Antiviral properties of supercritical CO₂ extracts from Oregano and Sage

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Running title: Oregano and sage as antiviral agents

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ABSTRACT

The antiviral properties of supercritical CO_2 extracts obtained from oregano and sage were evaluated against the herpes simplex virus type 1 at different stages during virus infection. All the extracts tested presented a moderate extracellular direct virucidal activity, although a pre-treatment of Vero cells with 10 µg/mL of sage extracts before virus addition inhibited 70% of the virus infection. Moreover, supercritical extracts of sage and oregano were able to significantly inhibit the in vitro virus replication, showing IC_{50} values of 1.88 and 5.33 µg/mL respectively. Carvacrol and thymol could be pointed out as the compounds responsible for the antiviral activity found in oregano supercritical extracts, meanwhile, borneol, camphor and 1,8-cineole could be proposed as antiviral compounds in supercritical sage extracts. Results demonstrated that supercritical extraction was an appropriate technique to obtain antiviral extracts from oregano and sage.

Keywords: Antiviral activity, Supercritical fluid extraction, Oregano, Sage, Herpes simplex virus type 1

INTRODUCCION

Herpes simplex virus type 1 (HSV-1) is a highly prevalent pathogen among children and adults, causing primary infections which are presented clinically as herpes labialis or as primary gingivostomatitis. The virus is also able to establish a latent infection in the nervous system that can be reactivated quite frequently (1). The major therapeutics agents for HSV infections are nucleoside analogues such as acyclovir and vidarabine. However, the increased and prolonged use of these compounds, especially in immunocompromised patients, has led to viral resistance against most of these drugs (2,3). The drug resistant HSVs retained their pathogenicity and could be associated with progressive and relapsing disease. Thus, it is necessary to explore and discover novel potential antiherpetic approaches.

Since a long time, medicinal plants have been used for the treatment of many infectious diseases, in most cases without a scientific background supporting their employment. On the contrary, at present, there is increasing emphasis on determining the scientific evidence and rationale use of preparations from medicinal plants. Thus, in last years, a large number of antiherpes screening experiments on medicinal plant extracts and plants derived secondary metabolites (e.g. polyphenolics, glycosides, terpenes, polysaccharides, polyketides, pheophorbides…) have been reported (4). Further, the antiherpes activity of several essential oils of different plant sources as well as of various constituents of essential oils was demonstrated (5-7). Many species of the Lamiaceae family are known for their antiviral activity. Among them, aqueous and ethanolic extracts of *Salvia officinalis* and *Salvia coccinia* revealed an important antiviral activity against HSV-1 and HSV-2 (8, 9). Also, extracts of *Melissa officinalis*

and its essential oil presented antiviral activity against herpes viruses (10, 11). Essential oils of *Mentha piperita* and *Thymus vulgaris* exhibited high levels of virucidal activity against herpes viruses (7, 12). This virucical effect was also presented by aqueous extracts of several species of this family (*Rosmarinus officinalis, Mentha piperita, Prunella vulgaris…)* (13).

In recent years, supercritical fluid extraction (SFE) has received increased attention as an important alternative to the traditional solvent extraction methods, since this technique provides a high speed and efficiency of extraction, eliminates concentration steps and avoids the use of organic solvents which are potentially harmful in terms of environmental impact. SFE is an extraction/fractionation method that exploits the unique properties of gases above their critical points to extract soluble components from a raw material. Carbon dioxide is an ideal solvent for the extraction of some classes of natural substances from food because is non-toxic, non-explosive, readily available and easy to remove from extracted products. In that way, the quality of supercritical fluid extracts is higher than those obtained by extraction solvents or by water or steam distillation, since these methods can induce thermal degradation or present the problem of toxic residual solvent in the products (14).

The goal of the present work was to study the in vitro antiviral activity of supercritical extracts obtained from oregano (*Origanum vulgare*) and sage (*Salvia officinalis*) against HSV-1. Simultaneously, the antiviral activity of the extracts at different steps during the viral infection cycle was also determined, and attempts were made to effectively correlate the antiviral activity with chemical profile of the extracts.

