Essential oil of Seseli tortuosum L. from Portugal: safety and anti-inflammatory potential evaluation

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Abstract: Several *Seseli* L. (Apiaceae) species are used in folk medicine for several healing effects, namely herbal remedy for human inflammation, swelling, rheumatism, pain and common cold. In Portugal, there are two taxa usually used in traditional medicine: *Seseli tortuosum* L. and *Seseli montanum* subsp. *peixotoanum* (Samp.). The aim of the present research was to evaluate the anti-inflammatory activity of *S. tortuosum* and to assess their safety profile in several mammalian cell types at concentrations presenting strong bioactivity. This oil is characterized by high percentage of α-pinene (21.1%), β-pinene (22.6%) and *cis-β*-ocimene (11.8). The anti-inflammatory potential was investigated in lipopolysaccharide (LPS)-triggered nitric oxide (NO) production by macrophages and microglia concomitantly treated with *S. tortuosum* essential oil. Our results demonstrated a significant decrease of LPS-induced NO production at concentrations up to 0.16 µL/mL, without affecting cell viability. Our findings confirm the safety of *S. tortuosum* oil in doses with anti-inflammatory activity. These results support further studies envisaging the use of *S. tortuosum* oil in pharmaceutical formulations for inhalation, topical application or oral administration.

Keywords: *Seseli tortuosum,* essential oil, cytotoxicity, anti-inflammatory activity, nitric oxide

1. Introduction

Several *Seseli* species are reported in ancient literature for various healing effects, namely herbal remedy for human inflammation, swelling, rheumatism, pain and common cold (Hu et al. 1990).

In Portugal, there are two taxa usually used in traditional medicine: *S. tortuosum* L., widespread in several provinces and *S. montanum* subsp. *peixotoanum* (Samp.) M. Lainz

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restricted to some ultrabasic habitats in Tras-os-Montes province (North of Portugal) (Castroviejo et al., 2003; Proença da Cunha et al., 2007).

Seseli tortuosum belongs to the family Apiaceae, which is composed by aromatic herbs and economically important species widespread used as foods, spices, condiments and ornamentals (Lawrence 1995; Crowden et al. 1969; Pimenov & Leonov 1993). This species is widespread in psammophilous bushes in the secondary dune, less frequent in maritime rocks and slopes of saline (Pimenov & Leonov 1993). In Turkish folk medicine, the fruit of *Seseli tortuosum* is used as emmenagogue and antiflatulent (Baytop 1999).

Some works have been carried out to evaluate several biological properties of *S. tortuosum* extracts namely the anti-inflammatory and antinociceptive properties of the ethyl acetate, methanol and *n*-hexane extracts (Küpeli et al. 2006; Tosun et al. 2006) and the antifungal activities of the essential oil (Gonçalves et al. 2012).

Therefore, the aim of the presented research was to characterize the *S. tortuosum* essential oil from plants collected in the sand dunes of Peniche (Central Portugal) and to unveil safe bioactive concentrations of the oil in several cell lines representative of different mammalian tissues. Concomitantly, this research aimed to disclose for the first time the anti-inflammatory activity of *S. tortuosum* essential oil on cell lines representative of peripheral and central nervous system, namely macrophages and microglia respectively, through evaluation of nitric oxide (NO) production. NO is a well-established pro-inflammatory molecule overproduced in inflammatory disorders and inhibition of its production is a good approach to screen anti-inflammatory molecules or phytochemicals.

2. Materials and methods

2.1 Isolation of essential oil

Aerial parts of *S. tortuosum* were collected in July 2013, during flowering stage, in Consolação beach, Peniche (Portugal). Essential oil was isolated by hydrodistillation for 3h using a Clevenger-type apparatus, according to the procedure described in the European Pharmacopoeia (Council of Europe, 1997).The plant was identified by a plant taxonomist and a voucher specimen deposited in the Herbarium of Medicinal Plants, Faculty of Pharmacy, University of Coimbra, Portugal.

