sesquiterpenes (61.1%) which characterizing the dry fruits oil, Spathulenol (28, 16.8%), α-cadinol (39, 16.8%) and T-cadinol (37, 14.2%) represented the major components. Among the 11 constituents of this class, only five were detected in the fresh fruits oil. However, we noted that the major constituent of fresh fruits essential oil was sesquiterpene hydrocarbons with 40.9%. They were mainly represented by bicyclogermacrene (20, 13.9%), 7-epi-silphiperfol-5-ene (9, 9.6%) and δ-cadinene (25, 6.8%). Among the 12 compounds belonging to this class, only seven were identified in the dry fruits oil. The third major class was the phenylpropanoids (20.1%), represented by one compound, elemicin (26, 20.1%), identified only in the dry fruits oil. Apocarotenoids were mainly represented only by γ-irone in the fresh and dry fruits oils (18, 14.4 and 2.3%, respectively).

### 3.2. Antioxidant activity

#### 3.2.1. Free radical scavenging activity (DPPH assay)

The antioxidant measurements by DPPH method of fresh and dry fruits oils are given in Table 2. All of the assessed essential oils were able to reduce the stable purple-colored radical DPPH to yellow-colored DPPH-H reaching 50% of reduction. Results showed that essential oil of dry fruits was the most potent radical scavenger with an $IC_{50}$ of 54.0 ± 1.3 μg/mL compared with a reference standard tert-butyltetrahydroxytoluene (BHT) (18.0 ± 1.0 μg/mL), followed by fresh fruits oil with an $IC_{50}$ of 92.0 ± 1.8 μg/mL. The elemicin which is present only in high amounts in dry fruits' oil can be partly responsible for such activity, but this activity is not attributed to a single compound since a synergistic effect between the different compounds can occur (Bouzouita et al., 2008).

3.2.2. ABTS radical-scavenging assay

Another method to evaluate the antioxidant ability is based on ABTS assay. According to the ABTS results assay (Table 2), the most active essential oil was the dry fruits oil with an $IC_{50}$ of 68.0 ± 2.0 μg/mL compared with BHT ($IC_{50}$ = 50.0 ± 1.0 μg/mL). However, results showed that essential oil of fresh fruits exhibited a moderate activity with an $IC_{50}$ = 111.0 ± 2.3 μg/mL.

3.2.3. Reducing power assay

This method evaluates the ability of essential oils to reduce potassium ferricyanide solution which is monitored by measuring the formation of Perl's Prussian blue $Fe_4[Fe(CN)_6]_3$ at 700 nm. Increasing the absorbance at 700 nm indicates an increase in reductive ability (Joshi et al., 2010). The values of the reducing power of fresh and dry fruits essential oils from *M. insulare* are given in Table 2. Results showed that essential oils of fresh and dry fruits exhibited a moderate ferric- reducing ability. However, essential oil of dry fruits presented the highest activity with an $IC_{50}$ of 194.0 ± 1.4 μg/mL compared with the fresh fruits oil ($IC_{50}$ = 321.0 ± 4.4 μg/mL).

3.2.4. Catalase assay

Results of catalase activity of fresh and dry fruits oils are given Table 2 and showed that the best activity was exhibited by essential oil of dry fruits with 504.0.28 ± 0.655 Units/mg protein compared with vitamin C (757.575 ± 0.002 u/mg protein), followed by fresh fruits essential oils (387.610 ± 0.413 u/mg protein) (Table 2). The Decrease of catalase activity can lead to the accumulation of the $O_2\%^-$, $H_2O_2$ or the accumulation of hydrogen peroxide that correlates with cancer metastasis (Tsai et al., 2014). Loss of catalase activity makes oxygen bigotry and provokes a number of adverse reactions such as oxidation of ADN and death of the cell (Halliwell and Gutteridge, 1999). Catalase plays a protective role towards cardiovascular disease.

3.2.5. Paraoxonase assay (PON1)

Essential oils of fresh and dry fruits are tested for their potential Paraoxonase activity. Results showed that essential oil of dry fruits exhibited a high activity with 77.51 ± 0.47 μM/min/L compared with the vitamin C (55.0 ± 0.2 μM/min/L) (Table 2). However, essential oil of fresh fruits showed a moderate effect on the activity of this enzyme with 33.0 ± 0.5 μM/min/L. These finding prompt us to deduce that essential oil of dry fruits from *M. insulare* can play an important role in the higher risk for several diseases, especially as PON1 has been implicated in many important affections health, including organophosphorus (Cole et al., 2010) sensitivity and in the etiology of various disorders such as diabetes, atherosclerosis, Parkinson's and Alzheimer diseases (Menini and Gugliucci, 2014).

