

An. R. Acad. Nac. Farm., 2010, 76 (3): 343-356

ARTÍCULO

Cytotoxic activity of α -humulene and transcaryophyllene from *Salvia officinalis* in animal and human tumor cells

Adil el Hadri^{1,4*}, María Ángeles Gómez del Río¹, Jesús Sanz², Azucena González Coloma³, Mohamed Idaomar⁴, Bartolomé Ribas Ozonas⁵, Juana Benedí González¹, María Isabel Sánchez Reus¹

¹ Department of Pharmacology, Faculty of Pharmacy, University Complutense Madrid, 28040-Madrid, Spain.

² Instituto de Química Orgánica General (CSIC), Juan de la Cierva, 3, 28006-Madrid, Spain.

³ Centro de Ciencias Medioambientales (CSIC), Serrano, 115, 28006 Madrid, Spain.

⁴ Laboratory of Biology and Health, Faculty of Sciences, University Abdelmalek Essâadi BP 2121, 93002-Tétouan, Morocco.

⁵ Royal National Academy of Pharmacy, Institute of Spain, Farmacia 11, 28004-Madrid, Spain.

Recibido el 29 de abril de 2010.

ABSTRACT

Background: The purpose of the present work is two-fold: the fractionation of *Salvia officinalis* essential oil and the cytotoxic study of this oil with its fractions “*in vitro*” tumor cell lines. **Materials and Methods:** *S. officinalis* essential oil was obtained by hydrodistillation and fractionated with column chromatography; the essential oil and its fractions were analyzed by gas chromatography (GC) coupled to mass spectrometry (MS). The cytotoxic activity was evaluated in cellular lines of breast cancer MCF-7, colon cancer HCT-116, and murine macrophage RAW264.7 cell lines by the MTT assay. **Results:** the sub-subfraction F1.1.1 of *S. officinalis* essential oil containing α -humulene present highest activity on RAW264.7 and HCT-116 with

IC₅₀ values of 41.9 and 77.3 µg/ml, respectively. The sub-subfraction F1.2.1 of *S. officinalis* essential oil with trans-caryophyllene showed less activity on RAW246.7 and HCT-116 with IC₅₀ values of 90.5 and 145.8 µg/ml. Conclusion: This paper suggests that the α-humulene and trans-caryophyllene extracted from *S.officinalis* essential oil inhibit tumor cell growth.

Keywords: *Salvia officinalis*; cytotoxicity; sesquiterpenes; α-humulene; trans-caryophyllene.

RESUMEN

Actividad citotóxica del α-humuleno y del tras-cariofileno de *Salvia officinalis* en dos líneas celulares tumorales animal y humana

Antecedentes: Este trabajo tiene dos objetivos: el fraccionamiento del aceite esencial de la especie *Salvia officinalis* y la determinación de la citotoxicidad del mencionado aceite esencial con sus fracciones en líneas celulares tumorales “*in vitro*”. Material y Métodos: El aceite esencial de *Salvia officinalis* fue obtenido por hidrodestilación y fraccionado mediante cromatografía en columna; el aceite esencial y sus fracciones fueron analizadas mediante cromatografía de gases (GC) acoplada a espectrometría de masas (MS). La actividad citotóxica fue evaluada en líneas celulares de cáncer de mama MCF-7; cáncer de colon HCT-116 y en macrófago murino RAW264.7 con el ensayo MTT. Resultados: La sub-subfracción F1.1.1 del aceite esencial de *Salvia officinalis* que contiene alfa-humuleno presenta la actividad mas acusada frente a las líneas celulares RAW264.7 y HCT-116, con valores de IC₅₀ de 41,9 y de 77,3 µg/ml respectivamente. La sub-subfracción F1.2.1 del aceite esencial de *Salvia officinalis* con trans-cariofileno, muestra menor actividad sobre células RAW246.7 y HCT-116 con valores de IC₅₀ de 90,5 y 145,8 µg/ml respectivamente. Conclusión: Estos resultados sugieren que el alfa-humuleno y el trans-cariofileno de los extractos del aceite esencial de *Salvia officinalis* inhiben el crecimiento de células tumorales.

Palabras Clave: *Salvia officinalis*; citotoxicidad; sesquiterpenos; alfa-humuleno; trans-cariofileno.

