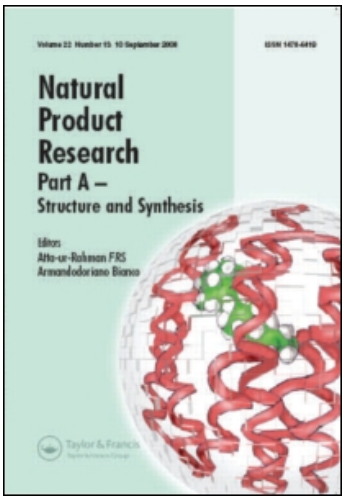

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Chemical composition of *Schinus molle* essential oil and its cytotoxic activity on tumour cell lines

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The leaf essential oil hydrodistilled from *Schinus molle* grown in Costa Rica was characterised in terms of its chemical composition, antioxidant activity, ability to induce cytotoxicity and the mechanism of cell death involved in the process. As a result, 42 constituents, accounting for 97.2% of the total oil, were identified. The major constituents of the oil were β-pinene and α-pinene. The antioxidant activity showed an IC_{50} of 36.3 $\mu g\,mL^{-1}$. The essential oil was cytotoxic in several cell lines, showing that it is more effective on breast carcinoma and leukemic cell lines. The LD_{50} for cytotoxicity at 48 h in K562 corresponded to 78.7 $\mu g\,mL^{-1}$, which was very similar to the LD_{50} obtained when apoptosis was measured. The essential oil did not induce significant necrosis up to 200 $\mu g\,mL^{-1}$, which together with the former results indicate that apoptosis is the main mechanism of toxicity induced by *S. molle* essential oil in this cell line.

In conclusion, the essential oil tested was weak antioxidant and induced cytotoxicity in different cell types by a mechanism related to apoptosis. It would be interesting to elucidate the role that different components of the oil play in the effect observed here, since some of them could have potential anti-tumoural effects, either alone or in combination.

Keywords: *Schinus molle*; Anacardiaceae; essential oil; cytotoxicity; tumour cells; apoptosis

1. Introduction

Although some plants have been widely studied in terms of the substances they contain, it has been suggested that only 10% of the ≈250,000 higher plant species have been characterised in chemical and pharmacological terms (Calixto, 2005). In most cases, there is no scientific validation of widely utilised natural products, especially in developing countries (García-González & Morales, 2005).

Drug discovery from medicinal plants has played an important role in the treatment of cancer and most of the new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer.

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Several currently used anti-tumoural agents, some of which are produced nowadays in laboratories, were originally obtained from plant extracts (Balunas & Kinghorn, 2005).

Among the substances with therapeutic potential are the essential oils, obtained from different parts of the plant and constituted by complex mixtures of volatile and lipophilic secondary metabolites, which are generally responsible for their particular odours. Plant volatiles, as they are also known, are lipophilic molecules with high vapour pressure and different ecological roles, frequently restricted to specific plant lineages (Pichersky, Noel, & Dudareva, 2006).

It has been shown that essential oils purified from several plants have anti-tumoural properties and some of them have anti-oxidant activity, which could be related to their anti-neoplastic activity (Edris, 2007). However, some compounds present in essential oils can also deplete cells from glutathione and several thiols, when added in higher doses (Yoo et al., 2005), showing that essential oils can also be pro-oxidative by increasing the production of oxygen free radicals.

In general terms, essential oils are composed by terpenoids (monoterpenes and sesquiterpenes) and phenylpropanoids, but they can also present certain amounts of several other substances with different chemical composition (Pichersky et al., 2006). Monoterpenes, one of the main groups of terpenoids present in essential oils, have been tested in different animal systems and it has been shown that they can inhibit tumour growth, specifically breast, kidney, skin, lung and stomach cancer. Some studies have also been done in cell cultures and some of these substances such as limonene and perillyl alcohol have been tested in clinical trials (Crowell, 1999).

Together with the ability to inhibit cellular growth, monoterpenes present in essential oils can also induce cell differentiation and death, mainly by apoptosis, during the phase of tumour promotion (Belanger, 1998).

The botanical family Anacardiaceae includes 73 genera and about 850 species worldwide, in tropical, subtropical, Mediterranean and temperate regions. *Schinus* comprises about 30 species of trees and shrubs that are native to South America (Argentina, Bolivia, Chile, Paraguay, Peru, Southern Brazil and Uruguay) (Barkley, 1944; Mabberley, 1990). *Schinus molle* L. (pepper tree) is known in Costa Rica as 'pirul' or 'chile'. It is a resinous evergreen small tree or shrub. The plant grows up to 7–10 m tall, with rather sparse pinnately compound leaves and pink to reddish fruits of 5 mm in diameter (Gupta, 1995; Holdridge, Poveda, & Jiménez, 1997). In Costa Rica, *S. molle* is occasionally used as an ornamental garden tree but in other countries this plant is utilised popularly for many ailments. It is used as anti-rheumatic, antiseptic, anti-inflammatory, antifungal, antibacterial, cicatrizant and also for the treatment of skin disorders (Gupta, 1995; Ruffa et al., 2002). Recently, it was reported that a hexanic extract of leaves and fruits could be repellent and insecticidal for Chagas disease vector *Triatoma infestans* (Ferrero, Werdin González, & Sánchez Chopa, 2006). Also, in ancient times, Incas in the Central Andes used *S. molle* fruits to produce the alcoholic beverage named 'chicha de molle' (Goldstein & Coleman, 2004).

