Daucus carota subsp. *gummifer* essential oil as a natural source of antifungal and anti-inflammatory drugs

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Abstract

The present study was conducted to elucidate the composition, antifungal and anti-inflammatory activities and cytotoxicity of *Daucus carota* subsp. *gummifer* essential oil. Aerial parts of the plants were hydrodistilled and the oil analysed by GC and GC-MS. The oil was characterized by high contents of monoterpenes (83.9%), the major compounds being geranyl acetate (37.0%) and α -pinene (30.9%). The daucane sesquiterpene, carotol, was also found in relatively high amounts (11.0%). For the antifungal activity, minimal inhibitory and minimal lethal concentrations (MIC and MLC, respectively) were determined against several pathogenic fungi strains. The oil was particularly active against dermatophytes and *Cryptococcus neoformans*, with MIC values ranging from 0.32 to 0.64 μ L/mL. Concerning the anti-inflammatory potential, the oil demonstrated a strong anti-inflammatory activity by inhibiting nitric oxide (NO) production in both lipopolysaccharide (LPS)-triggered macrophages and microglia cells. NO scavenging activity was also assessed using the NO donor S-nitroso-N-acetyl-D,L-penicillamine and a significant effect was disclosed. To assure that the bioactivity of the oil was achieved without detrimental effects to cells, the toxicity of *Daucus carota* subsp. *gummifer* oil was investigated using the MTT assay in several mammalian cell lines: macrophages (Raw 264.7), keratinocytes (HaCat), hepatocytes (HepG2) and

microglia (N9). Interestingly, we detected a cytotoxic effect only for the highest concentrations of the oil, thus assuring a safe toxicological profile at bioactive concentrations.

These results advise that *Daucus carota* subsp. *gummifer* essential oil should be explored as a natural source of antifungal and anti-inflammatory drugs with potential application both at the peripheral and central nervous system levels, thus supporting in vivo studies focused in the management of dermatophytosis and/or inflammatory-related diseases.

Key words: *Daucus carota* subsp. *gummifer*; essential oils; cytotoxicity; NO scavenging; inflammation; microglia

1. Introduction

Natural products and their derivatives are an important source of potential drugs like antibiotic, anti-cancer and anti-inflammatory ones, and represent more than 50% of all the drugs in modern therapeutics (Pan et al., 2013). Among these natural products, essential oils are aromatic and volatile secondary metabolites crucial for plant's survival as they often possess antimicrobial properties. They are mixtures of small lipophilic molecules with recognized antioxidant and anti-inflammatory activities, able to cross the blood-brain barrier and enter the central nervous system. In addition, many of the commercial plant based essential oils are included in the Generally Recognized As Safe (GRAS) list fully approved by the Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) in the USA for addition to food and beverages (Burt, 2004) and many of these aromatic plants have been considered efficient and safe by the Committee on Herbal Medicinal Products (HMPC). Furthermore, due to its excellent bioavailability, essential oils can easily be applied by inhalation, dermal application and oral administration being, therefore, very attractive molecules in the prevention of brain dysfunction, cancer, heart disease and immune system decline (Aruoma, 1998; Kamatou et al., 2010).

The Apiaceae family (Umbelliferae) is rich in a number of secondary metabolites with demonstrated therapeutic properties, primarily antibacterial, antioxidant, hepatoprotective, anti-tumoral and anti-inflammatory effects (Valente et al., 2013a, b; Miguel, 2010; Tavares et al., 2010; Figueiredo et al., 2008; Pae et al., 2002; Bernart et al., 1996). For example, aliphatic C17-polyacetylenes from this family have shown to be enzymes inhibitors (Metzger et al., 2008) and highly toxic towards fungi, bacteria and mammalian cells, also displaying anti-inflammatory and anti-platelet-

aggregator effects. The previously documented effects of these polyacetylenes towards human cancer cells as well as their bioavailability and capacity to reduce tumor formation highlight its potential therapeutic benefits for health (Christensen et al., 2006) justifying further investigations to explore in more depth the pharmacological potential of these species.

