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Ridolfia segetum (L.) Moris (Apiaceae) from Portugal: A source of safe antioxidant and anti-inflammatory essential oil

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ABSTRACT

Ridolfia segetum (L.) Moris essential oil was obtained by hydrodistillation and analysed by GC and GC–MS. The antioxidant properties were evaluated through the TBARS assay, and the *in vitro* anti-inflammatory activity was assessed through inhibition of NO production triggered by the *Toll*-like receptor 4 (TLR4) agonist, lipopolysaccharide (LPS), in mouse macrophages. Assessment of cell viability was made through a colorimetric assay using MTT on macrophages, keratinocytes, and hepatocytes.

The main compounds are α-phellandrene (53.0–63.3%), terpinolene (11.9–8.6%), ß-phellandrene (5.5–6.0%), and dillapiol (1.9–8.0%). The oil demonstrated high antioxidant capacity in the concentrations tested (0.05–0.20 μL/mL) and significantly inhibited NO production without cytotoxicity in concentrations up to 1.25 μL/mL.

The plants showed a high essential oil yield, which confers an industrial potential interest. Also, the high content of α-phellandrene (53.0–63.3%) makes it an important natural source of this compound.

These results provide evidence of the therapeutic effects of this oil, and suggest that the anti-inflammatory activity in doses without cytotoxicity on mammalian cells, combined with its antioxidant potential and pleasant smell, could be of great value in the development of new phytopharmaceuticals.

1. Introduction

Aromatic plants are used for their large therapeutic potential and benefits as natural preservatives (Bakkali et al., 2008) and since antiquity, essential oils have been traditionally used for their biological properties: antioxidant, bactericidal, fungicidal, virucidal, antiparasitical, insecticidal, anti-inflammatory (Timsina et al., 2012), being the antioxidant and anti-inflammatory activities of interest in the food, cosmetic and pharmaceutical industries (Miguel, 2010a,b,b). The biological properties of the essential oils seem to be the result of a synergism between these compounds.

The antioxidant activity of essential oils is of great interest because they may be useful to preserve foods from the toxic effects of oxidants (Maestri et al., 2006). Moreover, essential oils being able of scavenging free radicals may also play an important role in some disease prevention such as brain dysfunction, cancer, heart disease, and immune system decline. Increasing evidence has suggested that these diseases may result from cellular damage caused by free radicals (Aruoma, 1998; Kamatou and Viljoen, 2010). Indeed, the formation of reactive oxygen species (ROS) induce lipid peroxidation that, in some circumstances, can overcome the cellular defense system. The membrane structures are destroyed and the function of the cell organelles is loosed with the lipid oxidation. Induction of lipid peroxidation is linked to several diseases: rheumatoid arthritis, atherosclerosis, ischemia, carcinogenesis, and aging (Jadhav et al., 1995). If essential oils are able to scavenge some free radicals, they can also act as anti-inflammatory agents, because one of the triggers of the inflammatory responses is the oxidative burst that occurs in diverse cells (monocytes, neutrophils, eosinophils, and macrophages).

Macrophages produce a variety of pro-inflammatory mediators, such as nitric oxide, upon activation with the pro-inflammatory stimulus and TLR4 agonist LPS. Since NO is a well-established inflammatory marker, the inhibition of its production is a good approach to screen anti-inflammatory molecules or phytochemicals.

Plants of the Apiaceae family (carrot or parsley family) possess a range of compounds with many biological activities, such as the ability to induce apoptosis, the antimicrobial, hepatoprotective, and vaso-relaxant activities, cyclooxygenase inhibitory effect

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and anti-tumor action (Pae et al., 2002). This family is very well represented in the Portuguese flora and includes well-known aromatic and medicinal plants with characteristic hollow stems.

Ridolfia segetum (L.) Moris belongs to the Apiaceae, and it is the only species of the monotypic genus *Ridolfia* Moris. This species is commonly known as *corn parsley*, *false fennel*, or *false caraway*, and it is distributed throughout the Mediterranean region. In Portugal, it is commonly called *andrage* or *endrão*, and it occurs in roadsides and cultivated fields (Aedo, 2003). *Ridolfia segetum* is used in traditional medicine to prevent constipation, coughing, and respiratory tract infections. In a recent ethnobotanical survey in Sicily, this species was indicated as being eaten raw in salads, cooked and as medicinal-food in cases of gastric acidity (Lentini and Venza, 2007).