The methanolic extract of *S. molle* (leaves and bark) has been shown to be very toxic on human hepatoma HepG2 cell line, compared to the extracts obtained from other medicinal plants (Ruffa et al., 2002). Recently, Ibrahim, Fobbe, & Nolte (2004) reported that the leaf oil of *S. molle* from Egypt shows a strong activity against Ehrlich ascites carcinoma cell lines. In terms of its essential oil, Dikshit, Naqvi, & Husain (1986) studied the antifungal activity of the leaf oil and Gundidza (1993) had previously reported some effects, such as antimicrobial activity.

The chemical composition of leaf essential oil from *S. molle* has been also the subject of some studies (Baser, Kürkçüoglu, Demirçakmak, Ülker, & Beis, 1997; Ibrahim et al., 2004; Maffei & Chialva, 1990; Rossini, Menéndez, Dellacassa, & Moyna, 1996; Talenti, Ubiergo, & Taher, 1989).

Since essential oils can also be toxic for normal cells such as primary fibroblasts (Hayes & Markovic, 2002) and monocytes, neutrophils and epithelial cells (Hammer, Carson, Riley, & Nielsen, 2006), it is imperative to study the effect of them in normal cells also, to predict their *in vivo* effects and become aware of the doses that could be tolerated in potential treatments.

This study has the goal of characterising the chemical composition of *S. molle* leaf essential oil from Costa Rican origin as well as measuring the potential toxicity of this oil in several cell lines, some derived from tumours and others with non-tumourogenic characteristics. In addition, we intend to partially characterise the cytotoxic mechanism of this essential oil as well as to determine its antioxidant activity.

2. Materials and methods

2.1. *Plant material*

Leaves of *S. molle* L. were collected in the location of Tacares, Alajuela (Costa Rica) in June 2001. The plant material was identified by L.J. Poveda (Escuela de Ciencias Ambientales, UNA, Heredia). A voucher specimen was deposited in the Herbarium of the University of Costa Rica under accession number USJ-90489.

2.2. *Extraction of essential oil*

Fresh leaves (1 kg) were subjected to hydrodistillation for 2 h using a modified Clevenger-type apparatus. The distilled oil was collected and dried over anhydrous sodium sulfate, filtered and stored in a freezer (0–10°C). The colourless oil yield was 1.2% (v/w).

2.3. *Gas chromatography analyses*

The oil of *S. molle* was analysed by GC/FID using a Shimadzu GC-17 gas chromatograph. The data were obtained on a 5% phenyl methylpolysiloxane fused silica capillary column (30 m × 0.25 mm; film thickness 0.20 μm), Heliflex (Alltech) AT-5, with a Shimadzu Class-VP, version 4.3 software. Operating conditions were: carrier gas N_2, flow 1.0 mL min^{-1}; oven temperature programme: 60–220°C at 3°C min^{-1}, 220°C (10 min); sample injection port temperature 250°C; detector temperature 275°C; split 1 : 50.

The analysis by GC/MS was performed using a Shimadzu GC-17A gas chromatograph coupled to a GCMS-QP5050 apparatus and CLASS 5000 software with Wiley138 computer database. Data were obtained on a 5% phenyl methylpolysiloxane fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 μm). Operating conditions were: carrier gas He, flow 1.0 mL min^{-1}; oven temperature programme: 60–240°C at 3°C min^{-1}; sample injection port temperature 250°C; detector temperature 260°C; ionisation voltage: 70 eV; ionisation current 60 μA; scanning speed 0.5 s over 38–400 amu range; split 1 : 70.

Identification of the components of the oil was achieved using the retention indices on DB-5 type column, and by comparison of their mass spectra with those published in the literature (Adams, 2001; McLafferty, 1993) or those of our own database.

The quantification of the components was performed on the basis of their GC peak areas, without corrections for response factor.

2.4. *Determination of DPPH radical scavenging activity*

Radical scavenging activity (RSA) was evaluated measuring the scavenging activity of *S. molle* essential oil on 2.2 diphenyl 1-picrylhydrazil (DPPH). Two millilitres of several oil dilutions (10–50 $\mu g\,mL^{-1}$) were combined with 1 mL of DPPH (0.25 mM in methanol). The absorbance of the samples and control (2 mL methanol + 1 mL DPPH) was recorded after 1 h in the darkness at room temperature, at 517 nm. IC_{50} represents the oil concentration ($\mu g\,mL^{-1}$) that induces a RSA of 50%, obtained from a linear regression analysis. RSA was calculated with the following equation: % $RSA = 100*(A_{control} - A_{sample}/A_{control})$. The activities were compared with that of a commercial standard antioxidant (butylated hydroxytoluene, BHT) (Bozin, Mimica-Dukic, Simin, & Anackov, 2006).