In the Iberian Peninsula, 14 endemic Apiaceae species have been identified, many of them widely used as herbal remedies (Küperi et al., 2006; Castroviejo, 2003; Ekiert, 2000) and in continental Portugal alone, four spontaneous subspecies of *Daucus carota* can be found. As part of our ongoing studies on the valorization of wild carrot essential oils, the aim of this study was to assess the antifungal and anti-inflammatory potential of *Daucus carota* subsp. *gummifer* (Syme) Hook.f. essential oil from Portugal. Furthermore, its cytotoxicity was also evaluated on several mammalian cells: macrophages, keratinocytes, hepatocytes and microglia cells in order to identify its safe and bioactive concentrations. As far as we know, this is the first report on the biological potential and cytotoxicity of this species.

2. Material and methods

2.1. Plant material

Ripe umbels of *D. carota* subsp. *gummifer* commonly known as sea carrot were harvested at S. Pedro de Moel (Central Portugal). The plants were identified by Jorge Paiva, a taxonomist of the University of Coimbra and a voucher specimen was deposited at the Herbarium of the Faculty of Pharmacy of the University of Coimbra.

2.2. Essential oil isolation and analysis

Essential oils of ripe umbels of *D. carota* subsp. *gummifer* were isolated by hydrodistillation for 3 h from air-dried plant material, using a *Clevenger*-type apparatus (Council of Europe, 1997). Analysis of the volatile oil was carried out by combination of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) according to a standard analytical procedure used in our lab using two fused silica capillary columns with different stationary phases: SPB-1 (polydimethylsiloxane; 30 m × 0.20 mm i.d., film thickness 0.20 µm), and SupelcoWax 10 (polyethylene glycol; 30 m × 0.20 mm i.d., film thickness 0.20 µm) (Cavaleiro et al., 2004).

Compounds of the volatile oil were identified by both their retention indices and their mass spectra. Retention indices were calculated by linear interpolation relative to retention times of C8–C24 of n-alkanes and compared with those of reference compounds included in CEF's laboratory database or literature data (Adams, 2004). Mass spectra were matched with reference spectra from the CEF's laboratory own spectral database, Wiley/NIST database or literature data (Joulain and Köning, 1998; Adams, 2004; Wiley/NIST, 2006). Relative amounts of individual components were calculated based on GC peak areas without FID response factor correction.

2.3. Fungal strains

To evaluate the antifungal activity of the oil, several collection and clinical strains of yeast and filamentous fungi, responsible for human infections, were used: two *Candida* strains isolated from recurrent cases of vulvovaginal candidosis (*Candida krusei* H9 and *Candida guilliermondii* MAT 23), three type strains from the American Type Culture Collection (*Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 13803 and *Candida parapsilosis* ATCC 90018) and one type strain from the Colección Española de Cultivos Tipo (*Cryptococcus neoformans* CECT 1078); three dermatophyte

strains isolated from nails and skin (*Trichophyton mentagrophytes* FF7, *Microsporum canis* FF1 and *Epidermophyton floccosum* FF9), and two type strains from the Colección Española de Cultivos Tipo (*Trichophyton rubrum* CECT 2794 and *Microsporum gypseum* CECT 2908); and one *Aspergillus* strain isolated from bronchial secretions (*Aspergillus flavus* F44) and two type strains from the American Type Culture Collection (*Aspergillus niger* ATCC 16404 and *Aspergillus fumigatus* ATCC 46645). The strain *Candida parapsilosis* ATCC 90018 was used as control.

All fungal isolates were previously identified by standard microbiology methods and stored on Sabouraud broth with glycerol at -70 °C. Prior to antifungal susceptibility testing, growth characteristics and purity of all isolates were assured.

2.4. Antifungal activity

The minimal inhibitory concentrations (MICs) and minimal lethal concentrations (MLCs), according to NCCLS references M27-A3 (CLSI, 2008a) and M27-S3 (CLSI 2008b) for yeasts and M38-A2 (CLSI, 2008c) for filamentous fungi were determined using macrodilution broth assays.

