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Cytotoxic and antiviral activities of the essential oils from Tunisian Fern, *Osmunda regalis*

S. Bouazzi [a], H. Jmii [b], R. El Mokni [c,d], K. Faidi [a], D. Falconieri [e,f], A. Piras [f], H. Jaïdane [b], S. Porcedda [f], S. Hammami [a,*]

[a] Research Unit of Applied Chemistry and Environment (UR13ES63), University of Monastir, Faculty of Sciences of Monastir, Tunisia
[b] Laboratory of Transmissible Diseases LR99ES27, Faculty of Pharmacy, University of Monastir, Avenue Avicenne, 5000 Monastir, Tunisia
[c] Laboratory of Botany and Plant Ecology, Faculty of Sciences of Bizerta, University of Carthage, Jarzouna, 7021 Bizerta, Tunisia
[d] Laboratory of Plant Biology, Faculty of Pharmacy, University of Monastir, BP. 207, Avenue Avicenne, Monastir 5000, Tunisia
[e] Industrial Technical Institute "Michele Giua", Via Montecassino, 09134 Cagliari, Italy
[f] Department of Chemical and Geological Science, University of Cagliari, Cittadella Universitaria di Monserrato, S.P. Monserrato-Sestu km 0,700, 09042 Monserrato (CA), Italy

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ABSTRACT

This study was undertaken to assess the *in vitro* cytotoxic and antiviral activities of the essential oil (EO) from Tunisian fern, *Osmunda regalis*. The essential oil was obtained by hydrodistillation and its chemical composition was determined by gas chromatography and mass spectrometry (GC-FID and GC–MS) analyses that allowed detecting 85.35% of the components. The main compounds were hexahydrofarnesyl acetone (11.82%), 2,4-di-t-butylphenol (6.80%), and phytol (6.46%). Cytotoxicity of the essential oil was assessed on HEp-2 cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. Antiviral activity was also evaluated *in vitro* against Coxsackievirus B4 (CV-B4), an enterovirus implicated in a variety of diseases such as myocarditis, type 1 diabetes and central nervous system diseases, by measuring cell viability following viral infection (using MTT) and appreciating the reduction of cytopathic effect (CPE). Hence, the 50% cytotoxic concentration (CC_{50}), 50% inhibitory concentration (IC_{50}) and selectivity index (SI) were determined. The essential oil turned out to be non-toxic against the tested cell line (CC_{50} = 1772.41 ± 0.95) μg/mL, have a relevant anti-Coxsckievirus B4 activity (IC_{50} = 2.24 ± 0.99) μg/mL and a high SI (789.66). Results presented here suggest that *O. regalis* EO is a potentially promising new source as active antiviral agent.

1. Introduction

For thousands of years, humanity has used various plants to treat many devastating diseases and to relieve different sorts of suffering (Sadat-Hosseini et al., 2017). Essential oils, the secondary metabolites synthesized by medicinal and aromatic plants, have acquired a great renewed interest as a potential source of bioactive lead compounds for drug discovery. They are being studied for their possible use as an alternative for protection against cancer (Loizzo et al., 2007; Driss et al., 2016). A good example is that of the essential oils of Chinese propolis which have been cited to inhibit the proliferation of human colorectal cancer cells (Sena-Lopes et al., 2018). *Thymus vulgaris* essential oil inhibits human head and neck squamous cell carcinoma growth (Sertel et al., 2011). Moreover, the efficacy of many essential oils and their volatile constituents against a wide range of bacterial pathogens and viruses has been well documented and the stunning efficacy of almost of them against herpes lesions has been also demonstrated (Loizzo et al., 2008; Astani et al., 2010). In addition, many essential oils have been reported to possess a great antioxidant potential (Urbizu-González et al., 2017). *Osmunda regalis* L. known as royal fern (Magrini and Scoppola, 2012) is a member of the Osmundaceae family which is the most primitive fern comprising the genera: *Osmunda*, *Todea* and *Leptopteris*, with about 21 species (Moore et al., 2009). *O. regalis* is a cosmopolitan species, it is widely distributed throughout Southern Africa, America, Asia, New Zealand and Northern and Eastern Europe (Tian et al., 2008). In addition, *O. regalis* grows spontaneously in Tunisia, in humid slopes near water as a perennial plant with raised stems (20–35) cm. Previous phytochemical investigations of the surface lipids from the German *Osmunda regalis* fern, showed the presence of free fatty acids, such as linoleic and oleic acids (Gemmrich, 1977), alkanediols, ketoaldehydes and fatty acid esters (Jetter and Riederer, 1999).