2.5. *Cell culture and treatment*

Mouse macrophage J774, human hepatoma Hep3B and HepG2, human bladder carcinoma ECV-304, human leukemic K562, mouse myoblast C2C12 and mouse breast carcinoma EMT6 were obtained from American Type Culture Collection. Cells were maintained in Dulbecco essential medium supplemented with 10% foetal bovine serum, 2 mmol L^{-1} glutamine, 100 IU mL^{-1} penicillin and amphotericin B in a 37°C humidified incubator under an atmosphere of 7% CO_2 in air. For the experiments, adherent cells were cultured in 96-well plates to confluence and allowed to adhere overnight. Non-adherent cells were plated at 10,000 per well. Various concentrations of *S. molle* essential oil, dissolved in 95% ethanol, were added to the plates containing cells in 100 µL of fresh medium and were incubated for 48 or 72 h. (R)−(+) Limonene (Sigma-Aldrich) was used as a standard for cytotoxicity.

2.6. *Assessment of cytotoxicity by MTT assay*

After the cells were treated with increasing concentrations of the essential oil for 48 h (or 72 h), 10 µL of (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (MTT reagent, 5 mg mL^{-1} in PBS) was added to the culture medium and, after 2 h at 37°C, medium was carefully removed and 100 µL of 95% ethanol was added to the wells to dissolve the formazan crystals. Absorbances were read at 570 nm and results were expressed as viability percentages, using samples incubated with ethanol as 100% viability value. The LD_{50} values were calculated with Slidewrite plus 6.1 programme.

2.7. *Determination of apoptosis and necrosis by flow cytometry*

Cells were treated with the essential oil at several concentrations for 24 h and after removal from the plates by Trypsin-EDTA treatment, they were washed twice with PBS. For apoptosis determination, cells were resuspended in 1 mL of hypotonic propidium iodide solution (PI, 10 mg mL^{-1}, 0.1% sodium citrate, 0.1% Triton X-100)

for 1 h at 4°C. The percentage of hypodiploid cells present in each sample was then determined by flow cytometry by displaying cell cycle distribution according to DNA content.

For necrosis determination, cells were incubated with PI solution without membrane permeabilisation, using the same PI concentration mentioned above. Cells with disrupted plasma membrane and positive for PI were counted with the flow cytometer and the results are expressed as a percentage. The LD_{50} values were calculated with Slidewrite plus 6.1 programme.

3. Results

3.1. *Chemical composition of the essential oil*

The chemical composition of the leaf oil of *S. molle* growing in Costa Rica is presented in Table 1. In total, 42 constituents were identified, and more than 97.2% of the total oil was characterised. Monoterpenoids were the main constituents of the oil (58.9%), including β-pinene (31.1%) and α-pinene (22.7%) as the main compounds. The second group in quantity was constituted by sesquiterpenoids (29.9%), with γ-cadinene (6.0%), *epi*-α-cadinol (5.6%) and β-caryophyllene (4.7%) as the major constituents.

3.2. *Antioxidant activity of the essential oil*

From the linear regression analysis (Figure 1), we obtained the value for *S. molle* essential oil antioxidant activity; the concentration that reduces DPPH from purple to orange colour. The value obtained for the IC_{50} for this essential oil was 36.3 $\mu g\,mL^{-1}$.

3.3. *Cytotoxicity of the essential oil*

Experiments of cytotoxicity show that *S. molle* essential oil induces different degrees of damage in different cell lines at 48 h of treatment. Three lines derived from tumours (bladder and liver) were not very susceptible to the oil, showing a LD_{50} of 211.2 $\mu g\,mL^{-1}$ (Hep3B), 118.8 $\mu g\,mL^{-1}$ (ECV-304) and 108.9 $\mu g\,mL^{-1}$ (HepG2), the hepatocellular carcinoma cell line Hep3B being the most resistant to the effect (Figure 2). Breast carcinoma cell line showed more sensitivity to the oil, which presented a LD_{50} of 75.7 $\mu g\,mL^{-1}$ (Figure 2d).

When we tested non-tumour cell lines to the essential oil, we observed that there was also a variability between the two tested cell types. Macrophages were more resistant than myoblasts to the effect (Figure 3), but both cell lines were basically resistant. The LD_{50} values for J774 cell line corresponded to 252.8 $\mu g\,mL^{-1}$ and for C2C12 was 116.1 $\mu g\,mL^{-1}$.