Briefly, serial doubling dilutions (0.16 to 20 µL/mL) of the essential oil were prepared in dimethyl sulfoxide (DMSO; final concentration of DMSO never exceeded 2 %). Recent cultures of each strain were used to prepare the cell suspension ((1-2x10³ cells/mL for yeasts and 1-2x10⁴ cells/mL for filamentous fungi). For MICs assessment, the test tubes were incubated aerobically at 35 °C for 48 h/72 h (*Candida* spp. and *Aspergillus* spp./*Cryptococcus neoformans*) and at 30 °C for 7 days (dermatophytes) and then MICs were registered. To evaluate MLCs, 20 µL of broth were taken from each

negative tube, after MIC reading, and cultured in Sabouraud dextrose agar plates. Plates were then incubated as previously referred.

All antifungal tests were performed in RPMI medium. The growing conditions of each strain and the sterility of the medium were checked in two control tubes and the innocuity of DMSO was also evaluated at the highest concentration tested. All experiments were performed in duplicate and repeated at least three times.

2.5. Cell culture materials

Most of the reagents used were purchased from Sigma Chemical Co. (Saint Louis, MO). Specifically, Dulbecco's Modified Eagle Medium and fetal bovine serum were obtained from Gibco (Invitrogen, Paisley, UK).

2.6. Cell culture

The culture conditions for each cell line are described:

For Raw 264.7 (ATCC TIB-71) endotoxin-free Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) non-inactivated fetal bovine serum, 3.5g/L D-glucose, 1.5g/L sodium bicarbonate, 100 µg/mL streptomycin and 100 U/mL penicillin was used.

HaCat (DKFZ, Heidelberg) cells were cultured in DMEM medium supplemented with 10% (v/v) heat inactivated fetal bovine serum, 3.70g/L D-glucose, 3.70 g/L sodium bicarbonate, 100 µg/mL streptomycin and 100 U/mL .

HepG2 (ATCC HB-8065) were cultured in DMEM medium supplemented with 10% (v/v) heat inactivated fetal bovine serum, 1.5 g/L sodium bicarbonate, 100 µg/mL streptomycin and 100 U/mL penicillin.

For Microglia cells (N9) Roswell Park Memorial Institute medium (RPMI) supplemented with 5% fetal bovine serum, 23.8 mM sodium bicarbonate, 30 mM D-glucose, 100 U/mL penicillin and 100 µg/mL streptomycin was used.. All cells were maintained in an humidified atmosphere of 95 % air and 5% CO₂ at 37°C and microscopically monitored in order to detect any morphological changes.

2.7. Anti-inflammatory activity

To evaluate the anti-inflammatory potential of *D. carota* subsp. *gummifer* essential oil, macrophages (0.3x10⁶ cells/well) and microglia cells (0.15x10⁶ cells/well) were cultured in 48-well and 24-well microplates, respectively, and allowed to stabilize for 12 h. Following this period, cells were either maintained in culture medium (control) or pre-incubated with different concentrations of the essential oil for 1 h, and later activated with LPS (1 µg/mL) for 24 h. The production of NO was measured by the accumulation of nitrites in the culture supernatants, using the Griess reagent (Green et al. 1982). Briefly, 170 µL of medium were diluted with equal volumes of the Griess reagent and maintained during 30 min, in the dark. The absorbance at 550 nm was measured in an automated plate reader (SLT, Austria). Culture medium was used as blank and nitrite concentration was determined from a linear regression analysis using serial dilutions of sodium nitrite as standard.

2.8. Nitric oxide scavenging activity

The NO scavenging assay was used to evaluate the antioxidant potential of the essential oil. S-nitroso-N-acetyl-D,L-penicillamine (SNAP) was used as a NO donor. Several concentrations of the oil (0.04 – 1.25 µL/mL) were incubated with 0.9 µL of SNAP

solution (100 mM) in DMEM medium, in a final volume of 300 μ L, for 3 h. The NO scavenging activity was evaluated after this period by quantifying the nitrite levels in the medium using the Griess reaction, as previously referred.