1. INTRODUCTION

The genus *Salvia* L. (sage) of the family *Lamiaceae* is an aromatic and medicinal plant and includes about 900 species spread throughout the world. *Salvia officinalis* L. (*S. officinalis*), with the common arabic name *sâlma*, *sâlniya* or *swak en-nâbi*, is a native to the Mediterranean region that is now extensively cultivated all over the world (1). This plant has been known and utilized for hundreds of years in natural medicine, due to its curative properties and good performance in combating various diseases. Recent publications have shown some interesting features of its chemical composition, which is determined by its volatile (2) and nonvolatile compounds, and its applications in antibacterial (3), anti-inflammatory (4), antifungal (5), antiviral (6), antioxidant activities (7) and clinical treatment of Alzheimer's disease (8). Terpenoids and phenolics have been identified as the two major typical products of *S. officinalis* secondary metabolites (9). Among the terpenoids, volatile oils have been largely investigated (9, 10) because of their broad range of applications to culinary, cosmetic, pharmaceutical, and industrial fields. The essential oil composition of *Salvia* species is highly influenced by genetic and environmental factors, climate conditions (11), season, and culture site (10, 12). The strongest active constituents of sage are within its essential oil (1-2.8%), comprising the monoterpenes α - and β -thujone, camphor, 1,8-cineole, and borneol as well as the sesquiterpenes α -humulene and β -caryophyllene in larger amounts; whereas the leaf contains di- and triterpenes, as well (13). The compounds of the essential oils were divided into five groups according to the amount of the major constituents (13):

1. camphor > α -thujone > 1,8-cineole > β -thujone ((14) and our essential oil).
2. camphor > α -thujone > β -thujone > 1,8-cineole (15).
3. β -thujone > camphor > 1,8-cineole > α -thujone.
4. 1,8-cineole > camphor > α -thujone > β -thujone.
5. α -thujone > camphor > β -thujone > 1,8-cineole.

Moreover, many studies have reported antitumor activities of *Salvia* species, especially for *S. miltiorrhiza*, and *S. officinalis* under *in vitro* conditions (16, 17). In addition, *S. officinalis* has also a poten-

tial in treating cancer “*in vivo*” as it shows strong antitumorigenic activities in mice (18).

In this study, we report the chemical composition of *S. officinalis* collected in the north-west of Morocco. The leaf essential oil and its active fractions, subfractions and sub-subfractions obtained were investigated by gas chromatography (GC) and GC/mass spectrometry (GC-MS) analysis. They were tested against two human cancer cell lines (HCT-116, MCF-7) and murine macrophage cell line (RAW264.7) under *in vitro* conditions.

2. MATERIALS AND METHODS

2.1. Essential oil

The leaves of *S. officinalis* plant were collected in Tétouan area, north-west of Morocco in June 2008. The botanical identification of *Salvia* plant and the extraction of essential oil were realized in the Biology and Health laboratory of the Département de Biologie, Université Abdelmalek Essaâdi Tétouan. Two thousand grams of fresh material were submitted to hydrodistillation for 3 h using a Clevenger-type apparatus. The white-yellow essential oil was dried over anhydrous sodium sulphate and stored at 4 °C.

2.2. Fractionation of *S. officinalis* essential oil

Column chromatography was used for the fractionation of the essential oil. Fifteen grams of the *S. officinalis* essential oil was fractionated using a column chromatography (1.5 cm× 500 cm) packed with 100 g of silica gel 60, equilibrated with hexan. The column was eluted successively with 250 ml each of n-hexan (100), n-hexan-ethyl acetate (95/5, 90/10, 85/15, 75/25, 50/50, 20/80, 10/90), ethyl acetate (100), and methanol. The organic solvents were removed from the eluates by evaporation under reduced pressure to get nine fractions (F1-F9). The first fraction F1 showed a significant cytotoxic activity and was further fractionated (Table 2). First by preparative thin-layer chromatography (TLC) and achieved using silica gel 60-F₂₅₄-precoated plates

(Merck) with heptane (100) as the mobile phase. The plates were revealed by spraying with vanillin/ sulfuric acid reagent and subsequent heating at 105 °C. Three subfractions were obtained (F1.1, F1.2 and F1.3); the subfractions F1.1 and F1.2 showed a cytotoxic activity in the three cell lines (Table 2). In the same way, the two active subfractions were fractionated again with TLC analysis in silica plates with toluene/ethyl acetate (97/3) as mobile phase, and two sub-subfractions F1.1.1 and F1.2.1 were selected from the subfractions F1.1 and F1.2 respectively.