Although *R. segetum* is used as food and in traditional medicine, there is a lack of information regarding the validation of its uses through evaluation of biological activities and proofs of its safety.

The essential oil of this species has been the subject of previous studies of chemical characterization, being possible the distinction of two types of oils: those largely dominated by monoterpene hydrocarbons, usually α-phellandrene, terpinolene, and *p*-cymene (Bicchi et al., 2009; Fleisher and Fleisher, 1996; Marongiu et al., 2007; Palá-Paúl et al., 2002, 2005); and those which also contain phenylpropanoids as major compounds or in appreciable amounts, usually myristicin and dillapiol (Jannet and Mighri, 2007; Jabrane et al., 2010).

Concerning the evaluation of biological activities, Jabrane et al., 2010, evaluated the antioxidant and antibacterial activities. In 2007, Jannet and Mighri evaluated the antibacterial properties of the essential oil of Tunisian *R. segetum*. Latter, in 2009, Bicchi et al. reported the HIV-1-inhibiting activity of *R. segetum* from Sardinia, Italy.

Bearing in mind the traditional medicinal uses of *R. segetum*, the present study was designed to elucidate the antioxidant activity of its essential oil, as well as, the anti-inflammatory potential and the safety on three types of mammalian cells, macrophages, keratinocytes, and hepatocytes.

2. Materials and methods

2.1. Plant material and reagents

Aerial parts of *R. segetum* were collected during flowering stage in May 2012 (RS_Fl) and fruiting stage in July 2012 (RS_Fr), in Rabaçal (Central Portugal). Voucher specimens were identified by a plant taxonomist and deposited, under the numbers C. Cabral 12012 and C. Cabral 24012, in the Herbarium of Medicinal Plants, Faculty of Pharmacy, University of Coimbra.

Thiobarbituric acid (TBA), 2,2-azobis-(2-amidinopropane) dihydrochloride (ABAP), sodium dodecyl sulphate (SDS), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were purchaded to Sigma–Aldrich®. α-Phellandrene (>90%) was purchased to the Brithish Drug Houses, Lda.

2.2. Essential oil isolation

Essential oil was isolated by hydrodistillation for 3 h using a Clevenger-type apparatus, according to the procedure described in the European Pharmacopoeia (Council of Europe, 1997).

2.3. Gas chromatography (GC)

Analytical GC was carried out in a Hewlett-Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) chromatograph with a HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and two flame ionization detectors (FID). A graphpak divider (Agilent Technologies, part no. 5021-7148) was used for simultaneous sampling to two Supelco (Supelco, Bellefonte, PA, USA) fused silica capillary columns with different stationary phases: SPB-1 (polydimethylsiloxane 30 m × 0.20 mm, film thickness 0.20 μm), and SupelcoWax-10 (polyethyleneglycol 30 m × 0.20 mm, film thickness 0.20 μm). Oven temperature program: 70–220 °C (3 °C/min), 220 °C (15 min); injector temperature: 250 °C; carrier gas: helium, adjusted to a linear velocity of 30 cm s^{-1}; splitting ratio 1:40; detectors temperature: 250 °C.

2.4. Gas chromatography–mass spectrometry (GC–MS)

GC–MS was carried out in a Hewlett-Packard 6890 gas chromatograph fitted with a HP1 fused silica column (polydimethylsiloxane 30 m × 0.25 mm, film thickness 0.25 μm), interfaced with an Hewlett-Packard mass selective detector 5973 (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters as described above; interface temperature: 250 °C; MS source temperature: 230 °C; MS quadrupole temperature: 150 °C; ionization energy: 70 eV; ionization current: 60 μA; scan range: 35–350 units; scans per second: 4.51.

2.5. Qualitative and quantitative analyses

Components of the volatile oil were identified by their retention indices on both SPB-1 and SupelcoWax-10 columns, calculated by linear interpolation relative to retention times of C_8–C_{24} of *n*-alkanes and compared with those of reference compounds included in CEF laboratory database or literature data (Adams, 2004), and by their mass spectra by matching with reference spectra from the CEF laboratory own spectral database, Wiley/NIST database or literature data (Joulain and Konig, 1998; Adams, 2004; Wiley Registry, 2006). Relative amounts of individual components were calculated based on GC raw data areas without FID response factor correction.