2.9. Effect on cells viability

To evaluate the oil's cytotoxicity, four mammalian cell lines were used (macrophages, keratinocytes, hepatocytes and microglia cells) and their metabolical activity was assessed using the MTT reduction colorimetric assay, as previously reported (Mosmann, 1983). Macrophages (0.3×10^6 cells/well), keratinocytes (0.2×10^6 cells/well), hepatocytes (0.2×10^6 cells/well) and microglia cells (0.15×10^6 cells/well) were plated in 48-well microplates (macrophages, keratinocytes, hepatocytes) and 24-well microplates (microglia) and allowed to stabilize for 12 h. Then, cells were either maintained in culture medium or incubated with different concentrations of the oil for 24 h. After this period, the MTT solution (5 mg/mL in phosphate buffered saline) was added (1:10) and cells incubated at 37 °C for 15 min (macrophages and microglia), 30 min (keratinocytes) or 1 h (hepatocytes) in a humidified atmosphere of 95 % air and 5 % CO₂. Supernatants were discarded and the dark blue formazan crystals solubilized with 300 μ L acidic isopropanol (0.04N HCl in isopropanol). Quantification of formazan was performed using an ELISA microplate reader (SLT, Austria) at 570 nm, with a reference wavelength of 620 nm. In order to exclude non-specific effects of the oils on MTT, a cell-free control was also tested (data not shown).

2.10. Statistical analysis

Results are expressed as mean $\pm$ SEM values of at least three independent assays. Comparisons between control and treatment conditions were performed using two-sided

unpaired t-test. The effect of different concentrations of the essential oil were compared using one-way ANOVA followed by a Dunnett's multiple comparison test. The statistical tests were applied using GraphPad Prism, version 5.02 (GraphPad Software, San Diego, CA, USA).

3. Results and Discussion

3.1. Essential oil composition

The essential oil of *D. carota* subsp. *gummifer* from S. Pedro de Moel (Portugal) was obtained with a yield of 0.7 % (v/w) and a total of twenty-nine compounds were identified, attending 98.7% of the total oil composition (Table 1). The oil was characterized by high contents of monoterpene hydrocarbons (44.2 %) and oxygen containing monoterpenes (39.7%) being the main constituents geranyl acetate (37.0 %) and α -pinene (30.9%). The daucane sesquiterpene, carotol, also occurred in considerable amounts (11.0 %). The chemical composition of the essential oils of this species has been reported previously for plants growing in other Mediterranean regions. For example in Spain, Pinilla and collaborators (1995) pointed out geranyl acetate (51.7-76.9 %) as the main compound. In plants growing in Italy, sabinene (26.8-60.6%) and α -pinene (10.8-12.2%) were the major components of the oils (Flamini et al., 2003). More recently, plants from Algeria were also studied showing high amounts of α -pinene (34.1g/100g) and myrcene (13.1g/100g; Nawel et al., 2013). Taking into account these studies, the high amounts of geranyl acetate (37.0 %) distinguish Portuguese *D. carota* subsp. *gummifer* essential oils from the Italian and Algerian ones and show a similar pattern to those from plants growing in Spain.

3.2 Antifungal activity

The antifungal activity (MIC and MLC) of *D. carota* subsp. *gummifer* essential oil is summarized in Table 2. The oil was more active against dermatophyte strains and *Cryptococcus neoformans* with MIC values ranging from 0.32-0.64 $\mu\text{L/mL}$ (Table 2). Interestingly the oil was much more effective against *Candida guilliermondii* (MIC = 1.25 $\mu\text{L/mL}$) in comparison to other *Candida* strains (MIC ≥ 10 $\mu\text{L/mL}$) and for most *Candida* and dermatophyte strains, the MIC showed similar values to the MLC, suggesting a fungicidal effect of this oil.