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis of the essential oil *S. officinalis* and of its active fractions was carried out using a Hewlett-Packard 7890 gas chromatograph equipped with a methylpolysiloxane capillary column (30 m length, 0.25 mm internal diameter, 0.25 film thickness) coupled to a Hewlett Packard 5975C mass spectrometer. Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The carrier gas was helium (5psi, 0.7ml/min) and the temperature program was 70 °C for 2min, then 6 °C/min to 270 °C and held constant for 20 min. 1 μ l of a hexane solution at 0.2-1% was injected in the split mode. Volatile components were identified from their retention data and their mass spectra, which were compared with reference data (Wiley mass spectral library). Relative amount was estimated from total ion current peak area.

2.4. Cancer cell lines

The colorectal adenocarcinoma human cell line (HCT-116), human breast cancer cell (MCF-7) and murine macrophage cell line (RAW264.7) were cultured in RPMI-1640 medium (+)L-glutamin, supplemented with 10% foetal bovine serum, 1% penicillin/streptomycin, 2 mg of gentamicin, 1% sodium pyruvate 100 mM (Invitrogen-Gibco, Madrid, Spain) and 1% D-(+) glucose solution (45%) (Sigma-Aldrich, Madrid, Spain). Cells were cultured in a humidified atmosphere at 37 °C in 5% CO₂. The cultures were passed twice a week by trypsinization using a trypsin-EDTA (0.05%) solution (Invitrogen-Gibco, Madrid, Spain).

2.5. Chemicals

1,8-cineole, α -thujone, β -thujone, α -humulene, trans-caryophyllene, tamoxifen and different solvents were purchased from Sigma-Aldrich, Madrid, Spain.

2.6. Measurement of viability using the MTT Assay

Cytotoxicity was assessed using the microtitration colorimetric method of MTT reduction test as described by Mosmann in 1983 (19). The cells were trypsinized, counted and placed in 96-well plates at optimal plating density of each cell line which was determined over a range from 5×10^4 to 10×10^4 . After 24 h, the cells were treated with serial dilutions of the samples. Each sample was initially dissolved in dimethyl sulphoxide (DMSO) and further diluted in medium to produce different concentrations. One hundred microliters/ well of each dilution were added to the plates in six replicates to obtain the final concentrations ranging from 12.5 to 800 $\mu\text{g/ml}$ for whole essential oil, their active fractions, subfractions and sub-subfractions, from 1.56 to 100 $\mu\text{g/ml}$ for the commercially available identified constituents (1,8-cineole, β -thujone, α -humulene, trans-caryophyllene). All compounds were dissolved in DMSO and the final concentration of DMSO in the culture medium was maintained at 0.5% (v/v). Cytotoxic activity of these compounds was expressed as the concentration of drugs inhibiting cell growth by 50% (IC_{50}). Fluorescence was measured on the automated 96-well plate reader at 550 nm. Tamoxifen (Sigma-Aldrich, Madrid, Spain) was used as positive control.

2.7. Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means \pm S.D. Differences were evaluated by the one-way analysis of variance (ANOVA) test completed by a multicomparison Dunnett's test. Differences were considered significant at (* $P < 0.05$). The IC_{50} was calculated by a nonlinear regression curve with the use of Prism GraphPad Prism version 5.0 software for Windows. The dose-response curve was obtained by plotting the percentage of inhibition versus the concentrations.

3. RESULTS

3.1. Essential oil, active fractions, subfractions and sub-subfractions compositions

In order to identify active compounds present within the essential oil and its active subfractions, gas chromatography systems were applied. The chemical composition of the essential oil and its fractions, subfractions and sub-subfractions is shown in Table 1. *S. officinalis* essential oil was characterized by 23 compounds (98% of the total oil) of which 18 were monoterpenes, 4 sesquiterpenes and diterpene. The GC-MS analysis revealed the presence of camphor (21.23%), α -thujone (21.22%), 1,8-cineole (17.52%), β -thujone (13.45%), trans-caryophyllene (1.67%) and α -humulene (1.45%). The fractions F1, F2 and F3 represent respectively 92.67%, 96.67% and 91% of the total compounds identified. In the fraction F1, the main compounds were α -pinene (18.56%), trans-caryophyllene (18.32%), camphene (14.81%) and α -humulene (12.38%). In the F2 and F3 fractions the main compounds were α -thujone and 1,8-cineole with 67.05%, 10.88% and 49.06%, 20.72% respectively.