2.2 Chemical composition

2.2.1 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 (polyethylene glycol 30 m × 0.20 mm, film thickness 0.20 µm). Oven temperature program: 70–220ºC (3ºC/min), 220 ºC (15 min); injector temperature: 250ºC; carrier gas: helium, adjusted to a linear velocity of 30 cms^{-1}; splitting ratio 1:40; detectors temperature: 250ºC.

2.2.2 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: 250ºC; MS source temperature: 230ºC; MS quadrupole temperature: 150 ◦C; ionization energy: 70 eV; ionization current: 60µA; scan range: 35–350 units; scans per second: 4.51.

2.2.3 Identification of individual components

Components of the volatile oils 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, 2007), 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, 2007; Wiley Registry, 2006). Relative amounts of individual components were calculated based on GC raw data areas without FID response factor correction.

2.3 Anti-inflammatory activity evaluation

2.3.1 Cell culture and materials

The macrophage cell line (Raw 264.7), obtained from the American Type Culture Collection (TIB-71) was kindly supplied by Dr. Otília Vieira (Center for Neuroscience and Cell Biology, University of Coimbra, Portugal). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) non-inactivated fetal bovine serum, 3.02 g/L sodium bicarbonate, 100 µg/mL streptomycin and 100 U/mL penicillin at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

The microglia cell line (BV2) was purchased from Biological and Cell Banking Fatory Centro di Risorse Biologiche, Genova. Cells were grown in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% (v/v) non-inactivated fetal bovine serum, 3.02 g/L sodium bicarbonate, 100 µg/mL streptomycin and 100 U/mL penicillin at 37ºC in a humidified atmosphere of 95% air and 5% CO_2.

2.3.2 Nitric oxide (NO) measurement

The anti-inflammatory potential of the essential oil of *S. tortuosum* was evaluated in the macrophage cell line (RAW 264.7) and microglia (BV2).

The macrophage cells and microglia (0.6×10^6/0.3×10^6 respectively cells/well) were cultured in 48-well microplates, in a final volume of 600 µL 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 or its main compounds for 1 h, and later activated with LPS (1 µg/mL) for 24h. The production of NO was evaluated by quantifying the accumulation of nitrites in the culture supernatants, through the colorimetric reaction with the Griess reagent (Cruz et al., 2001; Green et al., 1982). Briefly, 170 µL of medium were diluted with equal volumes of the Griess reagent [0.1 % (w/v) N-(1-naphthyl) ethylene diamine dihydrochloride and 1% (w/v) sulphanilamide containing 5%(w/v) H_3PO_4 (v/v)] and maintained during 30 min, in the dark. The absorbance at 550 nm was measured in an automated plate reader.

Three independent experiments were performed with the essential oil of *S. tortuosum*. The results were expressed as the percentage of nitrite production by cells cultured with LPS.

2.4 *In vitro* cytotoxicity assessment

2.4.1 Cell culture

The human hepatocyte cell line (HepG2) was purchased from ATCC (number: 77400) and kindly provided by Dr. Conceição Pedroso Lima. Hepatocytes were cultured in Dulbecco's Modified Eagle Medium (high glucose) supplemented with 10% (v/v) inactivated fetal bovine serum, with 1.5 g/L sodium bicarbonate, 100 U/ml penicillin, 100 µg/ml streptomycin, and kept at 37ºC in a humidified atmosphere of 95% air and 5% CO_2.

The human alveolar epithelial cell line (A549) was purchased from ATCC (CCL-185 number). The alveolar epithelial cells were cultured in Dulbecco's Modified Eagle Medium (high glucose) supplemented with 10% (v/v) inactivated fetal bovine serum, 3.02 g/L sodium bicarbonate, 100 µg/mL streptomycin and 100 U/ml penicillin, at 37ºC in a humidified atmosphere of 95% air and 5% CO_2.