As a medicinal plant, *O. regalis* is endowed of a great source of active ingredients useful to treat some diseases and could be considered of a highly efficient remedy. Moreover, it has been used in folk medicine for the treatment of some joint disorders, bone fractures, rheumatic and arthritic, arthrosis or back pain. *O. regalis* is antojil wine, which has been traditionally employed for muscle-skeletal disorders,

* Corresponding author.
E-mail address: h_saoussen@yahoo.fr (S. Hammami).

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traumatic injuries such as bruises, dislocations, or sprains. It is also used as tonic, against rickets, digestive and respiratory disorders (Molina et al., 2009). *O. regalis* has also been used in reproductive health of tribal women; so, it is an abortifacient. Leaves are mixed with thin cured for the birth control (Singh and Singh, 2012). This plant is cultivated as an ornament, its fibers are exploited for orchid-growing and the hairs of young leaves are used for textile production (Jetter and Riederer, 1999). On the basis of its potential pharmacological benefits, we report herein, the chemical composition of *O. regalis* essential oil, its toxicity towards HEp-2 cells and its effect on Coxsackie B viruses which is positive sense single-stranded RNA virus belonging to enterovirus genus and picornaviridae family and Coxsackie B viruses are associated with a variety of diseases including myocarditis (Huber, 2006), diabetes (Jaïdane and Hober, 2008) and central nervous system pathologies especially among new-born and infants (Michos et al., 2007; Kumar et al., 2012).

2. Materials and methods

2.1. Plant material and extraction of the essential oil

Aerial parts of *Osmunda regalis* L. was collected during mature stage from Northwest of Tunisia on June 2011. The identification of the plant material was performed by one of the authors (R.E.M). Some voucher specimens [PTER-OSM/01-Osm.r; 00017/2011] were deposited both in the local Herbaria of the Faculty of Pharmacy of Monastir and in the Faculty of Sciences of Bizerta, Jarzouna, Tunisia (Fig. 1). Collected material was air-dried at room temperature till totally dehydration. Then, a fine powder was obtained with a mean particle size of 1 mm. *O. regalis* essential oil was obtained by hydrodistillation in a Clevenger-type apparatus according to the procedure given by Okoh et al. (Okoh et al., 2011). The essential oil yields were calculated on a dry-weight basis (w/w).

2.2. GC-FID GC–MS analyses

GC analyses of the extracts were performed using a gas chromatograph (Agilent 7890A, Palo Alto, CA, USA), equipped with a 30 m × 0.25 mm i.d. with 0.25 μm stationary film thickness DB-5 capillary column (Agilent J&W) and a flame ionization detector (FID). The following temperature program was used: from 60 °C to 246 °C at rate of 3 °C min^{-1} and then held at 246 °C for 20 min (total analysis time 82 min). Other operating conditions are the following: carrier gas helium (purity ≥99.9999% – Air Liquide Italy); flow rate, 1.0 mL min^{-1}; injector temperature, 250 °C; detector temperature, 300 °C. Injection of 1 μL of diluted sample (1:100 in hexane, w/w) was performed with 1:10 split ratio, using an autosampler (Agilent, Model 7683B). GC–MS analyses were carried out using a gas chromatograph (Agilent 6890 N) equipped with a 30 m × 0.25 mm i.d. with 0.25 μm stationary film thickness HP-5 ms capillary column (Agilent J&W) coupled with a mass selective detector having an electron ionization device, EI, and a quadrupole analyzer (Agilent 5973). The temperature program was the same used for GC. Other chromatographic operating conditions are the following: carrier gas helium (purity ≥99.9999%); flow rate 1.0 mL min^{-1}; injector temperature, 250 °C. Injection of 1 μL of diluted sample (1:100 in hexane, w/w) was performed with 1:20 split ratio, using an autosampler (Agilent, Model 7683B). The MS conditions were as follows: MS transfer line temperature, 240 °C; EI ion source temperature, 200 °C with ionization energy of 70 eV; quadrupole temperature, 150 °C; scan rate, 3.2 scan s^{-1} at *m/z* scan range: (30 to 480). To handle and process chromatograms and mass spectra was used the software MSD ChemStation (Agilent, rev. E.01.00.237).