2.6. Antioxidant activity evaluation

2.6.1. TBARS

Two sets of experiments were based on a modified thiobarbituric acid (TBA) reactive substances assay (TBARS) to measure the antioxidant ability of the samples, without (1) and with (2) a lipid peroxidation inducer.

(1) Egg yolk homogenate was used as the lipid-rich medium, obtained as described by Dorman et al. (1995), i.e., an aliquot of yolk material was made up to a concentration of 10% w/v in KCl (1.15% w/v). The yolk was then homogenized for 30 s followed by ultrasonication for further 5 min. Homogenate (500 μL) and 100 μL of sample or positive controls of BHA and BHT (in the concentrations 0.05, 0.1, 0.15, and 0.20 μg/mL), dissolved in methanol, were added to a test tube and made up to 1 mL with distilled water, followed by the addition of 1.5 mL 20% acetic acid (pH 3.5) and 1.5 mL 0.8% w/v TBA in 1.1% w/v sodium dodecyl sulphate (SDS). This mixture was stirred in a vortex and heated at 95 °C for 60 min. After cooling to room temperature (20 min in water), 5 mL of *n*-butanol was added to each tube, stirred and centrifuged at 4500 rpm for 15 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Cintra 101 GBC) in the software Cintral General Applications.

For set (2), 50 μL of 2,2-azobis-(2-amidinopropane) dihydrochloride (ABAP) (0.07 M) were added to induce lipid peroxidation, soon after the addition of sample, the remaining procedure being as reported above.

The percentage of inhibition was calculated using the following equation: % inhibition = $[(A_C - A_S)/A_C] \times 100$.where A_C is the absorbance of the control (without sample), and A_S is the

absorbance in the presence of the sample. Tests were carried out in triplicate.

2.7. Anti-inflammatory evaluation

2.7.1. Cell culture and materials

Raw 264.7 (ATCC number: TIB-71), a mouse macrophage cell line kindly supplied by Dr Otília Vieira (Centro de Neurociências e Biologia Celular, Universidade de Coimbra, Coimbra, Portugal), was cultured in Iscove's Modified Dulbecco's Eagle Medium supplemented with 10% non-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2.7.2. Nitric oxide (NO) measurement

The anti-inflammatory activity of the oil from samples RS_Fl and RS_Fr was evaluated in the mouse macrophage cell line Raw 264.7.

The production of NO was measured by the accumulation of nitrite in the culture supernatants, using a colorimetric reaction with the Griess reagent (Green et al., 1982). The cells were plated at 0.6×10^6 cells/well in 48-well culture plates, allowed to stabilize for 12 h, and then incubated with culture medium (control), or stimulated with 1 μg/mL LPS, or with 1 μg/mL LPS in the presence of different concentrations of the essential oil during 24 h. Briefly, 170 μL of culture supernatants were collected and diluted with equal volume of the Griess reagent [0.1% (w/v) *N*-(1-naphthyl) ethylenediamine dihydrochloride and 1% (w/v) sulphanilamide containing 5% (w/v) H_3PO_4] during 30 min, in the dark. The absorbance at 550 nm was measured using an automatic plate reader (SLT, Austria). Nitrite concentration was determined using a sodium nitrite standard curve.

2.8. Evaluation of cytotoxicity

2.8.1. Cell culture and materials

The human keratinocyte cell line HaCaT, obtained from DKFZ (Heidelberg), was kindly supplied by Dr Eugénia Carvalho (Centro de Neurociências e Biologia Celular, Universidade de Coimbra, Coimbra, Portugal). Keratinocytes were cultured in Dulbecco's Modified Eagle Medium (high glucose) supplemented with 4 mM glutamine, 10% heat inactivated fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

The human hepatocyte cell line HepG2 (ATCC number: 77400), kindly supplied by Professor Conceição Pedroso Lima, was cultured in Dulbecco's Modified Eagle Medium (low glucose) supplemented with 10% heat inactivated fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Along the experiments, cells were monitored by microscope observation in order to detect any morphological change.

2.8.2. MTT assay for cell viability

Cell viability was assessed for the oil of samples RS_Fl and RS_Fr using the mouse macrophage cell line Raw 264.7, the human keratinocyte cell line HaCaT and the cell line Hep G2.