MATERIAL AND METHODS

Samples and chemicals

The oregano (*Origanum vulgare)* and sage (*Salvia officinalis*) samples consisted of dried leaves obtained from a herbalist´s shop (Murcia, Spain). Cryogenic grinding of the samples was performed under carbon dioxide. The size of the particle (between 999 and 500 µm) was determined by passing the ground plant material through sieves of appropriate size. The whole sample was stored at – 20ºC until use.

1,8-cineole, camphor, borneol, sabinene hydrate, carvacrol and thymol standards were purchased from Sigma (Madrid, Spain). CO_2 (N38 quality) was supplied from Air Liquid (Madrid, Spain).

Extraction method

Extractions were carried out using a pilot-plant supercritical fluid extractor (Thar Technology, Pittsburgh, PA, USA, model SF2000) comprising a 2 L cylinder extraction cell and two different separators (S1 and S2), each of 0.5 L capacity, with independent control of temperature and pressure. For each experiment, the extraction vessel was packed with 0.6 kg of the plant. Extraction assays were performed at 30 MPa and 313 K, with a CO_2 flow rate of 50 g/min. Temperature was set to 313 K in both S1 and S2 separators. In the first separator (S1) the pressure was maintained at 10 MPa, while in the second separator (S2) the pressure was ambient pressure. The cascade decompression system produced two different extracts with different composition which were collected in separator 1 (S1) and separator 2 (S2). According to previous kinetic studies the overall extraction time was set to be 8 h (15).

Antiviral assays

Cells and viruses

Vero cells (African green monkey kidney cell line) were obtained from the Americam Type Culture Collections (ATCC number: CCL-81), Rockville, MD. They were used as host for HSV-1. The cells were grown using Eagle's Minimum Essential Medium (MEM) supplemented with 5% foetal bovine serum (FBS), 1% penicillin-streptomycin, 1% hepes buffer 1M, 1% non essential aminoacids and 1% L-glutamine. Maintenance medium for Vero cells was as described above but with 2% FBS.

Herpes virus simplex type 1, sensitive to acyclovir, (HSV-1) (KOS) was obtained from the Americam Type Culture Collections (ATCC number: VR-1493D), Rockville, MD, prepared in aliquots and stored at −80ºC until use. Virus titer was determined by plaque reduction assay in Vero cells and expressed as plaque forming units (pfu) per mL.

Cytotoxicity assays

The cytotoxic effect of the different extracts and standards on Vero cells was tested using MTT assay, according to a publish method (16). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma, Spain) is a yellow water soluble tetrazolium dye that is reduced by live cells, but not dead to a purple formazan product that is insoluble in aqueous solutions. Monolayers of Vero cells in 24-multiwell plates were incubated with MEM containing different concentrations of the extracts for 48h at 37ºC. Cells were then washed with PBS and 0.5 mg/ml of MTT were added to each well

and incubated 4h at 37ºC. Supernatants were discarded and formazan crystals dissolved in a extraction solution (10% sodium dodecyl sulphate in a mixture of dimethyl formamide and water 1:1 v/v, adjusted to pH 4.7 with acetic acid) overnight at 37ºC. Formazan quantification was performed by measuring the optical density at 570 nm using a multiscanner autoreader (Sunrise, Tecan) with the extraction solution as a blank. The data were plotted as dose-response curves, from which the concentration required to reduce 50% the number of viable Vero cells (CC_{50}) after 48 h of incubation with the different extracts were obtained.

Evaluation of virucidal activity

Virus samples containing 10^5 pfu/ml were mixed and incubated at 37ºC for 1h with MEM containing different extracts or standards concentrations or MEM alone (control). Samples were then diluted and used to infect confluent Vero cells for 1h at 37ºC. After incubation, the virus inocula was removed, the cells washed with PBS and then overlaid with maintenance medium (with 0.5% agarose) for 48 h at 37ºC. The infected cells were fixed acetone:methanol (50:50) at 4ºC, stained with a 1% solution of crystal violet and the number of the plaques counted. The percentage of inhibition of plaque formation was calculated as follows: [(mean number of plaques in control) −(mean number of plaques in test)] / (mean number of plaques in control) x 100