2.4.2 MTT assay for cell viability

Cell viability was assessed for the essential oil of *S. tortuosum* in the cell lines: macrophages (Raw 264.7), microglia (BV2), hepatocytes (HepG2) and alveolar epithelial cells (A549).

Evaluation of cell viability was performed by a colorimetric assay using the MTT reduction colorimetric assay, as previously reported (Mosmann, 1983). Macrophages (RAW 264.7), microglia (BV2), hepatocytes (HepG2) and alveolar epithelial cells (A549) were cultured at densities of $0.6x10^6$, $0.3x10^6$, $0.2x10^6$ and $0.2x10^6$ cells/well, respectively. Cells were cultured in 48-well microplates in a final volume of 600µL for 12 hours and were further cultured with different concentrations (0.02 to 0.32 µL/mL) of the essential oil.

After 24h, 60 µL of a MTT solution (5 mg/mL in PBS) were added per well to hepatocytes and alveolar epithelial cells and 43 µL of MTT solution to macrophages and microglia (BV2). Cells were further incubated at 37ºC in a humidified atmosphere of 95% air and 5% CO_2. Microglial cells, macrophages and hepatocytes were incubated for 1 hour and the alveolar epithelial cells for 2 hours and 30 minutes. After this time of incubation with MTT, the supernatants were discarded and 300 µL of acidic isopropanol (0.04 N HCl in isopropanol) were added to each well. Quantification of formazan crystals was performed using an ELISA microplate reader at 570 nm with a reference wavelength of 620 nm.

Three independent experiments were performed with the essential oil of *S. tortuosum*. The results were expressed as a percentage of MTT reduction and compared with control.

2.4.3 Data analysis

All the experiments were performed in duplicate, being the results expressed as mean ± SEM of three independent experiments. Statistical analyses were performed using one-way ANOVA, with a Dunnett's multiple comparison test. The statistical tests were applied using GraphPad Prism, version 6.00 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1 Essential oil composition

The essential oil was obtained with yield of 1.2%. 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.

Table 1. Composition of the essential oil of *Seseli tortuosum* from Peniche (Portugal)

RI SPB-1[a]	RI SW 10[b]	Compound	%
921	1028	tricyclene	t
922	1029	thujene	0.6
930	1029	*α*-pinene	21.1
943	1072	camphene	1.2
964	1123	sabinene	6.2
969	1116	*β*-pinene	22.6
977	1161	myrcene	2.3
1005	1152	*α*-phellandrene	0.1
1012	1272	*p*-cymene	1.3
1021	1201	limonene	5.8
1021	1210	*β*-phellandrene	t
1027	1233	*cis*-*β*-ocimene	11.8
1035	1249	*trans*-*β*-ocimene	2.8
1047	1249	*γ*-terpinene	9.1
1076	1288	terpinolene	0.6
1081	1544	*cis*-sabinene hydrate	0.1
1082	1542	linalool	0.1
1117	1396	allo-ocimene	t
1120	1639	pinocarveol	0.1
1158	1595	terpinen-4-ol	2.5
1169	1689	*α*-terpineol	0.4
1223	1600	carvacrol methyl ether	t
1369	2004	methyleugenol	t
1377	1519	*β*-bourbunene	0.1
1382	1580	*β*-elemene	0.1
1408	1590	*E*- caryophyllene	1.5
1442	1662	*α*-humulene	0.1
1467	1699	germacrene-D	1.6
1481	1717	bicyclogermacrene	0.2
1497	1746	*γ*-cadinene	t

1506	1746	*δ*-cadinene	0.1
1540	1818	germacrene B	0.1
1577	2004	carotol	0.1
1663	1709	*β* -bisabolol	0.2
		Total identified	93.1

Compounds listed in order of elution from the *Supelco SPB-1* column.
RI SPB-1: GC-retention indices relative to $C_9 - C_{23}$ *n*-alkanes on the *Supelco SPB-1* column.
RI SW 10: GC-retention indices relative to $C_9 - C_{23}$ *n*-alkanes on the *SupelcoWax-10* column.
t= traces (≤ 0.05 %).