As far as we know this is the first report on the antifungal activity of *D. carota* subsp. *gummifer*. Previously only the antibacterial effect of the oil was evaluated against Gram-positive and Gram-negative strains, being more effective against *L. monocytogenes*, *B. cereus*, *S. aureus* and *E. coli* (Nawel et al., 2013). The greater antifungal activity may be related to the presence of high concentrations of geranyl acetate and α -pinene. The first, also a major compound of *Thapsia minor* essential oil has been pointed out by our group to have high antifungal activity against dermatophyte strains, *Cryptococcus neoformans* and *Candida guilliermondii* (Gonçalves et al., 2012), similar to what was observed in the present study. Moreover, a study carried out by Zore et al. (2011) demonstrated that geranyl acetate exhibits excellent anti-*Candida* activity and significant synergistic activity with fluconazole at concentrations that did not affect the viability of HeLa cells. Regarding α -pinene, different studies have also shown effective antifungal activity (Zuzarte et al., 2011) and antibacterial activities (Magwa et al., 2006). Moreover, Jasicka-Misiak and collaborators (2004) identified the antifungal effect of carotol on a phytotoxic fungal strain.

3.3. Inhibition of NO production on LPS-stimulated macrophages and microglia

cells

As far as we know this is the first report on the anti-inflammatory activity of *D. carota* subsp. *gummifer*. The effect of the oil on NO production was analysed by quantifying the accumulation of nitrites in the supernatant of macrophages and microglia cultures stimulated with LPS. As shown in Fig. 1, incubation of macrophages (Fig. 1a) and microglia (Fig.1b) with LPS, for 24 h, resulted in a significant increase in nitrite production. Taking into account the toxicity of the oil present in Table 3 and discussed in the next section, inhibition of NO production was only considered for non-toxic concentrations of the oil. The inhibition of NO production in macrophages and microglia cells was more effective in the presence of 0.64 $\mu\text{L/mL}$ and 0.32 $\mu\text{L/mL}$ of the oil, for macrophages and microglia cells, respectively. Indeed, in macrophages nitrite production decreased 65.15%, relatively to LPS ($p<0.001$), without affecting cell viability in the presence of 0.64 $\mu\text{L/mL}$ of the oil. Lower concentrations of the oil were also effective reducing NO production by 41.35% (0.32 $\mu\text{L/mL}$; $p<0.001$), 26.55% (0.16 $\mu\text{L/mL}$; $p<0.001$) and 15.64% (0.08 $\mu\text{L/mL}$; $p<0.001$), relatively to LPS. Concerning microglia cells, a high inhibition (39.19%) was also achieved for the non-toxic concentration (0.32 $\mu\text{L/mL}$), relatively to LPS ($p<0.01$). Taking into account that high concentrations of NO are related to aggravated inflammatory responses involved in aging and in several diseases such as cancer (Porta et al., 2009; Khatami, 2012), rheumatoid arthritis, cardiovascular and neurodegenerative diseases (Hunter and Doddi, 2010), the strong NO inhibitory activity of *D. carota* subsp. *gummifer* oil justifies further studies as a therapeutic option in diseases associated with deregulated inflammatory responses. For instance, dysfunction or chronic activation of microglia

often results in the excessive production of neuro-inflammatory molecules, namely cytokines and NO, leading to inflammation-mediated neuronal cell death and brain injury, as observed in Alzheimer's disease, Parkinson's disease and multiple sclerosis. Herein we highlight the anti-inflammatory potential of *D. carota* subsp. *gummifer* oil at both peripheral and central nervous system levels suggesting its use to prevent or ameliorate neurodegenerative diseases. We may speculate that the anti-inflammatory properties of the oil could be partially due to its main compounds geranyl acetate and α -pinene. Accordingly, we and other authors previously demonstrated that these two compounds have anti-inflammatory properties (Gonçalves et al., 2012; Quintans-Júnior et al., 2013; Rufino et al., 2014). We are currently addressing both the participation of the main pure compounds in the bioactivity of the oil, as well as investigating the molecular mechanisms behind their anti-inflammatory potential.