Leukemic cell line K562 showed a LD_{50} of 78.7 $\mu g\,mL^{-1}$ at 48 h and 65.7 $\mu g\,mL^{-1}$ at 72 h of treatment (Figure 4), being one of the most susceptible cell types tested in the study. We also observed that the effect obtained by treating K562 cells with *S. molle* essential oil was stronger than the one obtained exposing the cells to pure limonene, which was used as a standard (LD_{50} of 242.9 $\mu g\,mL^{-1}$ at 48 h and 103.1 $\mu g\,mL^{-1}$ at 72 h) (Figure 4).

Table 1. Composition of the essential oil isolated from the leaves of *S. molle*.

Compound[a]	RI[b]	Composition (%)	Identification method[d]
tricyclene	927	0.1	1, 2
α-thujene	934	0.2	1, 2
α-pinene	938	22.7	1, 2, 3
camphene	953	0.5	1, 2
sabinene	974	0.2	1, 2
β-pinene	979	31.1	1, 2, 3
myrcene	991	0.3	1, 2
α-phellandrene	1007	0.1	1, 2
α-terpinene	1018	t[c]	1, 2
p-cymene	1025	0.1	1, 2
limonene and	1028		1, 2, 3
β –phellandrene	1029	2.6	1, 2
1,8-cineole	1033	t	1, 2, 3
(*Z*)-β-ocimene	1040	t	1, 2
(*E*)-β-ocimene	1049	0.6	1, 2
γ-terpinene	1062	0.1	1, 2
terpinolene	1089	t	1, 2
terpinen-4-ol	1177	0.2	1, 2
α-terpineol	1191	0.1	1, 2, 3
α-copaene	1377	0.1	1, 2
β-elemene	1391	0.2	1, 2
α-gurjunene	1412	0.3	1, 2
β-caryophyllene	1420	4.7	1, 2, 3
cis-muurola-3,5-diene	1449	t	1, 2
α-humulene	1452	0.6	1, 2
allo-aromadendrene	1459	4.0	1, 2
germacrene D	1487	3.9	1, 2, 3
viridiflorene	1495	0.1	1, 2
bicyclogermacrene	1499	3.0	1, 2
α-muurolene	1502	0.7	1, 2
γ-cadinene	1509	6.0	1, 2
δ-cadinene	1524	3.9	1, 2, 3
trans-cadina-1(2),4-diene	1534	0.1	1, 2
α-cadinene	1537	0.5	1, 2
spathulenol	1577	0.6	1, 2
globulol	1583	0.8	1, 2
viridiflorol	1591	0.4	1, 2
1,10-di-*epi*-cubenol	1615	1.7	1, 2
1-epicubenol	1627	0.2	1, 2
epi-α-cadinol	1639	5.6	1, 2
α-muurolol	1646	0.2	1, 2
α-cadinol	1658	0.8	1, 2
Monoterpenes		58.6	
Oxigenated monoterpenes		0.3	
Sesquiterpenes		28.1	
Oxigenated monoterpenes		10.3	

Notes: [a]Compounds listed in order of elution from 5% phenyl- 95% methylpolysiloxane column.
[b]RI = Retention Index (experimental), relative to *n*-alkanes on the 5% phenyl–95% methylpolysiloxane column.
[c]t = traces (< 0.05%).
[d]Method: 1 = Retention indices on 5% phenyl–95%methylpolysiloxane column; 2 = MS spectra; 3 = standard.

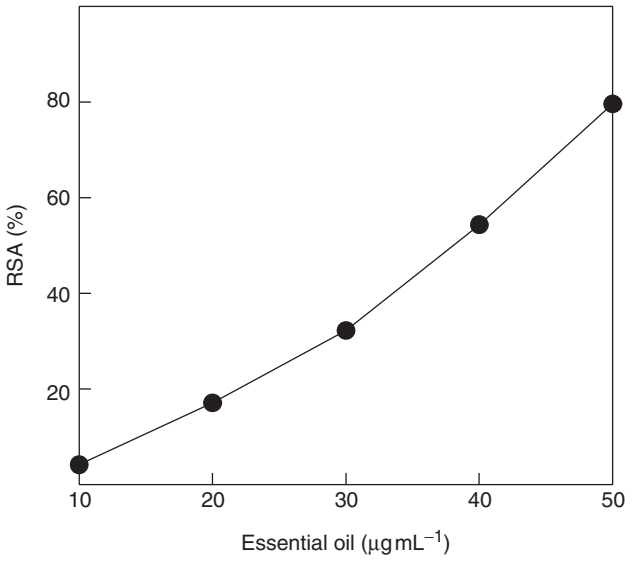

Figure 1. Antioxidant activity of *S. molle* essential oil.
Note: (Radical scavenging activity (%) of different concentrations ($\mu g\,mL^{-1}$) of the essential oil).

3.4. *Characterisation of the cytotoxic mechanism of the essential oil*

The essential oil from *S. molle* induced apoptosis in K562 cells when treated for 24 h. Figure 5 shows the effect of different concentrations of the oil, starting with 12.5 $\mu g\,mL^{-1}$ and ending with 400 $\mu g\,mL^{-1}$. At the first concentration tested, the results were very similar to the effect of ethanol, the solvent used to dilute the essential oil, but at 25 $\mu g\,mL$ the effect doubled (Figure 5).