3.2. Cytotoxicity

The cytotoxicity of the essential oil of *S. officinalis* and its fractions in the three cancer cell lines is shown in Figures 1, 2 and 3, and the IC_{50} values are given in Table 2. The first fraction F1 was the most active on the murine macrophage cells RAW264.7 (IC_{50} 118.4 μ g/ml). Less activity was found when the fraction F1 was applied to both cell cultures (279.3 and 265.5 μ g/ml for HCT-116 and MCF-7, respectively). The sub-subfraction (ssf) F1.1.1 obtained from subfraction (sf) F1.1 was more active on the RAW264.7 cells (IC_{50} 41.9 μ g/ml) than on the HCT-116 cells (IC_{50} 77.3 μ g/ml). Less activity was found when the ssf F1.1.1 was applied to MCF-7 cells (IC_{50} 125.7 μ g/ml). The ssf F1.2.1 selected from sf F1.2 was less cytotoxic in RAW264.7 (IC_{50} 90.5 μ g/ml), HCT-116 (IC_{50} 145.8 μ g/ml) and MCF-7 (IC_{50} 144.7 μ g/ml). Commercially available samples of some of the identified main compounds (1,8-cineole, β -thujone, trans-caryophyllene and α -humulene) were tested for their cytotoxic activity *in vitro* on the three

Table 1. Composition of *S. officinalis* essential oil and its fractions, subfractions and sub-subfractions

t _r	Compound	S. officinalis	F1	Sf F1.1	Ssf F1.1.1	F2	Sf F1.2	Ssf F1.2.1	F3
4.16	α-Thujene	0.31	1.76	-	-	1.01	-	-	-
4.3	α-Pinene	3.18	18.57	-	-	-	0.5	-	-
4.54	Camphene	3.67	14.81	-	-	-	1.12	-	-
4.88	Sabinene	0.41	-	-	-	-	-	-	-
4.97	β-Pinene	2.57	9.01	-	-	-	1.11	-	-
5.09	β-Myrcene	1.94	7.47	-	-	1.51	7.612	-	-
5.61	α-Terpinene	0.2	-	-	-	-	0.58	-	-
5.83	Limonene	1.7	7.21	-	-	0.72	1.17	-	-
5.91	1,8-Cineole	17.52	-	-	-	10.88	-	-	20.72
6.38	γ-Terpinene	0.42	2.06	-	-	0.25	2.152	-	-
6.97	γ-Terpinolene	0.28	-	-	-	-	1.28	-	-
7.39	α-Thujone	21.22	-	-	-	5.91	-	-	12.61
7.60	β-Thujone	13.45	-	-	-	67.06	-	-	49.06
8.24	Camphor	21.23	-	-	-	-	-	-	7.72
8.63	Borneol	1.67	-	-	-	-	-	-	-
8.85	Terpinen-4-ol	1.01	-	-	-	-	-	-	-
9.12	α-Terpineol	0.27	-	-	-	-	-	-	-
9.26	Myrtenol	0.28	-	-	-	-	-	-	-
11.18	Bornyl acetate	-	-	-	-	0.77	-	-	0.87
13.4	β-Bourbonene	-	0.1	-	-	-	-	-	-
14.14	trans-Caryophyllene	1.68	18.32	-	-	2.52	66.71	54.23	-
14.32	β-Cubebene	-	0.13	-	-	-	-	-	-
14.54	Aromadendrene	-	0.53	-	-	-	0.75	-	-
14.84	α-Humulene	1.45	12.38	-	81.48	6.05	0.87	-	-
15.26	γ-Cadinene	-	0.32	-	-	-	1.05	-	-
16.04	Calarene	-	-	-	-	-	1.46	-	-
16.5	Δ-Cadinene	-	-	-	-	-	1.31	-	-
17.45	Caryophyllene oxide	0.46	-	-	-	-	-	7.5	-
17.62	γ-Selinene	2.68	-	-	-	-	-	-	-
25.61	Manool	0.37	-	-	-	-	-	-	-
%	Identification	98	92.67	82.28	81.48	96.67	87.65	61.72	91