3.4. Nitric oxide scavenging activity

The NO scavenging potential of the oil was also assessed to elucidate whether the NO production inhibition was related to a scavenging activity of the compounds. The results revealed that the oil has a significant scavenging activity similar in all the tested concentrations (0.08-1.25 μ L/mL) as shown in Fig. 2. These results suggest that the oil has a NO scavenging potential, however, regarding the different degrees of inhibition of NO production shown in the presence of different oil concentrations (Fig. 1) an additional mechanism of action may be involved, mainly at the iNOS transcriptional level. Further studies are ongoing to discover the anti-inflammatory molecular targets of the oil and its main pure compounds.

3.4. Cytotoxicity

The safety profile of the oil for further applications to humans and animals was considered by testing the oil's cytotoxicity on different mammalian cells. Regarding macrophages (Table 3), the essential oil did not interfere with cell viability at concentrations lower than 1.25 $\mu\text{L/mL}$ and similar results were obtained on hepatocytes and keratinocytes. For microglia cells the oil was slightly more toxic affecting cell viability at concentrations higher than 0.64 $\mu\text{L/mL}$ (Table 3). These results are quite attractive, suggesting a potential safe use of the oil for the development of new pharmaceutical drugs, in oral and topical formulations.

4. Conclusions

Together, these findings provide novel insights into the antifungal, anti-inflammatory and NO scavenging activities of *D. carota* subsp. *gummifer* essential oil at concentrations demonstrating a safe toxicological profile to several mammalian cells. Remarkably, the anti-inflammatory effect was observed in both cells from peripheral and central nervous systems. More broadly, these findings may be useful in developing novel therapeutic strategies for the management of dermatophytosis and/or inflammatory-related diseases, thus justifying further in vivo studies.

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Figure 1. Effect of *Daucus carota* subsp. *gummifer* essential oil on NO production
triggered by LPS in macrophages (a) and microglia cells (b). Cells (0.3×10^6 cells/well
and 0.15×10^6 cells/well, respectively) were maintained in culture medium (control), or
incubated with 1 $\mu\text{g/mL}$ LPS in the absence or in the presence of different
concentrations of the oil for 24 h. Results are expressed as a percentage of nitrite
production by cells treated with LPS. Each value represents the mean $\pm$ SEM from at
least three independent experiments, performed in duplicate (* $p < 0.05$, ** $p < 0.01$ and
*** $p < 0.001$, compared to LPS; ###, compared to control; + bioactive concentrations
without toxicity).

Figure 2. Effect of *Daucus carota* subsp. *gummifer* essential oil on nitric oxide
scavenging activity. Different concentrations of essential oil (0.08-1.25 $\mu\text{L/mL}$) were
incubated with the NO donor, SNAP (100 mM), in culture medium for 3 h. Results are
expressed as percentage of NO release triggered by SNAP. Each value represents the
mean $\pm$ SEM from three experiments, performed in duplicate (*** $p < 0.001$, compared to
control).

a

b

Table 1 - Composition of the essential oil of *Daucus carota* subsp. *gummifer*

RI ^a	RI ^b	Compounds*	%
922	1030	α -Thujene	t
930	1030	α -Pinene	30.9
943	1073	Camphene	0.9
964	1128	Sabinene	t
970	1118	β -Pinene	3.8
980	1161	Myrcene	2.1
997	1171	α -Phellandrene	t
1020	1206	Limonene	5.8
1025	1235	<i>Z</i> - β -Ocimene	0.5
1035	1250	<i>E</i> - β -Ocimene	0.1
1050	1458	<i>trans</i> -Sabinene hydrate	t
1081	1543	Linalool	1.0
1158	1595	Terpinen-4-ol	0.2
1169	1692	α -Terpineol	0.1
1233	1838	Geraniol	1.0
1266	1574	Bornyl acetate	0.3
1328	1688	α -Terpinyl acetate	t
1362	1753	Geranyl acetate	37.0
1411	1590	<i>E</i> - β -Caryophyllene	1.1
1443	1660	α -Humulene	0.3
1448	1665	<i>trans</i> - β -Farnesene	0.2