Constituents of the samples were identified by comparing: mass spectra fragmentation patterns with those of a computer library (Adams, 2007; Stein et al., 2008) and linear retention indices (RI) based on a homologous series of C8-C26 n-alkanes with those reported in literature (Stein et al., 2008). The Table 1 shows the chromatographic results, expressed as GC peak area percentages.

Fig. 1. *Osmunda regalis* L.

2.3. Antiviral and cytotoxicity assays

2.3.1. Cell culture and virus preparation

HEp-2 cell line (Human epithelial cells) were used to propagate and titrate Coxsackie virus B4 (CV-B4), kindly provided by Prof. J. W. Yoon, Julia M.C. Farlane, Diabetes research center, Calgary, Alberta, Canada,

Table 1
Chemical compositions of *Osmunda regalis* L. essential oil (%).

Number	Compound	RI[a]	%RA[b]	Identification[c]
1	Camphor	1141	3.30	RI,MS
2	1-Dodecene	1187	0.71	RI,MS
3	Dodecane	1200	0.42	RI,MS
4	1-Tetradecene	1392	2.22	RI,MS
5	Tetradecane	1400	0.81	RI,MS
6	(E)-α-Ionone	1428	0.38	RI,MS
7	2,4-di-t-Butylphenol	1512	6.80	RI,MS
8	Dihydroactinidiolide	1525	0.86	RI,MS
9	1-Hexadecene	1588	4.12	RI,MS
10	Hexadecane	1600	0.97	RI,MS
11	Benzophenone	1626	0.52	RI,MS
12	1-Octadecene	1792	4.42	RI,MS
13	Octadecane	1800	1.37	RI,MS
14	Neophytadiene	1843	4.64	RI,MS
15	**Hexahydrofarnesyl acetone**	**1844**	**11.82**	**RI,MS**
16	Neophyltadiene isomer 1	1862	1.26	RI,MS
17	Neophytadiene isomer 2	1881	2.11	RI,MS
18	Nonadecane	1900	0.43	RI,MS
19	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	1929	1.54	RI,MS
20	Isophytol	1949	0.84	RI,MS
21	1-Eicosene	1992	4.38	RI,MS
22	Eicosane	2000	0.50	RI,MS
23	γ-Palmitolactone	2105	1.74	RI,MS
24	Phytol	2111	6.46	RI,MS
25	Nonacosanal	2118	2.31	RI,MS
26	1-Docosene	2189	2.81	RI,MS
27	Docosane	2200	0.73	RI,MS
28	Tricosane	2300	2.33	RI,MS
29	1-Tetracosene	2396	1.89	RI,MS
30	Tetracosane	2400	0.87	RI,MS
31	Pentacosane	2500	1.97	RI,MS
32	1-Hexacosene	2593	0.97	RI,MS
33	Tretracosanal	2631	2.53	RI,MS
34	1-Heptacosene	2693	1.61	RI,MS
35	Heptacosane	2700	1.27	RI,MS
36	1-Hexacosanol	2798	0.81	RI,MS
37	Squalene	2823	1.11	RI,MS
38	Hexacosanal	2830	1.52	RI,MS
	Identified components (%)		85.35	
	Unidentified components (%)		14.65	

[a] Retention index relative to n-alkanes on DB-5 capillary column.
[b] Relative area (peak area relative to the total peak area).
[c] Identification: MS, comparison of mass spectra with MS libraries.