3.3. Antimicrobial assays

Essential oils of fresh and dry fruits from *M. insulare* were evaluated for her antibacterial activity using disc-diffusion method against 3 gram-negative strains (*Pseudomonas syringae* pv. *syringae*, *Pseudomonas savastanoi* and *Agrobacterium tumefaciens*) and their potency was qualitatively assessed by the presence or absence of inhibition zones (Table 3).

Results showed that essential oil of fresh fruits exhibited a strong antibacterial activity against the tested Gram-negative bacteria with inhibition zone values of 25.0 ± 0.8 against *P. syringae* pv. *syringae*, 18.0 ± 0.6 against *A. tumefasciens* and 15.5 ± 0.6 against *P. savastanoi*. However, we noticed that essential oil of dry fruits, showed an interesting activity against *A. tumefasciens* (inhibition zone values of 17.0 ± 0.8) but had no effect on the growth of *P. syringae* pv. *syringae* and *P. savastanoi*. Moreover, the antifungal activity of essential oils of fresh and dry fruits from M. insulare was also tested against three fungal strains: *Aspergillus niger, Penicillium digitatum* and *Penicillium italicum*. According to the results given in Table 4, essential oil of fresh fruits exhibited an interesting antifungal activity against all fungal agents used (inhibition zone values of 24.5 ± 0.30 against *A. niger*, 21.0 ± 1.7 against *P. digitatum* and 16.0 ± 0.8 against *P. italicum*). However, results showed that essential oil of dry fruits was particularly effective against *P. digitatum* with a diameter of inhibition about 12.0 ± 1.0 mm. Antimicrobial activity of essential oils are difficult to correlate with a specific compounds for their variability and complexity. However, some researchers reported a relationship between chemical composition of each essential oil and antimicrobial activity (Frag et al., 1989; Cox et al., 2000). This relationship may be attributed to the presence of oxygenated sesquiterpenes components such as α-cadinol (39), β-caryophyllene (13), δ-cadinene (25) and germacrene D (19) with pronounced antimicrobial activities (Kubo et al., 1992; Chang et al., 2000; Cheng et al., 2004; Goren et al., 2011; Pérez-Lopez et al., 2011; Sakka Rouis-Soussi et al., 2014).

### 3.4. Anti-tyrosinase activity

Essential oils of dry and fresh fruits from *M. insulare* were also tested for her anti tyrosinase activity. Results are given in Table 5. Essential oils inhibited anti tyrosinase activity by the oxidation of L-DOPA catalyzed by mushroom tyrosinase enzyme. The enzyme activity was not suppressed but rather decreased rapidly. Results showed that essential oil of dry fruits exhibited a significant anti-tyrosinase activity with 81% compared with kojic acid (85.2 ± 2.0%, 50 μg/mL). However, essential oil of fresh fruits showed a moderate anti-tyrosinase activity (55 ± 2.3%, 100 μg/mL). Previous studies released with some essential oils showed that acyclique terpene alcohols such as linalool and β-caryophyllene have a significant antityrosinase activity (Nakatsu et al., 2000; Yang et al., 2015).

### 3.5. Cytotoxic activity

Cytotoxicity of essential oils of fresh and dry fruits from *M. insulare* was also evaluated against A549 lung epithelial carcinoma and Hela cervix cells lines. Results are given in Table 5 and showed that essential oil of dry fruits presented the best cytotoxic effect against the A549 and Hela with an IC50 of 68.50±0.70 and 78.20±1.75 μg/ml, respectively. However, essential oil of fresh seeds found to be less active against the A549 and Hela cells lines with an $IC_{50}$ of 74.20 ± 1.60 and 98.87 ± 1.31 μg/mL, respectively. It is well known that the biological activities of an essential oil may depend on the main constituents or a synergy between the major and some minor compounds (Wright et al., 2007). Cytotoxic activity of essential oils of fresh and dry seeds of this plant may be attributed to specific components. In fact, some com- pounds present in the *M. insulare* essential oils have previously been tested for their cytotoxicity such as α-cadinol, α-humulene, β-caryophyllene and germacrene D (Table 1) against MCF-7, MDA-MB-231, Hs 578T, PC-3 and Hep-G2 cell lines (Setzer et al., 2006; Palazzo et al., 2009; Salvador et al., 2011).

## 4. Conclusion

To the best of our knowledge, this is the first study reporting the chemical composition and the in vitro antimicrobial, antioxidant, anti-tyrosinase and cytotoxic properties of essential oils of fresh and dry fruits from *M. insulare*. The chemical constitutes of the isolated essential oils were analyzed by GC and GC–MS. A total of 39 compounds have been identified. The dry fruits essential oils voiced a significant antioxidant activity, an interesting tyrosinase inhibitory and a moderate cytotoxic activity against A549 lung epithelial carcinoma cells lines. However, the essential oils of fresh fruits exhibited a high antimicrobial effect against some bacterial and fungal strains. This work constitutes a contribution to a better valorization of medicinal species from *M. insulare* growing in Tunisia. Further, this study is encourages us to identify the main active compounds that are responsible for the observed biological effects of the essential oils.