1466	1699	Germacrene D	1.7
1484	1708	α -Selinene	0.2
1498	1720	β -Bisabolene	0.1
1557	1968	Caryophyllene oxide	t
1581	2001	Carotol	11.0
1630	2216	α -Cadinol	t
1630	2219	T-Cadinol	t
1663	1709	β -Bisabolol	t
		Monoterpene hydrocarbons	44.2
		Oxygen containing monoterpenes	39.7
		Sesquiterpene hydrocarbons	3.6
		Oxygen containing sesquiterpenes	11.2
		Total	98.7

*

Compounds listed in order to their elution on the SPB-1 column.

t = traces ($\leq 0.05\%$)

RI^a = Retention indices on the SPB-1 column relative to C₈ to C₂₄ *n*-alkanes.

RI^b = Retention indices on the SupelcoWax-10 column relative to C₈ to C₂₄ *n*-alkanes.

Table 2. Antifungal activity (MIC and MLC) of *Daucus carota* subsp. *gummifer* essential oil for *Candida*, dermatophyte and *Aspergillus* strains.

Strains	<i>Daucus carota</i> subsp. <i>gummifer</i>	
	MIC ^a	MLC ^a
<i>Candida albicans</i> ATCC 10231	>20	>20
<i>Candida tropicalis</i> ATCC 13803	10	10
<i>Candida krusei</i> H9	>20	>20
<i>Candida guilliermondii</i> MAT23	1.25	2.5
<i>Candida parapsilosis</i> ATCC 90018	>20	>20
<i>Cryptococcus neoformans</i> CECT 1078	0.64	0.64
<i>Epidermophyton floccosum</i> FF9	0.32	0.32
<i>Microsporum canis</i> FF1	0.64	1.25
<i>Microsporum gypseum</i> CECT 2908	0.64	0.64-1.25
<i>Trichophyton mentagrophytes</i> FF7	0.64	0.64
<i>Trichophyton rubrum</i> CECT 2794	0.32	0.32
<i>Aspergillus flavus</i> F44	>20	>20
<i>Aspergillus fumigatus</i> ATCC 46645	2.5	>20
<i>Aspergillus niger</i> ATCC16404	10	>20

Results were obtained from three independent experiments performed in duplicate.

^a MIC and MLC are expressed in $\mu\text{L/mL}$ (V/V).

Table 3. Effect of *Daucus carota* subsp. *gummifer* essential oil on mammalian cells

Oil concentration	Macrophages (Raw 264.7)	Keratinocytes (HaCat)	Hepatocytes (HepG2)	Microglia (N9)
1.25 $\mu\text{L/mL}$	23.00 $\pm$ 3.78***	44.23 $\pm$ 12.78*	88.99 $\pm$ 7.46**	15.55 $\pm$ 8.23***
0.64 $\mu\text{L/mL}$	83.14 $\pm$ 8.24	71.23 $\pm$ 17.18	102.6 $\pm$ 10.96	51.09 $\pm$ 10.86***
0.32 $\mu\text{L/mL}$	88.39 $\pm$ 5.47	90.70 $\pm$ 25.67	106.4 $\pm$ 11.39	98.65 $\pm$ 6.42
0.16 $\mu\text{L/mL}$	95.61 $\pm$ 6.35	91.99 $\pm$ 11.87	111.6 $\pm$ 7.37	98.36 $\pm$ 2.74

viability

Results are expressed as percentage of MTT reduction by control cells maintained in culture medium. Each value represents the mean $\pm$ S.E.M. from at least three experiments, performed in duplicate. **p<0.01 and *** p<0.001, compared to control (100%).