In total, 34 compounds were identified accounting for 93.1%, being the main compounds α-pinene (21.1%), β-pinene (22.6%) and *cis-β*-ocimene (11.8).

These results are in agreement with previous work from Portugal by Gonçalves et al. 2012, where the main compounds of the two samples studied were α-pinene (24.9 and 24.8%), β-pinene (23.9 and 23.5%) and *cis-β*-ocimene (13.3 and 16%). Similarly, high amounts of pinenes were also detected in the oils of *S. tortuosum* from Turkey (>42%) (Kaya et al., 2003), Iran (>35%) (Zohreh et al., 2003) and Italy (>31%) (Bader et al., 2003). However, the oil of *S. tortuosum* from Portugal is quite different from the Italian oil, which is characterized by high amounts of myrcene (29.2%) (Bader et al., 2003), and to the oil from Turkey, reported by Dogan et al., 2006, that has (E)-sesquilavandulol (37.0%) as major compound.

3.2 Anti-inflammatory activity

The anti-inflammatory activity (inhibition of NO production) of *S. tortuosum* essential oil was assessed *in vitro*, using cells with a key role in peripheral and central inflammatory disorders, namely macrophages (Raw 264.7) and microglia (BV2).

The effect of the oil on NO production was analyzed by measuring the accumulation of nitrites in the culture medium of cells stimulated with the *Toll-like* receptor 4 agonist, LPS. NO is synthesized from L-arginine by inducible nitric oxide synthase expressed in numerous mammalian cells, such as macrophages, and large amounts of NO have been found in several inflammatory-related diseases, namely atherosclerosis, obesity, diabetes and neurodegenerative diseases (Weinberg et al., 2007). For this reason, NO is a well-established marker of inflammation and inhibition of its production upon activation with an inflammatory stimulus, such as LPS, might be a useful strategy to disclose new anti-inflammatory drugs. As shown in Figure 1, NO production in macrophages decreased in the presence of the five tested concentrations of the essential oil to 26.24±1.49 (0.32 µL/mL), 65.71±2.22 (0.16 µL/mL), 84.10±2.04 (0.08 µL/ml), 94.25±0.90 (0.04 µL/ml), and 99.97±2.58 (0.02 µL/mL).

The NO inhibitory profile triggered by the oil in microglia was higher than in macrophages. Indeed, co-treatment of the cells with LPS in the presence of the five concentrations of the essential oil (Figure 2) decreased nitric oxide production to 15.73±0.52 (0.32 µL/mL), 26.34±2.03 (0.16 µL/mL), 59.27±1.30 (0.08 µL/ml), 80.62±0.79 (0.04 µL/ml), and 86.99±0.64 (0.02 µL/mL).

NO plays a crucial role in the pathophysiology of a wide range of human diseases, including respiratory pathologies, diarrhea, diabetes, neurodegenerative diseases, among others (Gaginella et al., 1995; Spinas, 1999; Gelb et al., 2012). Our results clearly demonstrate that *S. tortuosum* essential oil inhibited LPS-induced nitric oxide production in macrophages and microglia.

3.3 Evaluation of cell viability

The safety of *S. tortuosum* essential oil was assessed by the MTT assay in macrophages (RAW 264.7), microglia (BV2), hepatocytes (HepG2) and alveolar epithelial cells (A549).

Figure 3 demonstrates that the essential oil of *S. tortuosum* showed no significant cytotoxicity in macrophages (Raw 264.7) for all tested concentrations up to 0.16 µL/mL. Also, in Figure 4 it is represented the evaluation of cell viability in microglial cells (BV2), and the essential oil of *S. tortuosum* also showed no significant cytotoxicity for concentrations up to 0.16 µL/mL. In Figure 5, it is demonstrated that the essential oil showed no significant cytotoxicity in hepatocytes (HepG2) for concentrations up to 0.32 µL/mL and Figure 6 demonstrates the absence of cytotoxicity in alveolar epithelial cells (A549) for concentrations up to 0.32 µL/mL.