The LD_{50} for the apoptotic effect was 73.9 $\mu g\,mL^{-1}$, and the results indicate that a necrotic effect (membrane disruption) was observed only at higher concentrations, showing a LD_{50} of 242.2 $\mu g\,mL^{-1}$ (Figure 6). Apoptotic effect correlates very closely with the cytotoxic effect measured by MTT assay, which indicates that apoptosis is the main mechanism of cell death induced by this essential oil.

4. Discussion

Some of the essential oils obtained from *S. molle* growing in different countries present α-phellandrene (45.7-30.2%) as the main constituent (Baser et al., 1997; Ibrahim et al., 2004; Maffei & Chialva, 1990). The oil of the leaves from Uruguayan origin (Rossini et al., 1996), was rich in sesquiterpenes: bicyclogermacrene (29.3%) and germacrene D (12.1%), whereas the oil of Argentinean origin presents several compounds: α-pinene (11.7%), β-pinene (10.8%), α-phellandrene (10.4%), limonene (9.3%), phellandral (8.4%), geranyl acetate (8.2%) and β-phellandrene (6.3%) (Talenti et al., 1989). When we analysed the chemical composition of the oil from the plants growing in Costa Rica, we found that the main compounds were the monoterpenoids β- and α-pinene, which together represented more than 50% of the oil. It is interesting to notice that the

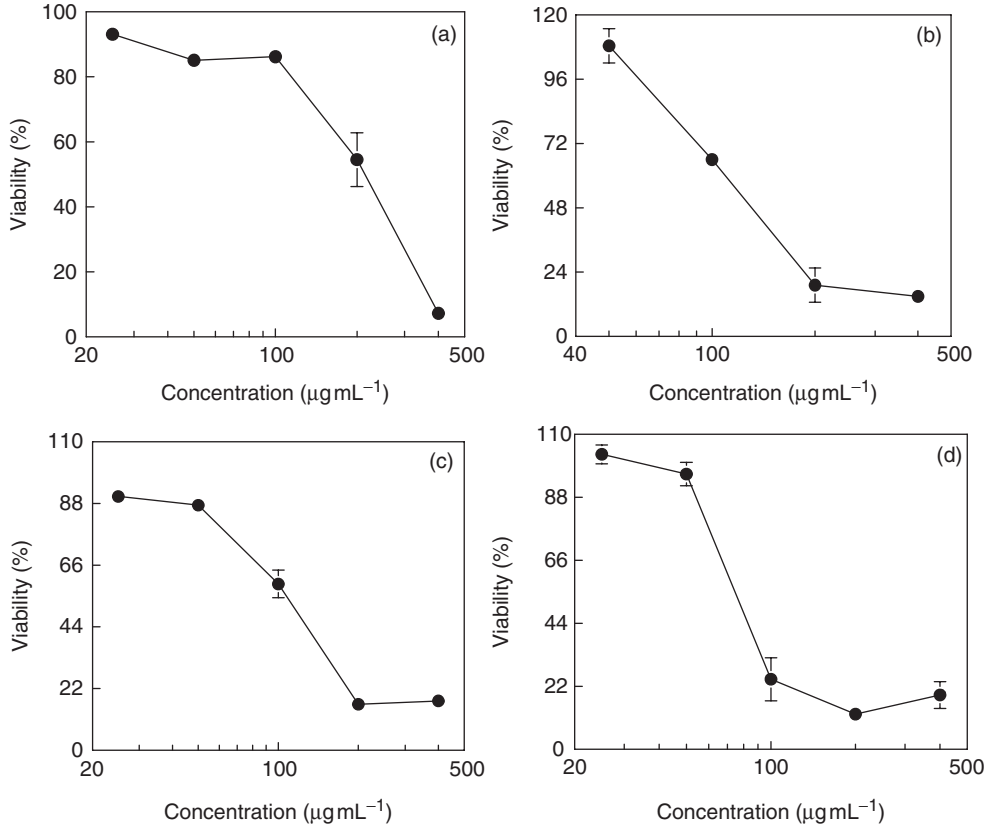

Figure 2. Cytotoxicity of *S. molle* essential oil on tumour cell lines. (a) Hepatoma Hep3B cell line; (b) Bladder carcinoma ECV-304 cell line; (c) Hepatoma HepG2 cell line; (d) Breast carcinoma EMT-6 cell line.
Notes: Cells were treated for 48 h with different concentrations of the oil dissolved in ethanol. MTT activity was determined at 570 nm and expressed as viability percentage using solvent as a negative control. Results are presented as mean±SE of triplicates of one representative experiment.

former compound only represents about 14% of the oil obtained from the plants grown in Uruguay (Rossini et al., 1996). Regarding sesquiterpenes found in the oil of the Costa Rican plants, we detected mainly γ-cadinene, *epi*-α-cadinol and β-caryophyllene in minor amounts, none of which were found in high concentrations in *S. molle* leaf oils reported in the literature. The sesquiterpene elemol that is present in the sample of Maffei and Chialva (1990) in about 13%, was absent in our sample. These results mean that both qualitative and quantitative differences can be observed in the chemical composition of the oils from plants grown in different regions. This variability could reflect the influence of extrinsic conditions based on geographical origin (different climatic and soil growing conditions) and the effect of infra-specific differences. This could suggest that different varieties or chemotypes of *S. molle* could exist around the world (Rossini et al., 1996).