(*S. officinalis*) *Salvia officinalis* essential oil, (F1) Fraction F1, (F2) Fraction F2, (F3) Fraction F3, (Sf.F1.1) Subfraction F1.1, (Sf.F1.2) Subfraction F1.2, (Ssf.F1.1.1) Sub-subfraction F1.1.1, (Ssf.F1.2.1) Sub-subfraction F1.2.1. t_r: Retention time (as min). % identification: peak area identified relative to total peak area %.

cancer cells, in order to identify and compare the active oil constituents. Interestingly, trans-caryophyllene exerted cytotoxic activity on HCT-116 (IC_{50} 65.2 μ g/ml) and was more active against RAW264.7 (IC_{50} 35.2 μ g/ml), but in the MCF-7 cells it showed less cytotoxicity (IC_{50} > 100 μ g/ml). The results also indicated that α -humulene was active in a similar manner against HCT-116 and MCF-7 cell lines with IC_{50} values of 64.2 μ g/ml and 81.9 μ g/ml, and the highest cytotoxic activity of this constituent (IC_{50} 38.4 μ g/ml) was found in the RAW264.7 cells.

Table 2. Cytotoxic activity of *S. officinalis*, its fraction and subfraction samples. IC_{50} in μ g/ml.

Essential oil, fractions and subfractions	Cell line		
	HCT-116	MCF-7	RAW264.7
<i>Salvia officinalis</i> L.	394.6 \pm 1.4 *	554.4 \pm 1.5	207.5 \pm 0.8 **
Fraction F1	279.3 \pm 3.6 **	265.5 \pm 4.4 **	139.5 \pm 0.6 ***
Subfraction F1.1	217.4 \pm 7.4 **	213.1 \pm 7.1 **	118.4 \pm 2.5 ***
Subfraction F1.2	145.2 \pm 0.6 **	139.7 \pm 6 **	101.8 \pm 4.7 ***
Subfraction F1.3	371 \pm 4.4 *	> 400	264.9 \pm 3.1 *
Fraction F2	316.6 \pm 6.6 **	444.3 \pm 12.6 *	163.7 \pm 2.5 ***
Fraction F3	423 \pm 6.3 *	514.4 \pm 11	143.8 \pm 3.8 ***
Fraction F4	> 800	556.9 \pm 13	> 400
Fraction F5	> 800	> 800	> 400
Fraction F6	> 800	> 800	> 400
Fraction F7	> 800	> 800	> 400
Fraction F8	> 800	> 800	> 400
Fraction F9	> 800	> 800	> 400

Data are given as the mean \pm S.D (three independent experiments). Significantly different from control at * p < 0.05, ** p < 0.01 and *** p < 0.001, respectively.

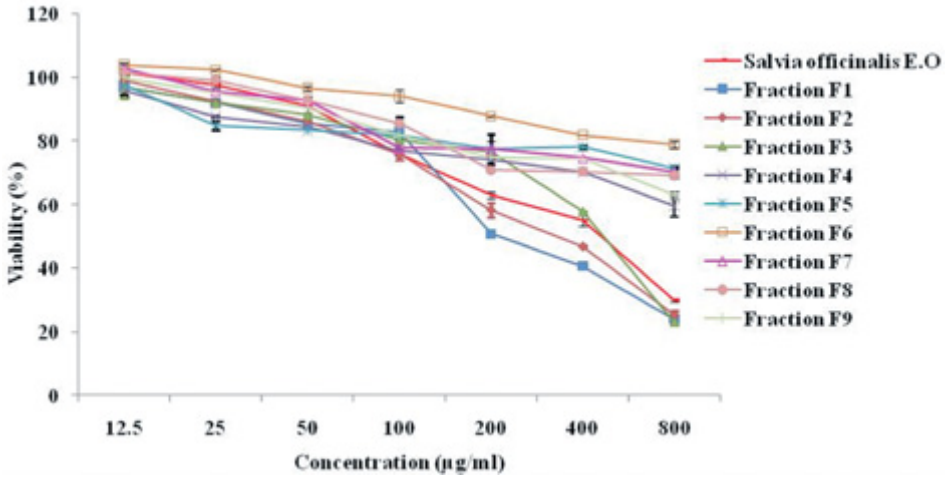


Figure 1. Cytotoxic activity (%) of *S. officinalis* essential oil and its fractions in a dose-dependent pattern (12.5- 800 µg/ml) for the HCT-116 colon cancer cell line. Values are expressed as mean ± S.D of three independent experiments in triplicate.