Influence of various treatment periods on the anti-HSV-1activity of the extracts

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Vero cells and viruses were incubated with the extracts at different stages during the viral infection cycle in order to determine the mode of antiviral action. (1) Cells pretreatment: monolayers of Vero cells in 24-multiwell plates were pretreated with MEM containing different concentrations of the extract or standard for 3h at 37ºC. Cells were then washed with PBS and infected with 120 pfu of HSV-1. After incubation for 1h at 37ºC, the virus inocula was removed, the cells washed with PBS and then overlaid with maintenance medium (with 0.5% agarose) for 48 h at 37ºC. The infected cells were fixed, stained and the number of the plaques counted. Control consisted on untreated cells infected with HSV-1. (2) Adsorption period: cells were infected with 120 pfu of HSV-1 in presence of different concentrations of the extracts for 1h at 37ºC. Then, the virus inocula and the extract were removed, the cells washed with PBS and then overlaid with maintenance medium (with 0.5% agarose) for 48 h at 37ºC. The infected cells were fixed, stained and the number of the plaques counted. Control consisted on cells infected without extract. (3) Intracelullar replication: cells were infected with 120 pfu of HSV-1. After incubation for 1h at 37ºC, the virus inocula was removed, the cells washed with PBS and then overlaid with maintenance medium (with 0.5% agarose) containing different concentrations of the extracts. After incubation for 48 h at 37ºC, the infected cells were fixed, stained and the number of the plaques counted. The concentration of a substance required to reduce plaque number in Vero cells by 50% (IC_{50}) as compared to control, was calculated from the dose-response curves generated from the data.

GC-MS analysis

Characterization of the supercritical sage and oregano extracts was carried out by a GC-2010 (Shimadzu, Japan), equipped with a split/splitless injector, electronic pressure control, AOC-20i auto injector, GCMS-QP2010 Plus mass spectrometer detector, and a GCMS Solution software. The column used was a ZB-5 (Zebron) capillary column, 30 m x 0.32 mm I.D. and 0.25 μm phase thickness. Helium, 99.996% was used as a carrier gas at a flow of 1 mL/min. Oven temperature programming was 60 ℃ isothermal for 4 min, increased to 64 ℃ at 1 ℃/min, then increased to 106 ℃ at 2.5 ℃/min. Oven temperature was then increased from 106 ℃ to 130 ℃ at 1 ℃/min, and then to 200 ℃ at 5 ℃/min, and then to a final temperature of 250 ℃/min at 8 ℃/min which was kept constant for 10 min. Sample injections (1 μL) were performed in split mode (1:20). The inlet pressure of the carrier gas was 57.5 KPa. Injector temperature was of 250 ℃ and MS ion source and interface temperatures were 230 and 280 ℃, respectively. The mass spectrometer was used in TIC mode, and samples were scanned from 40 to 500 amu. Compounds thymol, carvacrol, borneol and linalool were identified by comparison with standard mass spectra obtained in the same conditions and compared with the mass spectra from library Wiley 229. The rest of the compounds were identified by comparison with the mass spectra from Wiley 229 library and by their linear retention index.

Statistical analysis

One-way analysis of variance was performed using a StatGraphics Centurion XVI for Windows software (Statistical Graphics Corporation, Maryland, USA). The mean comparison test used was Fisher's least significant differences procedure. All the presented values are the mean of four determinations ± standard deviation.

RESULTS AND DISCUSSION

Extraction of oregano and sage leaves by using supercritical carbon dioxide was carried out on a pilot scale plant. Usually, the first step was to determine both, the working pressure and temperature, since the optimisation of the experimental conditions represent a critical step in the development of a SFE process. In this work, the extraction pressure and temperature were set to 30 MPa and 313 K respectively, using pure CO_2. These experimental conditions were selected based on previous studies done in our laboratory with rosemary, oregano and laurel leaves (17-20). As already mentioned, the extraction system employed in the present study allows a cascade depressurization providing two different extracts: S1 and S2. The main difference between S1 and S2 extracts was the fractionation pressure, which brought about a gradual precipitation of the extracted compounds on the basis of their solubility in the extracting agent at the conditions set in each separator. These two extracts are expected to present different characteristics in terms of both, composition and functional activity. Therefore, in this work, the antiviral activity of S1 and S2 extracts of sage and oregano were investigated.