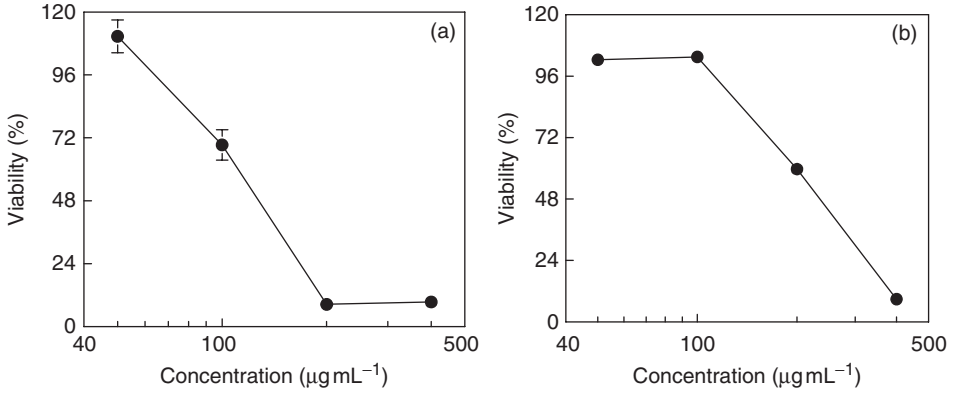

Figure 3. Cytotoxic effect of *S. molle* essential oil on two non-tumourogenic cell lines. (a) Myoblastic cell line C2C12. (b) Macrophage cell line J774.
Note: Cells were treated for 48 h with different concentrations of the oil dissolved in ethanol (see Figure 2 for details).

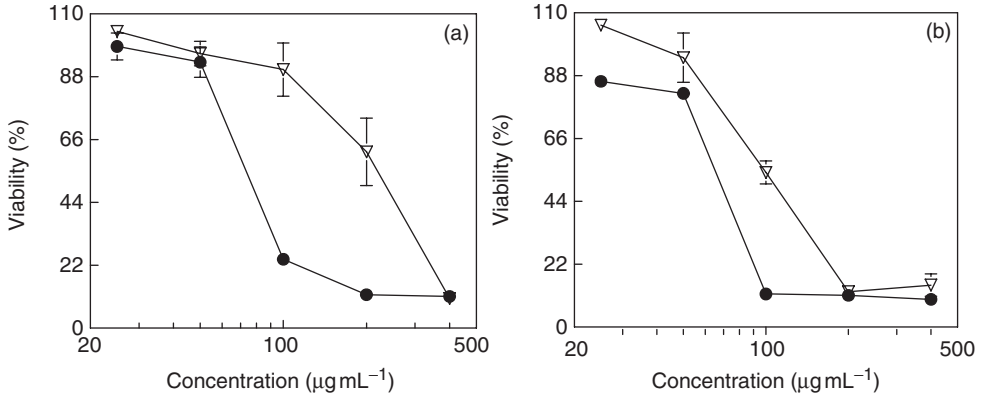

Figure 4. Cytotoxic effect of *S. molle* essential oil on leukemic cell line K562. (a) 48 h; (b) 72 h. Note: Circles represent *S. molle* essential oil and triangles represent the effect of limonene (see Figure 2 for details).

Regarding the cytotoxic effect that *S. molle* leaf essential oil produces in mammalian cells, we were not able to find any reports in the literature. However, an interesting study, which examined the methanolic extract of the leaves showed that *S. molle* was very toxic for the human hepatoma cell line HepG2, compared to the extracts obtained from other medicinal plants (Ruffa et al., 2002). The authors suggested that more studies were necessary, since this medicinal plant could have potential anti-tumoural activities. Taking this study into consideration, we decided to test the cytotoxic activity of the essential oil obtained from this plant on different cell lines.