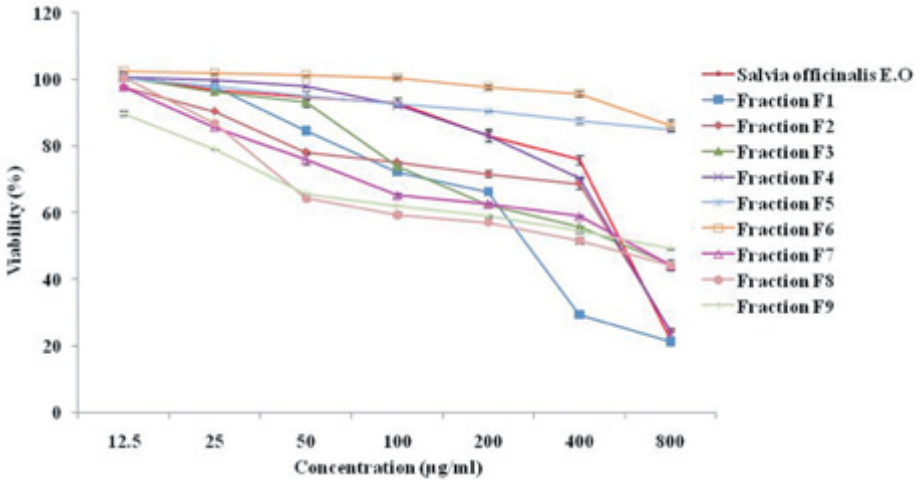


Figure 2. Cytotoxic activity (%) of *S. officinalis* essential oil and its fractions in a dose-dependent pattern (12.5- 800 µg/ml) for the MCF-7 breast cancer cell line. Values are expressed as mean ± S.D of three independent experiments in triplicate.

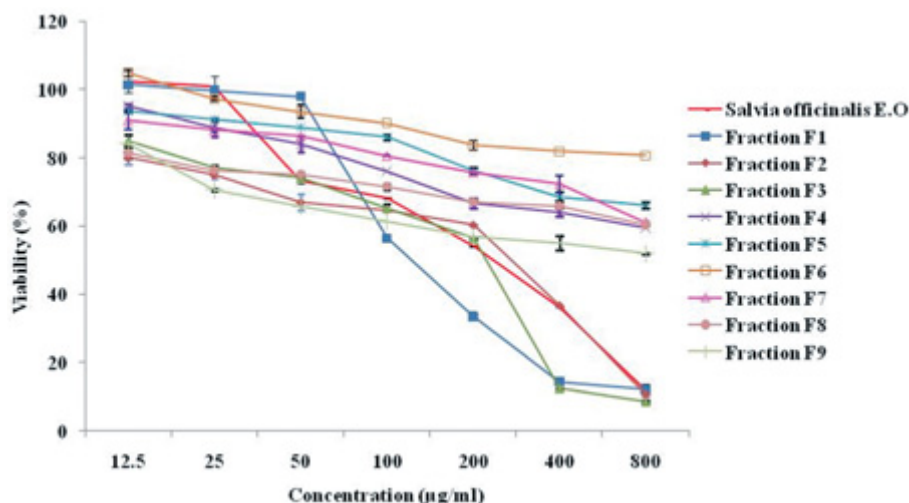


Figure 3. Cytotoxic activity (%) of *S. officinalis* essential oil and its fractions in a dose-dependent pattern (12.5- 800 µg/ml) for the RAW264.7 breast cancer cell line. Values are expressed as mean \pm S.D of three independent experiments in triplicate.

4. DISCUSSION

In our study *S. officinalis* leaf essential oil was analysed with CG-MS and fractionated to identify the mainly cytotoxic compounds in cancer cell lines. This species has been extensively investigated for its chemical composition and pharmacological profile. Some diterpenoid quinones (royleanone-SAR 3, horminone-SAR 26, and horminone-SAR 43) isolated from the roots of *S. officinalis* are able to induce cytotoxic and DNA-damaging activity in human colon carcinoma Caco-2 cells and human hepatoma HepG2 cells *in vitro* conditions (20).