Assessment of cell viability was made through a colorimetric assay, using MTT (Mosmann, 1983). HaCaT and HepG2 cells (0.2×10^6 cells/well, cultured in 48-well microplates) were incubated in a final volume of 600 μL, allowed to stabilize for 12 h, and then incubated for 24 h with different concentrations of the essential oil. After adding 60 μL of MTT solution (5 mg/mL in PBS) to each well, the cells were further incubated at 37 °C for 15 and 60 min for HaCaT cells and Hep G2 cells, respectively, in a humidified atmosphere of 95% air/5% CO_2. Supernatants were then discarded and 300 μL of acidified isopropanol (0.04N HCl in isopropanol) were added to the cultures and mixed thoroughly to dissolve the dark blue crystals of formazan. Formazan quantification was performed using an ELISA automatic microplate reader (SLT, Austria) at 570 nm, with a reference wavelength of 620 nm.

Concerning the cell line Raw 264.7, and after collection of 170 μL of culture supernatants for NO measurement, 43 μL of MTT solution (5 mg/mL in PBS) was added and cells were further incubated at 37 °C for 15 min, in a humidified atmosphere of 95% air and 5% CO_2. Supernatants were then discarded and 300 μL of acidified isopropanol (0.04N HCl in isopropanol) were added to the cultures and mixed thoroughly to dissolve the dark blue crystals of formazan. Formazan quantification was performed as described above.

2.9. Data analysis

All the experiments were performed in duplicate, being the results expressed as mean ± SEM of three independent experiments. The means were statistically compared using two-way ANOVA, with a Dunnett's multiple comparison test. The differences between the means were considered significant for values of $p < 0.001$. The statistical tests were applied using GraphPad Prism, version 6.01 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Essential oil composition

The essential oil was obtained with yields of 1.6% in flowering sample (RS_Fl) and 1.9% in fruiting sample (RS_Fr). The qualitative and quantitative composition of the oil is presented in Table 1, where the compounds are listed by order of their elution on a polydimethylsiloxane column.

In total, 26 compounds were identified accounting for 96.0–98.7%. The oil did not show a significant difference in composition between flowering and fruiting stages, with the monoterpene hydrocarbons representing the major percentage (95.2 and 85.0%, respectively), with some variations in the amounts of the main compounds: α-phellandrene (63.3 *vs* 53.0%), terpinolene (11.9 *vs* 8.6), ß-phellandrene (6.0 *vs* 5.5), and dillapiol (1.9 *vs* 8.0%).

3.2. Antioxidant activity

The antioxidant activity was evaluated through the thiobarbituric acid reactive substances (TBARS) assay. Different concentrations of the essential oil and one of its main compounds (α-phellandrene), BHA and BHT (two standard antioxidants) were tested in the absence and in the presence of the radical inducer 2,2′-azobis-(2-amidinopropane) dihydrochloride (ABAP). The results shown in Table 2 demonstrate that both essential oil samples possess a strong antioxidant activity, possibly due in part to the action of α-phellandrene.

3.3. Effect of the essential oil on nitric oxide production induced by LPS

The effect of essential oil on NO production triggered by LPS was evaluated in the mouse macrophage cell line Raw 264.7. After macrophages stimulation with LPS in the presence of the oil, nitric oxide production induced by LPS was significantly reduced with the four concentrations tested (Fig. 1). For α-phellandrene (α-phell), only the higher concentration tested (1.25 μL/mL), reduced significantly the nitric oxide production, thus, demonstrating that the anti-inflammatory action of the essential oil is not only due to the action of α-phellandrene (Fig. 1).

Table 1
Chemical composition of *R. segetum* essential oils from Portugal (RS_Fl - flowering stage; RS_Fr–fruiting stage).