Cytotoxicity

Oregano and sage supercritical extracts (S1 and S2) were initially evaluated for cytotoxicity on preformed monolayers of Vero cells by MTT method. The CC_{50} data obtained (Table 1) indicated similar cytotoxicity for all the extracts tested.

Virucidal activity of oregano and sage supercritical extracts

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In order to elucidate the possibility that oregano and sage supercritical extracts may act directly on the virus particle, a suspension of the virus was treated at 37ºC for 1h with different concentrations of these extracts. As shown in figure 1, pre-incubation of HSV-1 with extracts resulted in a dose-dependent reduction of remaining infectivity of the virus when compared with the untreated control. Data also indicated that sage supercritical extracts presented a higher virucidal activity than oregano extracts, being S1 a little more effective than S2 in both cases. However, these samples exerted a moderate virucidal activity since 3 mg/mL of sage S1 extract were necessary to reduce 60% virus infectivity.

Influence of various treatment periods on the anti-HSV-1 activity of oregano and sage extracts

Vero cells were pre-treated for 3h at 37ºC with different concentrations of oregano and sage S1 and S2 extracts. Afterward, extracts were removed and cells washed and infected with the HSV-1 virus. Results indicated that cells pre-treatment with oregano and sage extracts produced an important reduction of virus infectivity (figure 2), although sage extracts were more effective than oregano extracts. Thus 10 μg/mL of sage S1 extract inhibits virus infection by approximately 60%, whereas 20 μg/mL of oregano S1 extract reduced the virus infectivity by only 50%. Also, in both cases, extracts obtained in separator 1 were more effective than those collected in separator 2. These data suggested that all extracts interfered with the HSV-1 infection process at the initial infection steps, perhaps by blocking virus attachment or adsorption to Vero cells.

In order to investigate the influence of extracts on virus adsorption, cells were infected with HSV-1 in presence of different concentrations of the extracts for 1 h at 37 ℃. Then, the virus inocula and the extract were removed, the cells washed with PBS and maintained for 48 h at 37 ℃. Addition of 10 μg/mL of sage S1 and 20 μg/mL of oregano S1 extracts reduced virus infectivity by 70% and 60% respectively (Figure 3). In that case, also, oregano extracts were less effective than sage ones. Comparing data obtained during the adsorption stage with those found in the pre-treatment step, when oregano and sage extracts were applied during the adsorption stage, at the same concentration, the reduction of the virus infectivity was increased by 10%, indicating that extracts were more effective when applied during adsorption period.

The antiviral activity on the intracellular replication of the virus was evaluated by adding different concentrations of the extracts to previously HSV-1 infected Vero cells and incubated for 48 h at 37 ℃. All the samples showed a dose-dependent inhibition of virus replication. In this assay, sage extracts were more efficient against HSV-1 replication than oregano ones, showing the lowest IC_{50} values (1.88 μg/mL) (Table 1). However, oregano extracts also showed an important inhibition of virus intracellular replication (IC_{50} = 5.33 μg/mL). Comparing these data with those obtained during adsorption, sage and oregano extracts seemed to be much more active when added after virus infection. Thus, sage and oregano supercritical extracts were effective as inhibitors of the intracellular virus replication as well as agents able to disrupt virus attachment to the cell. These results were different to those obtained by other authors when employing aqueous and ethanolic extracts or essentials oils from several plants and herbs. Schnitzler et al. (9) studied the antiviral activity of aqueous and ethanolic extracts of *Salvia officinalis* and reported that HSV-1 were considerably inactivated after treatment

with the extracts prior to cell infection, meanwhile when the host cells were pretreated with the extracts prior to virus infection the activity of the extracts was lower and when the extracts were added to infected cells no or only moderate reduction of plaques was detected. In the same way, Nolkemper et al. (13) indicated that aqueous extracts from species of Lamiaceae family, including *Salvia officinalis*, exerted their antiviral effect on the free HSV and had no effect on the intracellular virus replication. Also, Koch et al. (7) reported that essential oils obtained from different plats and herbs significantly reduced plaque formation when HSV-2 was preincubated with essential oils. The different behaviour of supercritical extracts from oregano and sage obtained in this work might be related with the composition of the extracts, since the extraction technique employed in this work was completely different to those used in the literature.