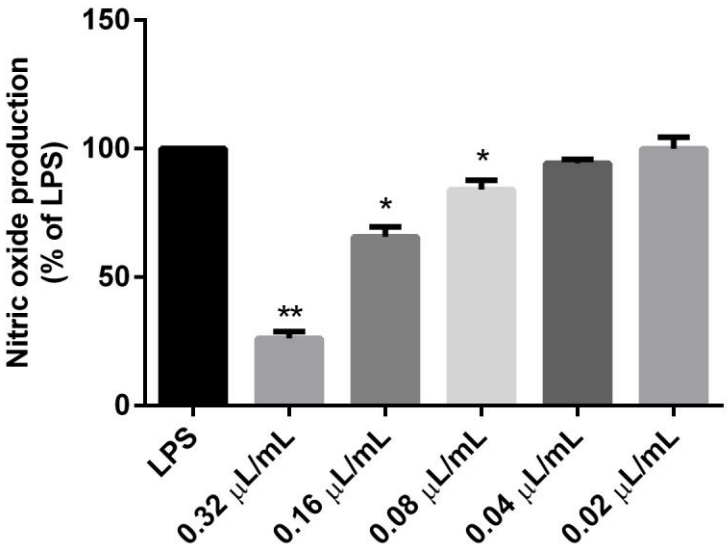

Figure 1. Effect of essential oil of *S. tortuosum* on NO production in macrophages (Raw 264.7). Results are expressed as the percentage of nitrite production by cells cultured in the presence of LPS. Each value represents the mean ± SEM of three independent experiments performed in duplicate (** p <0.01, * p <0.05, compared to LPS).

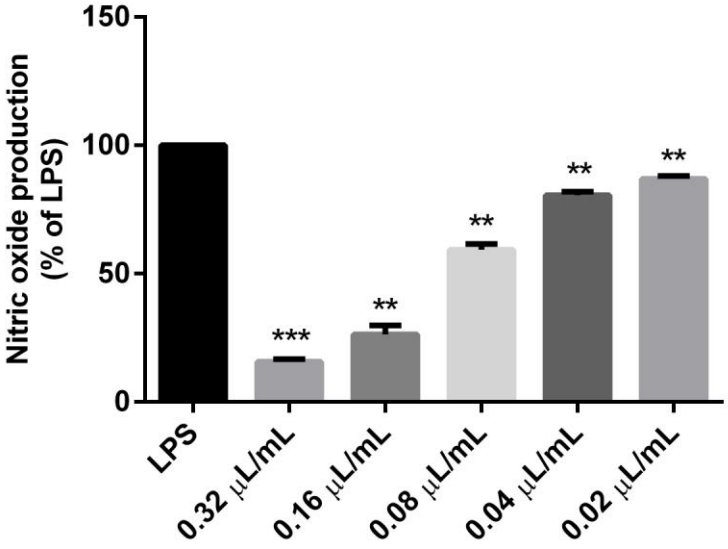

Figure 2. Effect of essential oil of *S. tortuosum* on NO production in microglia cells (BV2). Results are expressed as the percentage of nitrite production by cells cultured in the presence of LPS. Each value represents the mean ±SEM of three independent experiments performed in duplicate (*** p <0.001, ** p <0.01, compared to LPS).