RI[a]	RI[b]	Compound[c]	Percentage in samples %	
			RS_Fl	RS_Fr
921	1026	α-Thujene	*t*	0.4
928	1026	α-Pinene	2.5	2.6
944	1073	Camphene	*t*	*t*
962	1125	Sabinene	0.3	0.3
967	1115	β-Pinene	1.5	2.5
980	1159	Myrcene	1	1
998	1171	α-Phellandrene	63.3	53
1009	1188	α-Terpinene	*t*	0.3
1012	1272	*p*-Cymene	0.5	1.5
1020	1213	β-Phellandrene	6	5.5
1020	1204	Limonene	3.5	2.8
1024	1232	*Z*-β-Ocimene	4	6
1035	1250	*E*-β-Ocimene	0.5	0.4
1046	1246	γ-Terpinene	0.1	0.1
1076	1284	Terpinolene	11.9	8.6
1105	1555	*Z*-*p*-Menth-2-ene-1-ol	*t*	*t*
1111	1378	allo-Ocimene	*t*	0.2
1160	1595	Terpinene-4-ol	*t*	0.1
1183	n.d.	*trans*-Piperitenol	*t*	*t*
1230	1719	Piperitone	*t*	*t*
1267	1573	Bornyl acetate	*t*	*t*
1328	1948	Piperitenone oxyde	1.1	2.3
1408	1595	E-Caryophyllene	0.1	*t*
1442	1662	α-Humulene	0.1	0.1
1466	1701	Germacrene D	0.1	*t*
1586	2348	Dillapiol	1.9	8
Monoterpene hydrocarbons			95.2	85.2
Oxygen containing monoterpenes			1.3	2.6
Sesquiterpene hydrocarbons			0.3	0.2
Phenylpropanoids			1.9	8
Total identified			98.7	96

t = traces (≤0.05%).
[a] Retention indices on the SPB-1 column relative to C_8– C_{24} *n*-alkanes.
[b] Retention indices on the SupelcoWax-10 column relative to C_8–C_{24} *n*-alkanes.
[c] Compounds listed in order to their elution on the SPB-1 column.

3.4. Evaluation of cell viability

3.4.1. Effect of the essential oil on macrophages viability

To evaluate the possible cytotoxic activity of the oil in different mammalian cell types, the MTT assay was used in macrophages, keratinocytes, and hepatocytes. Twenty four hours of LPS exposure had no significant effect on macrophages viability. As shown in Fig. 2, the oil did not show significant cytotoxicity in macrophages compared with control. However, α-phellandrene, the main compound of the EO, demonstrats a slight cytotoxicity.

3.4.2. Effect of the essential oil on keratinocytes viability

As shown in Fig. 3, the oil did not show significant cytotoxicity in keratinocytes compared with control. α-Phellandrene demonstrateds a slight cytotoxicity. A cell-free control was performed in order to exclude non-specific effects of the oil on MTT (data not shown).

3.4.3. Effect of the essential oil on hepatocytes viability

As shown in Fig. 4, the oil and α-phellandrene did not show significant cytotoxicity in hepatocytes compared with control.

Table 2
Antioxidant index (%) of the essential oils, BHA, BHT, and α-phellandrene, in different concentrations using TBARS assay in the absence or presence of ABAP.

	Standard/EO	Concentration, mean ± SEM (μg/mL)			
		0.2	0.15	0.1	0.05
Absence of ABAP	BHA	64.10 ± 1.27	60.57 ± 1.11	58.03 ± 1.21	52.81 ± 0.38
	BHT	95.35 ± 0.43	93.20 ± 0.51	89.62 ± 0.51	84.65 ± 0.92
	RS_Fl	78.41 ± 1.81	74.9 ± 1.90	71.54 ± 1.28	65.05 ± 1.16
	RS_Fr	72.11 ± 1.51	69.13 ± 0.64	62.57 ± 3.46	51.84 ± 3.08
	α-phel	75.78 ± 1.08	70.94 ± 1.36	66.97 ± 1.52	57.70 ± 0.67
Presence of ABAP	BHA	89.94 ± 0.96	88.56 ± 1.27	86.25 ± 1.57	79.94 ± 0.80
	BHT	71.77 ± 0.79	68.34 ± 0.50	66.14 ± 0.64	63.99 ± 0.87
	RS_Fl	77.37 ± 1.34	75.14 ± 0.74	71.60 ± 0.80	64.68 ± 1.61
	RS_Fr	79.23 ± 0.74	77.40 ± 0.76	73.43 ± 0.58	64.91 ± 1.14
	α-Phel	70.39 ± 0.89	68.51 ± 0.66	62.43 ± 1.16	50.11 ± 0.88

SEM (standard error of the mean).