In order to better define the anti-HSV-1 compounds presented in supercritical extracts, they have been analysed by GC-MS to identify potential antiviral components.

GC-MS characterization of supercritical extracts from oregano

In an attempt to identify the compounds responsible of the antiviral activity found in supercritical S1 and S2 extracts from *Origanum vulgare,* a characterization by GC-MS of these samples was performed. Results obtained are shown in Table 2, where a tentative identification has been performed based on the comparison of mass spectra and retention index (RI). As can be observed, 16 compounds were identified. Some of them were detected in large amounts like trans-sabinene hydrate, thymol and carvacrol. The sum of these three compounds represented a 77.3% of the S1 extract; meanwhile extract obtained on separator 2 contained a smaller quantity (72.03%) of these compounds. In

that sense, several authors (21-23) have reported the antimicrobial and antifungal activity of thymol and carvacrol, meanwhile few information has been published regarding their antiviral activity (24). Thus, in order to correlate the antiviral activity found in the supercritical extracts with their chemical composition, the cytotoxicity and antiviral activity of pure standards of these three main components of the extracts (sabinene hydrate, thymol and carvacrol) were also examined at the same conditions. The cytotoxicity assays revealed that sabinene hydrate was less cytotoxic than the supercritical extracts, meanwhile thymol and carvacrol standards presented higher cytotoxicity values (Table1). Pre-incubation of HSV-1 with pure standards indicated that these standards presented a higher virucidal activity than oregano S1 and S2 extracts, being carvacrol and thymol more effective than sabinene hydrate (figure 1). When Vero cells were pre-treated with 20 µg/mL of carvacrol and thymol, the HSV-1 infection was inhibited by approximately 90% (figure 2), being sabinene hydrate less effective (60% virus inhibition). In contrast, when supercritical samples were applied at this concentration, only 50-40% inhibition was achieved. Moreover, if 20 µg/mL of carvacrol and thymol were applied during the virus adsorption period, the infectivity was reduced to 100% (figure 3), as compared with a reduction of 50-60% when supercritical extracts were used. The antiviral activity of standards on the intercellular replication of the virus was also evaluated, showing carvacrol and thymol IC_{50} values smaller than sabinene hydrate and oregano extracts (table 1). Consequently, carvacrol and thymol could be pointed out as the compounds responsible for the antiviral activity found in oregano supercritical extracts, although sabinene hydrate also contributes to this activity. Besides, the higher antiviral activity found in S1 extract could be explained since this extract presented a higher percentage of thymol than in S2, being the percentages of carvacrol and sabinene hydrate quite similar in the two extracts.

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GC-MS characterization of supercritical extracts from sage

A characterization by GC-MS of supercritical sage extracts was also performed. As can be observed in table 3, 16 compounds were identified. The most abundant were camphor, 1,8 cineole and borneol, representing a 62.4% of S1 extract and a 48.1% of S2 extract. As previously reported for oregano, the antimicrobial activity of these compounds has been extensively described (25, 26), meanwhile few studies reported their antiviral activity (4). With regard to potential compounds responsible for the antiviral activity detected in both sage samples, the determination of antiviral activity of pure standards of these three compounds, in the same conditions of previously was carried out. Data obtained (table 1) indicated that camphor, 1,8-cineole and borneol presented a cytotoxicity values higher than those of the supercritical extracts. Virucidal assays showed that these standards presented a higher virus inhibition when compared to those presented by supercritical extracts (figure 1). Pre-treatment assays indicated that camphor, borneol and 1-8 cineole presented a similar virus inhibition, increasing in a 10-15% the values obtained using the supercritical samples (figure 2). Moreover, during the virus adsorption period, 10 µg/mL of these substances reduced the infectivity by 85-80% (figure 3), meanwhile when sage extracts were applied the reduction was only a 60-70%. Regarding to the intracellular replication of the virus, camphor, borneol and 1,8 cineole showed similar values of the IC_{50}, slightly better the those obtained with the extracts. After analyzed all the data, borneol, camphor and 1,8-cineole could be proposed as the compounds responsible of the antiviral activity found in supercritical sage S1 and S2 extracts. Data also explained the higher antiviral activity found in

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extract obtained in S1, since this fraction contained a higher quantity of borneol, camphor and 1,8-cineole than extract S2.