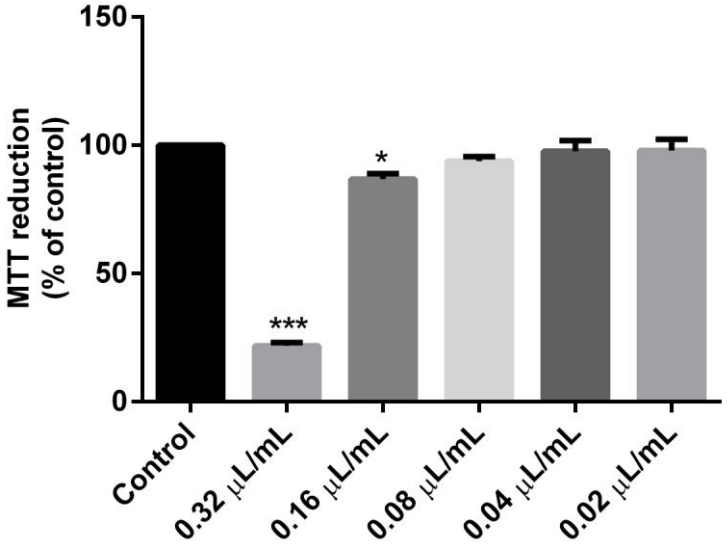

Figure 3. Effect of essential oil of *S. tortuosum* on macrophages (Raw 264.7) viability by the MTT assay. Results are expressed as the percentage of MTT reduction by control cells. Each value represents the mean ± SEM of three independent experiments performed in duplicate (*** p <0.001, * p <0.05, compared to control).

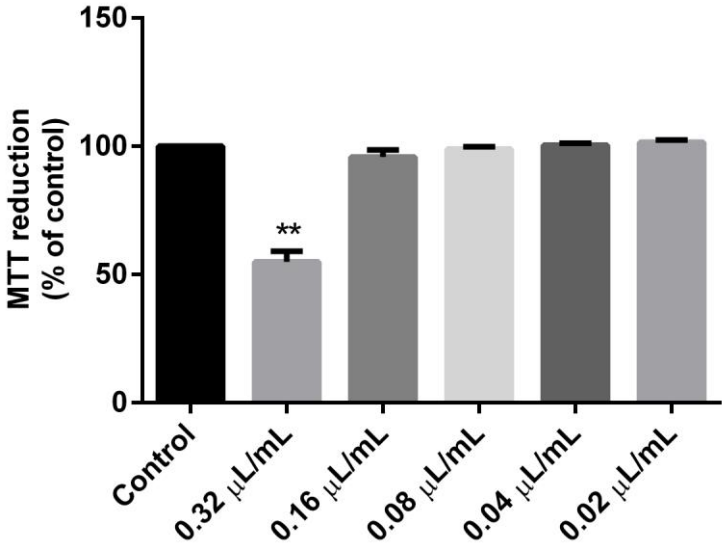

Figure 4. Effect of essential oil of *S. tortuosum* on microglia viability (BV2) by MTT assay. Results are expressed as the percentage of MTT reduction by control cells. Each value represents the mean ± SEM of three independent experiments performed in duplicate (** p <0.01, compared to control).

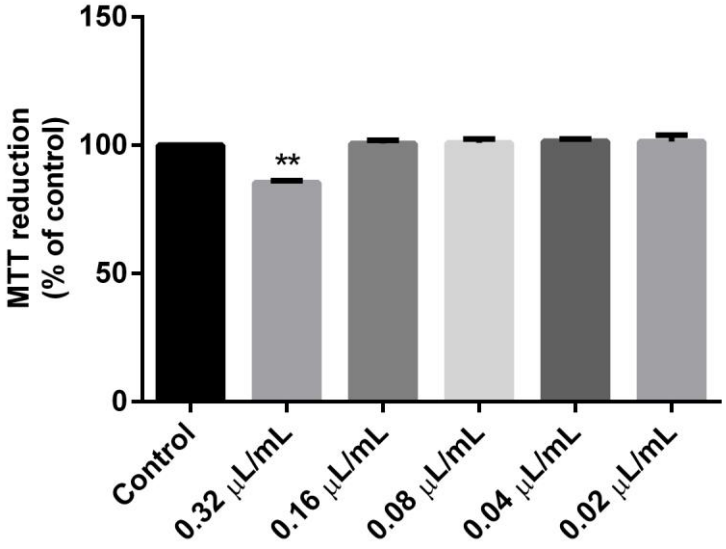

Figure *5*. Effect of essential oil of *S. tortuosum* on hepatocytes (HepG2) viability by MTT assay. Results are expressed as the percentage of MTT reduction by control cells. Each value represents the mean ± SEM of three independent experiments performed in duplicate (** p <0.01, compared to control).