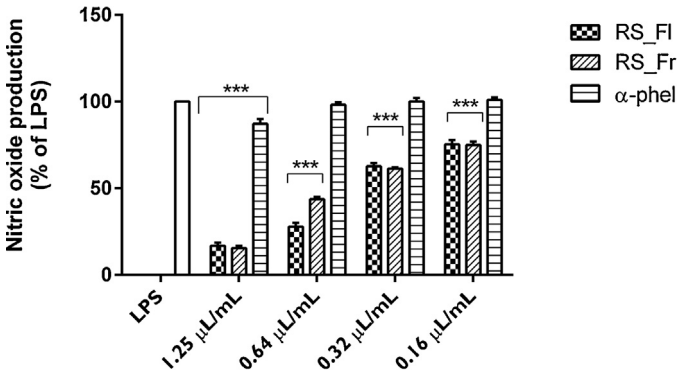

Fig. 1. Effect of essential oil of *Ridolfia segetum* (RS_Fl and RS_Fr) and α-phellandrene on NO production in macrophages. Results are expressed as a percentage of nitrite production by cells cultured in the presence of LPS. Each value represents the mean ± SEM from three experiments, performed in duplicate (***$p < 0.001$, compared to LPS).

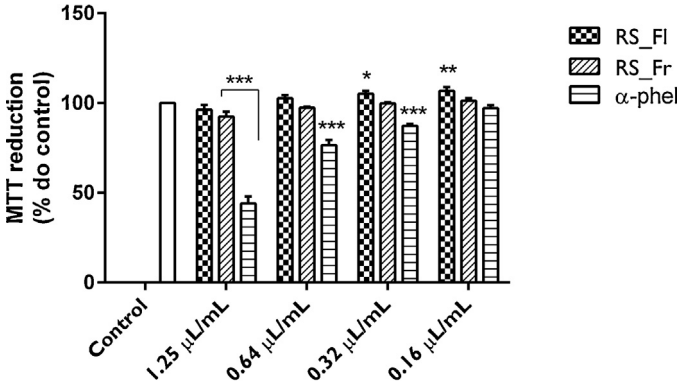

Fig. 2. Effect of essential oil of *R. segetum* (RS_Fl and RS_Fr) and α-phellandrene on macrophages viability (MTT assay). Results are expressed as a percentage of MTT reduction by control cells maintained in culture medium. Each value represents the mean ± SEM from three experiments, performed in duplicate (***$p < 0.001$, compared to control).

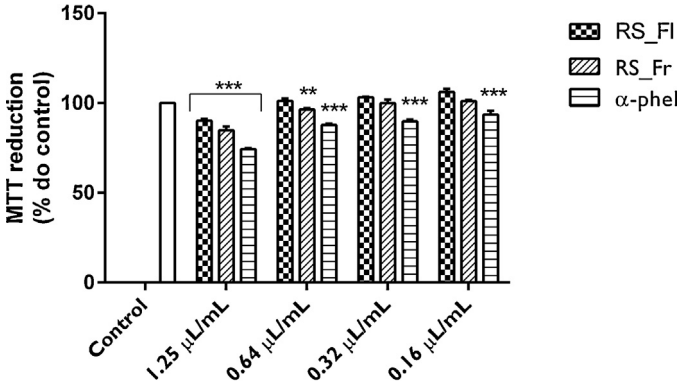

Fig. 3. Effect of essential oil of *R. segetum* (RS_Fl and RS_Fr) and α-phellandrene on keratinocytes viability (MTT assay). Results are expressed as a percentage of MTT reduction by control cells maintained in culture medium. Each value represents the mean ± SEM from three experiments, performed in duplicate (***$p < 0.001$, compared to control).

A cell-free control was performed in order to exclude non-specific effects of the oil on MTT (data not shown).

4. Discussion and conclusions

The essential oil of *Ridolfia segetum* from Portugal, in flowering and fruiting stage, was obtained with very high yields