When we tested the oil in mammalian cell lines (from mouse and human origin), some of which were originated from tumours, we found that the most sensitive cell types were

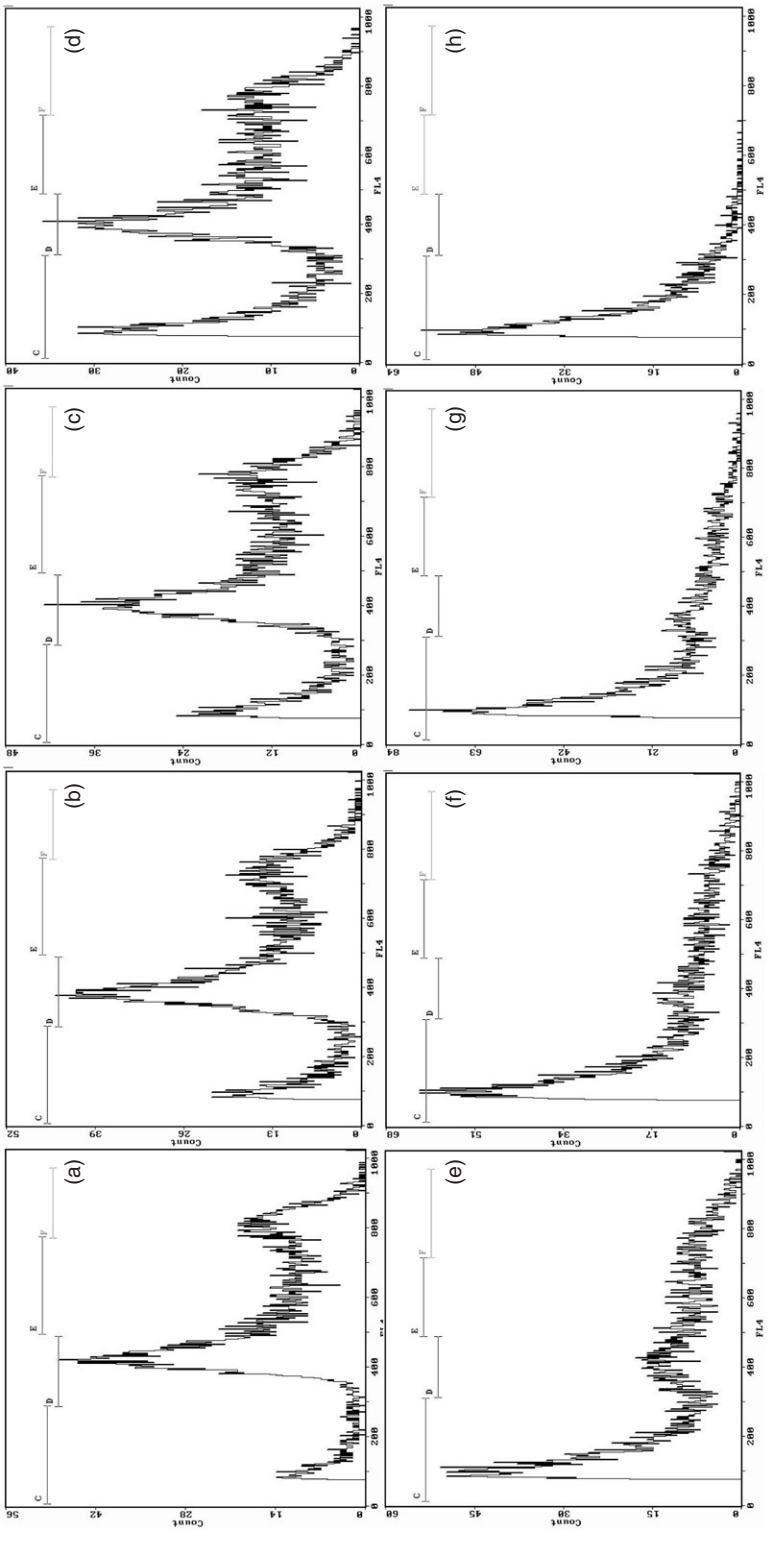

Figure 5. Flow cytometry analysis of the effect induced by *S. molle* essential oil on K562 cells treated for 24 h.
Notes: Apoptotic index (percentage of hypodiploid cells) is presented in parenthesis. (a) control (9.0); (b) ethanol (14.4); (c) 12.5 μg mL (16.0); (d) 25 μg mL^{-1} (24.7); (e) 50 μg mL^{-1} (45.8); (f) 100 μg mL^{-1} (57.0); (g) 200 μg mL^{-1} (61.4); (h) 400 μg mL^{-1} (88.5). Results show one of three representative experiments.