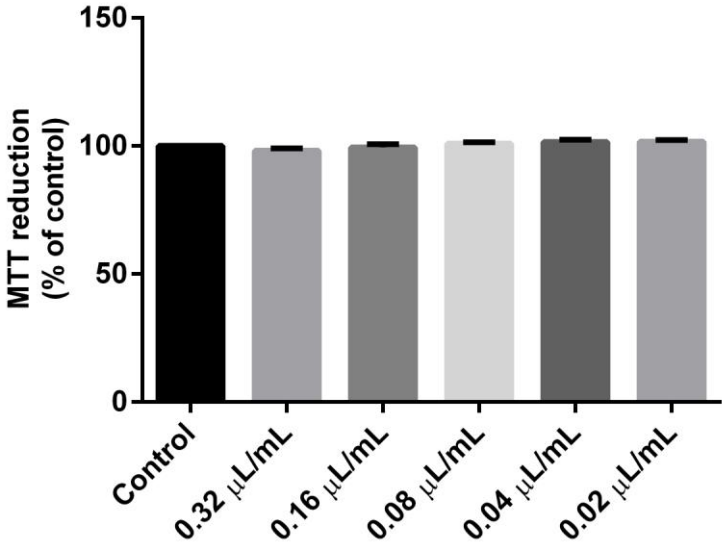

Figure *6*. Effect of essential oil of *S. tortuosum* on alveolar epithelial cells (A549) viability by MTT assay. Results are expressed as the percentage of MTT reduction by control cells.. Each value represents the mean ± SEM of three independent experiments performed in duplicate.

4 Conclusions

This work highlights, for the first time, the anti-inflammatory activity of *S. tortuosum* essential oil assessed in cells with a key role in peripheral and central inflammatory disorders, namely macrophages and microglia.

Our results point out the safety profile of *S. tortuosum* oil in bioactive doses on both macrophages and microglia. We may therefore conclude that it is safe to use *S. tortuosum* essential oil in concentrations up to 0.16 µL/mL, as confirmed by the absence of toxicity to macrophages, microglia, hepatocytes and alveolar epithelial cells.

Importantly, the high yield in essential oil together with its ability to disrupt the inflammatory response strongly encouraged *S. tortuosum* industrial exploitation, namely concerning its essential oil use in pharmaceutical formulations for inhalation, topical application or oral administration.

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References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. 4th edition. Allured Publishing Corporation, Carol Stream, IL.

Bader, A., Caponi, C., Cioni, L.P., Flamini, G., Morelli, I., 2003. Acorenone in the essential oil of flowering aerial parts of *Seseli tortuosum* L. *Flavour Fragr. J.* 18, 57–58.

Baytop, T. 1999. Therapy with medicinal plants in Turkey past and present, 2nd ed. Nobel Tıp Kitabevi, Istanbul.

Castroviejo, S., Lainz, M., Lopez Gonzalez, G., Montserrat, P., Munoz Garmendia, F.,

Crowden, R. K. Harborne, J. B. Heywood, V. H. 1969. Chemosystematics of the umbelliferae - a general survey. *Phytochemistry* 8 (10), 1963-1984.

Council of Europe, 1997. European Pharmacopoeia, third ed. Council of Europe. Strasbourg.

Cruz, M.T., Duarte, C.B., Gonçalo, M., Figueiredo, A., Carvalho, P., Lopes, M.C., 2001. Granulocyte-macrophage colony-stimulating factor activates the transcription of nuclear factor kappa B and induces the expression of nitric oxide synthase in a skin dendritic cell line. *Immunol. Cell Biol.* 79(6), 590–596.