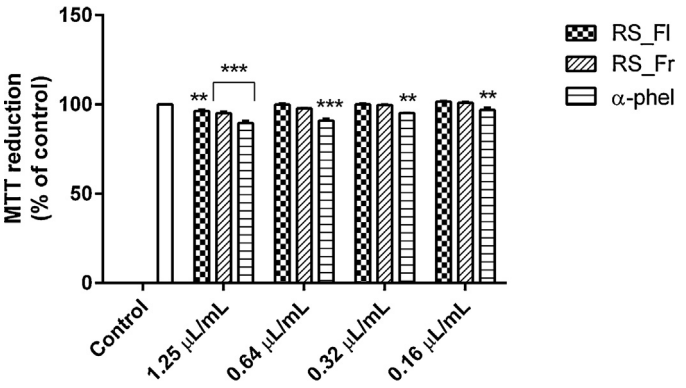

Fig. 4. Effect of essential oil of *R. segetum* (RS_Fl and RS_Fr) and α-phellandrene on hepatocytes viability (MTT assay). Results are expressed as a percentage of MTT reduction by control cells maintained in culture medium. Each value represents the mean ± SEM from three experiments, performed in duplicate (***$p < 0.001$, compared to control).

(1.6–1.9%) and with high percentage of monoterpene hydrocarbons, especially α-phellandrene (53.0–63.3%). These results are in accordance with the type of essential oil described by various authors (Bicchi et al., 2009; Fleisher and Fleisher, 1996; Marongiu et al., 2007), that is dominated by monoterpene hydrocarbons, usually α-phellandrene, terpinolene and *p*-cymene. Concerning the antioxidant properties of the essential oil, the results presented here demonstrate that both essential oil samples and α-phellandrene have significant antioxidant activity, even higher than BHA in the absence of the peroxidation inducer (ABAP), and higher than BHT in the presence of the peroxidation inducer.

The different behavior of the synthetic antioxidants in the absence/presence of ABAP suggests that BHA is able to operate as good antioxidant when the levels of peroxyl radicals are relatively high, whereas BHT does not seem to possess such ability (Miguel et al., 2004). Usually, the essential oil with high content of phenylpropanoids or monoterpene phenols (thymol

and carvacrol) possess better antioxidant activity (Dandlen et al., 2010). However, *R. segetum* essential oil, in which the main compound is α-phellandrene, a monoterpene hydrocarbon, showed a very high antioxidant activity, thus highlighting that the presence of phenolic compounds in the essential oils is not mandatory for the antioxidant activity, as already suggested by other authors (Miguel et al., 2004; Viuda-Martos et al., 2010).

The oil significantly inhibited NO production elicited by LPS in macrophages which demonstrates its strong anti-inflammatory potential. However, α-phellandrene, that revealed an antioxidant activity similar to that of the oil tested, showed a quite low inhibition of NO production, which suggests that the essential oil of *R. segetum* is more active than its main compound. In fact, essential oils are complex mixtures of multiple constituents, and their biological properties seem to be the result of a synergism between these compounds. Bearing in mind this assumption, it makes sense that α-phellandrene presents a lower effect in the reduction of NO production than the essential oil. This volatile oil is a mixture of various compounds specially α-phellandrene (53–63.3%), terpinolene (8.6–11.9), ß-phellandrene (5.5 *vs* 6.0%), and dillapiol (1.9–8.0%), Terpinolene has a broad spectrum of biological activities such as anticancer (Aydin et al., 2013; Harada et al., 2012), antioxidant (Aydin et al., 2013; Dorman et al., 2000), antifungal (Hammer et al., 2004), and larvacidal (Conti et al., 2012), which contributes for the activity of the oil. *Ridolfia segetum* oil did not affect macrophages, keratinocytes and hepatocytes viability at concentrations at least up to 1.25 μL/mL. Often essential oils possess a significant toxicity at 1.25 μL/mL or even at lower concentrations (Bouzabata et al., 2013; Cabral et al., 2013). The absence of ker-

atinocyte toxicity is crucial to make an essential oil suitable for inclusion in topical formulations. Also, the absence of toxicity on both macrophages and hepatocytes reveal the safety for internal use of this oil. Cytotoxicity evaluation of α-phellandrene demonstrated higher toxicity when compared to *R. segetum* oil at the same concentrations. These results suggest that the complex mixture of molecules present in the essential oil attenuates the cytotoxicity shown by its constituents individually tested; thus, emphasizing the benefic use of the essential oil.

Considering that *R. segetum* showed a high yield in essential oil, which confers an industrial potential interest, and its volatile oil has a pleasant smell and are safe in bioactive concentrations, the results herein presented emphasize its potential use as antioxidant and anti-inflammatory for the food, cosmetic and nutraceutic industries. Also, the high content of α-phellandrene makes this species an important natural source of this compound.

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