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**Table 1**
Chemical composition of essential oils of fresh and dry fruits from *M. insulare* R. Br.

| No. | Compounds | HP−5[a] | HP-WAX[b] | Composition (%)[c] | | Identification |
|---|---|---|---|---|---|---|
| | | | | Fresh fruits | Dry fruits | |
| 1 | α-Pinene* | 941 | 1031 | 0.7 | 0.4 | GC–MS, RI |
| 2 | β-Pinene* | 982 | 1130 | 0.4 | -[d] | GC–MS, RI |
| 3 | 1-Octen−3-ol* | 983 | 1452 | 0.9 | – | GC–MS, RI |
| 4 | (*E*)- β-Ocimene | 1052 | 1250 | 4.7 | 0.1 | GC–MS, RI |
| 5 | Linalool* | 1101 | 1545 | 3.7 | 0.9 | GC–MS, RI |
| 6 | *Cis-p*-mentha-2,8-dien-1-ol | 1137 | 1666 | 1.4 | - | GC–MS, RI |
| 7 | (*E*)- Myroxide | 1144 | 1470 | 0.1 | - | GC–MS, RI |
| 8 | Silphiperfol-5-ene | 1328 | 1428 | 0.9 | - | GC–MS, RI |
| 9 | 7-*epi*-Silphiperfol-5-ene | 1346 | 1454 | **9.6** | - | GC–MS, RI |
| 10 | Silphiperfol-6-ene | 1377 | 1501 | 0.1 | - | GC–MS, RI |
| 11 | β-Elemene* | 1392 | 1585 | 0.1 | - | GC–MS, RI |
| 12 | *n*-Tetradecane* | 1400 | 1400 | - | 0.2 | GC–MS, RI |
| 13 | β-Caryophyllene* | 1419 | 1625 | 2.8 | 0.9 | GC–MS, RI |
| 14 | 2,5-Dimethoxy-*p*-cymene | 1424 | 1868 | 2.3 | - | GC–MS, RI |
| 15 | α-Humulene* | 1455 | 1635 | 0.9 | - | GC–MS, RI |
| 16 | 4-Methyltetradecane | 1456 | – | - | 0.2 | GC–MS, RI |
| 17 | γ-Muurolene | 1478 | 1676 | - | 0.2 | GC–MS, RI |
| 18 | γ-Irone | 1479 | 1834 | **14.4** | 2.3 | GC–MS, RI |
| 19 | Germacrene D | 1482 | 1724 | 2.5 | 0.6 | GC–MS, RI |
| 20 | Bicyclogermacrene | 1496 | 1735 | **13.9** | 3.2 | GC–MS, RI |
| 21 | α-Muurolene | 1499 | 1711 | 0.5 | 0.6 | GC–MS, RI |
| 22 | α-Bulnesene | 1507 | 1613 | 2.0 | 1.3 | GC–MS, RI |
| 23 | γ-Cadinene | 1514 | 1772 | 0.8 | 0.8 | GC–MS, RI |
| 24 | Cubebol | 1515 | 1947 | – | 0.6 | GC–MS, RI |
| 25 | δ-Cadinene | 1524 | 1756 | **6.8** | 2.8 | GC–MS, RI |
| 26 | Elemicin* | 2245 | 1556 | - | **20.1** | GC–MS, RI |
| 27 | Germacrene D-4-ol | 1584 | 2060 | 1.7 | - | GC–MS, RI |
| 28 | Spathulenol* | 1591 | 2144 | 3.1 | **16.8** | GC–MS, RI |
| 29 | Globulol* | 1600 | 2088 | 1.2 | 3.9 | GC–MS, RI |
| 30 | Viridiflorol* | 1603 | 2099 | 0.8 | 1.3 | GC–MS, RI |
| 31 | *n*-Hexadecane* | 1606 | 1600 | - | 1.5 | GC–MS, RI |
| 32 | 5-*epi*-7-*epi*- α-Eudesmol | 1607 | 2158 | – | 1.1 | GC–MS, RI |
| 33 | β-Cedren-9-α-ol | 1615 | 2113 | 0.9 | - | GC–MS, RI |
| 34 | β-Oplopenone | 1629 | 2092 | - | 2.0 | GC–MS, RI |
| 35 | 1,10-di-*epi*-Cubenol | 1641 | 2056 | - | 0.6 | GC–MS, RI |
| 36 | 1-*epi*-Cubenol | 1646 | 2072 | - | 2.0 | GC–MS, RI |
| 37 | T-Cadinol | 1652 | 2167 | **8.5** | **14.2** | GC–MS, RI |
| 38 | α-Muurolol | 1584 | – | - | 1.8 | GC–MS, RI |
| 39 | α-Cadinol | 1591 | 2237 | **13.2** | **16.8** | GC–MS, RI |
| Monoterpene hydrocarbons | | | | 5.8 | 0.5 | |
| Oxygenated monoterpenes | | | | 7.5 | 0.9 | |
| Sesquiterpene hydrocarbons | | | | 40.9 | 10.4 | |
| Oxygenated sesquiterpenes | | | | 29.4 | 61.1 | |
| Phenylpropanoids | | | | - | 20.1 | |
| Apocarotenes | | | | 14.4 | 2.3 | |
| Non-terpene derivatives | | | | 0.9 | 1.9 | |
| Total identified | | | | **98.9** | **97.2** | |
| Yield% (w/w) | | | | 0.025 | 0.04 | |