Dogan, E., Duman, H., Tosun, A., Kurkcuoglu, M., Baser, K.H.C., 2006. Essential oil composition of the fruits of *Seseli resinosum* Freyn et Sint. and *Seseli tortuosum* L. growing in Turkey. *J. Essent. Oil Res*. 18, 57–59.

Gaginella, T.S., Kachur, J.F., Tamai, H., Keshavarzian, A., 1995. Reactive oxygen and nitrogen metabolites as mediators of secretory diarrhea. *Gastroenterology* 109, 2019-2028.

Gelb, A.F., Barnes, P.J., George, S.C., Ricciardolo, F.L., DiMaria, G., Zamel, N., 2012 Review of exhaled nitric oxide in chronic obstructive pulmonary disease. *J. Breath Res*. 6, 047101.

Gonçalves, M.J. Tavares, A.C, Cavaleiro, C. Cruz, M.T. Lopes, M.C. Canhoto, J. Salgueiro, L. 2012. Composition, antifungal activity and cytotoxicity of the essential oils of *Seseli tortuosum* L. and *Seseli montanum* subsp. *peixotoanum* (Samp.) M. Laínz from Portugal. *Ind. Crop. Prod.* 39, 204–209.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., 1982. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal. Biochem*. 126, 131–138.

Hu, C.-Q., Chang, J.-J., Lee, K.-H., 1990. Antitumor agents, 115. Seselidiol, a new cytotoxic polyacetylene from *Seseli mairei*. *J. Nat. Prod.*53, 932–935.

Joulain, D., Konig, W.A., 1998. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. E.B.- Verlag, Hamburg.

Kaya, A., Demirci, B., Baser, K.H.C., 2003. The essential oil of *Seseli tortuosum* L. growing in Turkey. *Flavour Fragr. J*. 18, 159–161.

Küpeli, E. Tosun, A. Yesilada, E. 2006. Anti-inflammatory and antinociceptive activities of *Seseli* L. species (Apiaceae) growing in Turkey. *J. Ethnopharmacol.* 104(3), 310–314.

Lawrence, B.M. 1995. The isolation of aromatic materials from natural plant products. In *Manual of the Essential Oil Industry*, K. Tuley De Silva (ed.), 57–154.

Paiva, J., Villar, L., 2003. Flora Iberica: Plantas vasculares de la Peninsula Iberica e Islas Baleares, vol. X. Araliaceae-Umbelliferae. Real Jardin Botanico, C.S.I.C., Madrid.

Pimenov, M. G. Leonov, M. V. 1993. The genera of the Umbelliferae. A nomenclator. Kew: Royal Botanic Gardens. 156.

Proença da Cunha, A., Ribeiro, J., Roque, O., 2007. Plantas aromáticas em Portugal, caracterização e utilizações. Fundação Calouste Gulbenkian, Lisboa.

Spinas, G.A., 1999. The dual role of nitric oxide in islet beta-cells. *News Physiol. Sci*. 14, 49-54.

Tosun, A. Baba, M. Bahadir, O. Okuyama, T. 2006. Coumarins isolated from the roots of *Seseli resinosum* in Turkey. *Pharm. Biol.* 44, 528–533.

Weinberg, J.B., Fermor, B., Guilak, F., 2007. Nitric oxide synthase and cyclooxygenase interactions in cartilage and meniscus - Relationships to joint physiology, arthritis, and tissue repair, in: Harris, R.E. (Ed.), Inflammation in the Pathogenesis of Chronic Diseases: The COX-2 Controversy, Subcellular Biochemistry Series. Springer, New York, pp. 31-62.

Wiley Registry, 2006. NIST 05 Mass Spectral Library, 8th ed. John Wiley & Sons, Hoboken, NJ.

Zohreh, H., Shiva, M., Abdolhossein, R., 2003. Chemical composition of the essential oil of *Seseli tortuosum* ssp. Kiabii Akhani. from Iran. *J. Essent. Oil Res.* 15, 412–415.