In the ssf F1.1.1, the main compound identified was α -humulene (81.47%) which exhibited high cytotoxic activity in the murine macrophage cells, colorectal adenocarcinoma cells and breast melanoma cells. These results were in accordance with other data which showed that the sesquiterpene fraction of *S. officinalis* with the presence of α -humulene, demonstrate a strong cytotoxic activity in human prostate carcinoma LNCaP cells (21). In the same way,

a previous study reported the cytotoxic activity of α -humulene on PC-3, A-549, DLD-1 and M4BEU tumor cells through glutathione depletion and reactive oxygen species production (22). Similarly, our results showed the production of reactive oxygen species in sesquiterpene sub-subfractions (F1.1.1, F1.2.1) and commercial compounds: α -humulene and trans-caryophyllene (data not shown). The ssf F1.2.1 contained trans-caryophyllene (54.23%) as the main compound and presented less cytotoxicity than ssf F1.1.1 in the three cell lines tested. In some works, the sesquiterpenes α -humulene and trans-caryophyllene are the main active compounds responsible for the anti-inflammatory actions displayed by the essential oil of *Cordia verbenacea* (23). However, the *in vivo* study of α -humulene administered orally to mice was rapidly transported to several important tissues and it was found markedly reduced in some possible inactive mono and di-epoxides (24).

In the present study, the observed variation in cytotoxic activities of the fractions, subfractions and sub-subfractions, can be explained by the sensitivity of the cell line studied. The comparison of results from all *S. officinalis* samples indicates that MCF-7 cells are more resistant than HCT-116 cells, whereas RAW264.7 cells are still more sensitive. Furthermore, the comparison of cytotoxic activity data with the chemical composition of the fractions F1, F2 and F3 of essential oil *S. officinalis* showed that 1,8-cineole and β -thujone were absent in the first fraction F1 which was highly cytotoxic, and both compounds were present in the fractions F2 and F3 (10.87%, 67.05% and 20.72%, 49.06% respectively) with less cytotoxic activity. This observation confirmed that 1,8-cineole and β -thujone were not responsible for the cytotoxicity of our essential oil in all cell lines studied.

5. CONCLUSION

The sesquiterpenes α -humulene and trans-caryophyllene were the main compounds of our sub-subfractions from the essential oil *S. officinalis* and had the ability to inhibit cancer cell growth. In addition, these data suggest that more analysis of the pharmacokinetic parameters of active compounds are necessary before starting the clinical trials.

6. ACKNOWLEDGEMENTS

This research was supported by a grant from the Spanish Agency for International Cooperation and Development (AECID) (2007-2009), Madrid, Spain.

7. REFERENCES

1. Bellakhdar, J. (1997) La pharmacopée marocaine traditionnelle: médecine arabe ancienne et savoirs populaires. 352.
2. Mirjalili, M. H., Salehi, P., Sonboli, A. & Vala, M. M. (2006) Essential oil variation of *Salvia officinalis* aerial parts during its phenological cycle. *Chem. Nat. Comp.* 42: 19-23.
3. Longaray-Delamare, A. P., Moschen-Pistorello, I. T., Artico, L., Atti-Serafini, L. & Echeverrigaray, S. (2007) Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. *Food Chem.* 100: 603-608.
4. Baricevic, D., Sosa, S., Loggia, R. D., Tubaro, A., Simonovska, B., Krasna, A. & Zupancic, A. (2001) Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: the relevance of ursolic acid. *J. Ethnopharmacol.* 75: 125-132.
5. Daferera, D. J., Ziogas, B. N. & Polissiou, M. G. (2000) GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *J. Agric. Food Chem.* 48: 2576-2581.
6. Watanabe, M., Kobayashi, Y., Ogihara, J., Kato, J. & Oishi, K. (2000) HIV-1 reverse transcriptase inhibitory compound in *Salvia officinalis*. *Food Sci. Tech. Res.* 6, 216-220.
7. Ben Farhat, M., Jordan, M. J., Chaouech-hamada, R., Landoulsi, A. & Sotomayor, J. A. (2009) Variations in essential oil, phenolic compounds, and antioxidant activity of Tunisian cultivated *Salvia officinalis* L. *J. Agric. Food Chem.* 57: 10349-10356.
8. Perry, N. S. L., Bollen, C., Perry, E. K. & Ballard, C. (2003) *Salvia* for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. *Pharm. Bioch. Beh.* 75: 651-659.
9. Avato, P., Fortunato, I. M., Ruta, C. & D'Elia, R. (2005) Glandular hairs and essential oils in micropropagated plants of *Salvia officinalis* L. *Plant Sci.* 169: 29-36.
10. Santos-Gomes, P. C. & Fernandes-Ferreira, M. (2001) Organ- and season-dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. *J. Agric. Food Chem.* 49: 2908-2916.
11. Maksimovic, M., Vidic, D., Milos, M., Solic, M. E., Abadzic, S. & Siljak-Yakovlev, S. (2007) Effect of the environmental conditions on essential oil profile in two Dinaric *Salvia* species: *S. brachyodon* Vandas and *S. officinalis* L. *Bioch. Syst. Ecol.* 35: 473-478.