CONCLUSIONS

Supercritical extracts from edible herbs like oregano and sage presented important antiviral activities against herpes simplex type 1, overall extracts obtained in separator 1. These extracts mainly inhibit HSV-1 intracellular replication, although they were also able to disrupt the virus attachment step. Carvacrol and thymol could be pointed out as the compounds responsible for the antiviral activity found in oregano supercritical extracts, although sabinene hydrate also contributes to this activity. Meanwhile, borneol, camphor and 1,8-cineole could be proposed as antiviral compounds in supercritical sage extracts. These results demonstrated that supercritical extraction was an appropriate technique to obtain antiviral extracts from oregano and sage.

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Table 1. Antiviral activities of supercritical extracts obtained from aromatic plants and pure standards against herpes simplex virus type 1.

Sample	CC_{50} (μg/mL)	IC_{50} (μg/mL)	SI
Oregano S1	128.20 ± 5.36^b	5.33 ± 0.07^b	24.05
Oregano S2	121.20 ± 6.32^b	5.50 ± 0.05^a	22.03
Salvia S1	119.42 ± 4.02^b	1.88 ± 0.09^e	63.52
Salvia S2	122.34 ± 4.69^b	2.09 ± 0.07^d	58.53
1,8-cineole	46.02 ± 2.35^f	$1.53 \pm 0.06^{f,g}$	30.08
Camphor	54.43 ± 3.21^e	1.41 ± 0.11^g	38.60
Borneol	65.92 ± 4.73^d	1.65 ± 0.09^f	39.95
Sabinene hydrate	254.91 ± 7.23^a	5.19 ± 0.07^c	49.12
Carvacrol	83.54 ± 3.78^c	1.80 ± 0.09^e	46.41
Thymol	76.45 ± 4.87^c	1.50 ± 0.07^g	50.97

CC_{50} (cytotoxic concentration 50%): concentration required to reduce 50% the number of viable Vero cells after 48 h of incubation with the compounds. IC_{50} (inhibitory concentration 50%): concentration required to reduce plaque number in Vero cells by 50%. SI (selectivity index): ratio CC_{50}/IC_{50}.

Each value is the mean of four determinations ± standard deviation. SI (selectivity index): ratio CC_{50}/IC_{50}. [a,b,c,d,e,f,g] Different superscript letters within a column indicated significant differences ($p<0.05$) among data.

Table 2. GC-MS identification, peak area contribution (normalized area percent), and retention indices (RI) of compounds found in oregano supercritical extracts. NI: non-identified compound, n.d.: non detected.

Retention time (min)	R.I.	Compound	S1 normalized area (%)	S2 normalized area (%)
10.20	971	Sabinene	n.d.	1.04
12.52	1015	Alpha-terpinene	n.d.	0.74
12.94	1023	ρ-cymene	5.70	1.78
13.19	1027	Limonene	n.d.	0.57
14.94	1057	γ-terpinene	2.48	3.74
15.39	1065	Cis-sabinene hydrate	2.76	3.67
17.17	1096	Trans-sabinene hydrate	45.21	45.05
17.35	1100	Linalool	2.29	1.62
21.74	1175	4-terpineol	2.60	5.14
22.52	1189	α- terpineol	2.37	2.84
25.09	1231	NI	n.d.	0.73
25.61	1240	Thymyl methyl ether	1.33	2.09
26.17	1250	Trans-sabinene hydrate acetate	1.55	0.87
26.40	1254	Linalyl acetate	1.62	1.51
28.65	1291	Thymol	24.10	19.81
29.23	1300	Carvacrol	7.99	7.17
37.80	1412	Trans-caryophyllene	n.d.	1.63

Table 3. GC-MS identification, peak area contribution (normalized area percent), and retention indices (RI) of compounds found in supercritical extracts (S1 and S2) from sage (*Salvia Officinalis*). NI: non-identified compound, n.d.: non detected.