Note: Bold type indicates major component.
[a]LRI, linear retention indices on the HP-5 column.
[b]LRI, linear retention indices on the HP-WAX column
[c]Relative abundances calculated by GC-FID on non-polar capillary column HP5

**Table 2**
Antioxidant activities of the fresh and dry seeds essential oils of *M. insulare* R. Br.

| Sample | DPPH radical $IC_{50}$[a] (μg/mL) | $ABTS^{\cdot+}$ radical $IC_{50}$[a] (μg/mL) | Reducing power $IC_{50}$[a] (μg/mL) | Catalase (u/mg (protein)[c] | Praoxonase (IU/L)[d] |
|---|---|---|---|---|---|
| Fresh seeds | 92.0 ± 1.8 | 111 ± 2.3 | 321.0 ± 0.0 | 387.610 ± 0.413 | 33.00 ± 0.50 |
| Dry seeds | 54.0 ± 1.3 | 68 ± 2.0 | 194.0 ± 1.4 | 504.028 ± 0.655 | 77.51 ± 0.47 |
| BHT[b] | 18.0 ± 1.0 | 50 ± 1.0 | 20.0 ± 1.0 | – | – |
| Vitamine C | – | – | – | 757.575 ± 0.002 | 55.00 ± 0.20 |

Values were expressed as mean ± SE.
[a] $IC_{50}$ (μg/mL) the concentration at which 50% is inhibited.
[b] BHT butylatedhydroxytoluene.
[c] u/mg protein: μmol of $H_2O_2$ destroyed/min/mg protein.
[d] IU: μM/min.

**Table 3**
Antibacterial activities of the fresh and dry seeds essential oils of *M. insulare* R. Br.

| Sample | Inhibition zone diameter (mm) | | |
|---|---|---|---|
| | *Pseudomonas syringaepvsyringae* | *Pseudomonas savastanoi* | *Agrobacterium tumefasciens* |
| Fresh seeds | 25.0 ± 0.8 | 15.5 ± 0.6 | 18.0 ± 0.6 |
| Dry seeds | – | – | 17.0 ± 0.8 |
| Ampicilin | 19.0 ± 1.4 | 12.5 ± 0.6 | 17.5 ± 1.5 |
| Hexane | – | – | – |

- no inhibition zone observed.

**Table 4**
Antifungal activities of the fresh and dry seeds essential oils of *M. insulare* R. Br.

| Sample | Inhibition zone diameter (mm) | | |
|---|---|---|---|
| | *Aspergillus niger* | *Penicillium digitatum* | *Penicillium italicum* |
| Fresh seeds | 24.5 ± 0.3 | 21.0 ± 1.7 | 16.0 ± 0.8 |
| Dry seeds | – | 12.0 ± 1.0 | – |
| FONG | 30.0 ± 1.5 | 42.0 ± 1.7 | 40.0 ± 0.6 |
| Hexane | – | – | – |

- no inhibition zone observed.

**Table 5**
Anti-tyrosinase and cytotoxic (Hela and A549 cells lines) activities of fresh and dry fruits essential oils from *M. insulare* R. Br.

| Sample | % of inhibition | Cytotoxic activity[a] | |
|---|---|---|---|
| | | Hela | A549 |
| Fresh seeds | 55.0 ± 2.3 (cc = 100 µg/mL) | 98.87 ± 1.31 | 74.20 ± 1.60 |
| Dry seeds | 81.0 ± 1.9 (cc = 100 µg/mL) | 78.20 ± 1.75 | 68.50 ± 0.70 |
| Kojic acid | 85.2 ± 2.0 (cc = 50 µg/mL) | | |
| Doxorubicin | | 0.36 ± 0.03[b] | |
| Ellipticine | | | 0.31 ± 0.04[b] |

[a] $IC_{50}$ (µg/mL).
[b] $IC_{50}$ is expressed in µg/mL.