12. Perry, N. B., Anderson, R. E., Brennan, N. J., Douglas, M. H., Heaney, A. J., McGimpsey, J. A., & Smallfield, B. M. (1999) Essential oils from dalmatian sage (*Salvia officinalis* L.): variations among individuals, plant parts, seasons, and sites. *J. Agric. Food Chem.* 47: 2048-2054.
13. Hethelyi, A. N., Farkas, A., Horvath, G., Lemberkovics, N. & Szoke, E. (2009) Chemical and genetic relationships among sage (*Salvia officinalis* L.) cultivars and sudean sage (*Salvia judaica* Boiss.). *J. Agric. Food Chem.* 57: 4663-4667.
14. Dob, T., Berramdane, T., Dahmane, D., Benabdelkader, T. & Chelghoum, C. (2007) Chemical composition of the essential oil of *Salvia officinalis* from Algeria. *Chem. Nat. Comp.* 43: 491-494.
15. Bayrak, A. & Akgul, A. (1987) Composition of essential oils from Turkish *Salvia* species. *Phytochem.* 26: 846-847.
16. Sung, H. J., Choi, S. M., Yoon, Y. & An, K. S. (1999) Tanshinone IIA, an ingredient of *Salvia miltiorrhiza* Bunge, induces apoptosis in human leukemia cell lines through the activation of caspase-3. *Exper. Mol. Med.* 31: 174-178.
17. Liu, J., Shen, H.M. & Ong, C.N. (2000) *Salvia miltiorrhiza* inhibits cell growth and induces apoptosis in human hepatoma HepG2 cells. *Cancer Lett.* 153, 85-93.
18. Ho, C. T., Wang, M., Wei, G. J., Huang, T. C. & Huang, M. T. (2000) Chemistry and antioxidative factors in rosemary and sage. *Biofactors.* 13: 161-166.
19. Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol Meth.* 65: 55-63.
20. Slamenova, D., Masterova, I., Labaj, J., Horvathova, E., Kubala, P., Jakubikova, J. & Wsolova, L. (2004) Cytotoxic and DNA-damaging effects of diterpenoid quinones from the roots of *Salvia officinalis* L. on colonic and hepatic human cells cultured in vitro. *Basic Clin. Pharm. Toxicol.* 94: 282-290.
21. Loizzo, M. R., Tundis, R., Menichini, F., Saab, A. M., Statti, G. A. & Menichini, F. (2007) Cytotoxic activity of essential oils from Labiatae and Lauraceae families against in vitro human tumor models. *Anticancer Res.* 27: 3293-3300.
22. Legault, J., Wivecke, D., Debiton, E., Pichette, A. & Madelmont, J. C. (2003) Antitumor activity of balsam fir oil: production of reactive oxygen species induced by α -humulene as possible mechanism of action. *Planta Med.* 69: 402-407.
23. Fernandes, E. S., Passos, G. F., Medeiros, R., Cunha, F. M. D., Ferreira, J., Campos, M. M., Pianowski, L. F. & Calixto, J. B. (2007) Anti-inflammatory effects of compounds α -humulene and (-)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *Eur. J. Pharm.* 569: 228-236.
24. Chavec, J. S., Leal, P. C., Planowsky, L. & Calixto, J. B. (2008) Pharmacokinetics and tissue distribution of the sesquiterpene α -humulene in mice. *Planta Med.* 74: 1678-1683.

***Información de Contacto:**

Adil el Hadri.

Department of Pharmacology, Faculty of Pharmacy, University Complutense Madrid, 28040 Madrid, Spain.

Tel.: +34617068468.

Fax: +34913941726.

e-mail: ahadri@ucm.farm.es