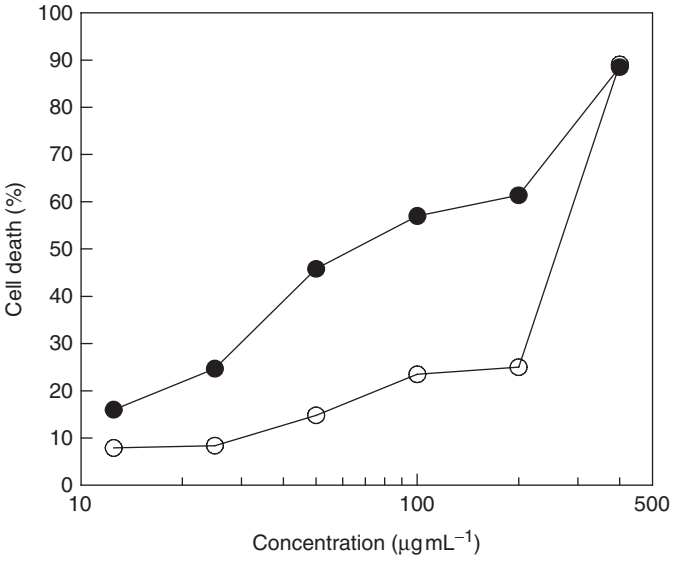

Figure 6. Characterisation of cell death induced by *S. molle* essential oil on K562 cells treated for 24 h.
Notes: Full circles represent apoptosis and open circles represent necrosis percentages, both measured by flow cytometry of cells stained with propidium iodide. Results are presented as mean±SE of triplicates of one representative experiment.

a mouse breast carcinoma and a human leukemic cell line. In both cases the effect was very rapid and more toxic than the effect produced by pure limonene, which was used as a standard since it has been shown to induce cytotoxicity in several cell types (Crowell, 1999; Edris, 2007; Tsuda et al., 2004).

Regarding the effect of the oil in other cell lines, we observed that hepatocellular carcinoma cell line Hep3B was very resistant whereas a less transformed hepatoma cell line (HepG2) and ECV-304, a cell line that originated from a bladder carcinoma, present an intermediate susceptibility. The non-tumour cell lines C2C12 and J774 were also somehow resistant to the treatment, strengthening the possibility that some components of the essential oil could be potentially useful for treating some specific types of tumours, as long as concentrations that have low effect in normal cells, are chosen.

It has been shown that some essential oils induce cytotoxicity specifically in leukemic cells. This is the case of some *Citrus* essential oils, which are particularly rich in limonene (Hata et al., 2003). The same is also observed with substances purified from some essential oils, such as eugenol (Yoo et al., 2005) and 1,8-cineole (Moteki et al., 2002), which are not found in *S. molle* leaf oil. In the case of the effects obtained with the *Citrus* oils, cytotoxicity is not completely attributed to the presence of limonene, since the apoptotic effects obtained with sweet orange, grapefruit and lemon oils were higher than those obtained with pure limonene (Hata et al., 2003). This indicates that other compounds present in minor quantities in the oils also have anti-tumoural potential and should be studied carefully.

When we determined the LD_{50} of the essential oil, we observed that *S. molle* toxicity was among average. For example, the IC_{50} of the oil obtained from *Malaleuca alternifolia*,

when cells such as HeLa, K562, HepG2, HL-60, fibroblasts and epithelial cells were exposed for 24 h, ranged from 20 to 2700 $\mu g\,mL^{-1}$ (Hammer et al., 2006). Essential oils from *Angelica archangelica* (Sigurdsson, Ogmundottir, & Gudbjarnason, 2005) and *Tetraclinis articulata* (Buhagiar, Camilleri Podesta, Wilson, Micallef, & Ali, 1999) applied on several tumour cell lines for 24 h, ranged from 48.0 to 108.3 and 125.0 to 210.0 $\mu g\,mL^{-1}$, respectively, showed values very similar to the ones obtained in this study.

Further characterisation of the mechanism of cell death induced by this essential oil, revealed that it is apoptosis. K562 cell membranes remained intact for a longer period of time while DNA fragmentation was taking place, which indicates that necrosis is probably a secondary mechanism. Apoptosis has been the mechanism of death suggested for several essential oils and some of its constituents and this activity would be related to their therapeutic potential (Edris, 2007).

Since the antioxidant activity is important for protective effects against several agents and anti-tumoural activity (mainly chemoprevention), we decided to measure the activity of this oil using DPPH. The activity was 36.3 $\mu g\,mL^{-1}$, which seems low when compared to the values obtained from other essential oils, such as the ones reported for *Melissa officinalis* ($IC_{50} = 7.58$), *Origanum vulgaris* ($IC_{50} = 0.17\,\mu g\,mL^{-1}$) and *M. alternifolia* ($IC_{50} = 1\,\mu g\,mL^{-1}$) (Bozin et al., 2006; Kim et al., 2004; Kim, Chen, Wang, Chung, & Jin, 2005), and the one obtained for standard BHT ($IC_{50} = 11.4\,\mu g\,mL^{-1}$). This implies that *S. molle* essential oil has low scavenging activity, however, it was still higher than the one reported for *Artemisia dracunculus* (Kordali et al., 2005).

In conclusion, this study demonstrates that the essential oil of *S. molle* has some anti-tumoural potential, at least for nonsolid tumours such as some types of leukemia. However, the effect on mouse breast carcinoma cells and a human gastric carcinoma cell line (preliminary results not shown) suggests that it can also have strong effect on solid tumours. Since several currently used anti-tumoural agents were originally obtained from plant extracts, it would be interesting to determine whether the effect of this oil is due to a combination of the effects of different constituents or based on the main components present. More studies are necessary to continue the characterisation of different plant essential oils, some of which could have potential in the treatment of diseases.

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