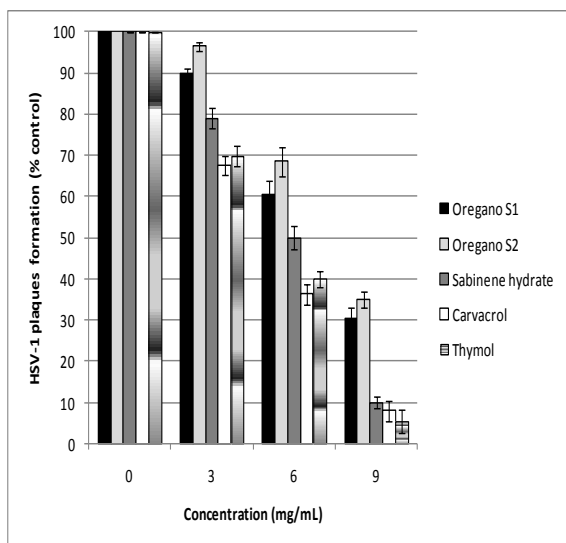| Retention time (min)	R.I.	Compound	S1 normalized area (%)	S2 normalized area (%)
13.33	1029	1,8 Cineole	13.34	4.64
17.38	1099	Linalool	1.16	1.10
19.64	1138	Cis-sabinol	2.05	n.d.
19.78	1140	Camphor	41.78	33.9
21.07	1163	Borneol	7.28	9.56
22.55	1188	Alpha-terpineol	1.16	1.81
26.44	1253	Linalyl acetate	4.86	5.81
28.19	1282	Endobornyl acetate	3.19	3.46
28.70	1291	Sabinyl acetate	5.00	5.94
32.60	1345	Alpha-terpinenyl	3.68	4.71
37.84	1412	E-cariophyllene	2.33	2.97
40.65	1484	Alpha-humulene	1.75	2.41
43.05	1535	Geranyl propionate	1.25	1.91
51.19	1575	Spathulenol	1.01	2.05
51.48	1579	Cariophyllene oxide	1.03	1.61
52.04	1586	viridiflorol	2.00	4.02
56.61	1685	N-I	2.11	5.06

FIGURE LEYENDS

Figure 1. Virucidal effects of supercritical extracts against HSV-1. (A) Oregano extracts and its main components (carvacrol, thymol and sabinene hydrate); (B) Sage extracts and its main components (Camphor, borneol and 1,8-cineole). Each bar is the mean of four determinations ± standard deviation
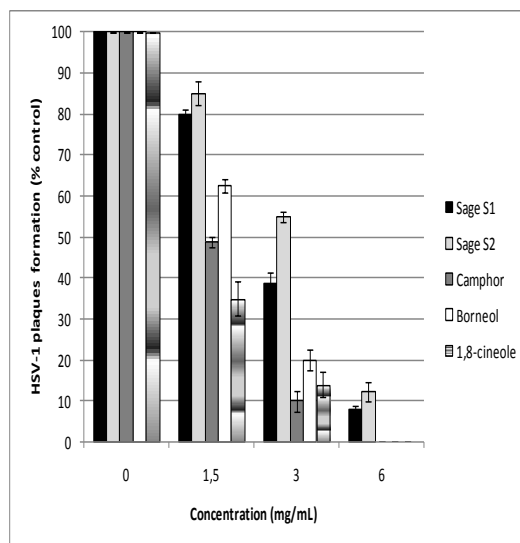
Figure 2. Effect of cell pre-treatment with supercritical extracts and pure standards on HSV-1 infectivity. (A) Oregano extracts and its main components (carvacrol, thymol and sabinene hydrate); (B) Sage extracts and its main components (Camphor, borneol and 1,8-cineole). Each bar is the mean of four determinations ± standard deviation.

Figure 3. Effect of supercritical extracts and pure standards on HSV-1 adsorption period. (A) Oregano extracts and its main components (carvacrol, thymol and sabinene hydrate); (B) Sage extracts and its main components (Camphor, borneol and 1,8-cineole). Each bar is the mean of four determinations ± standard deviation.
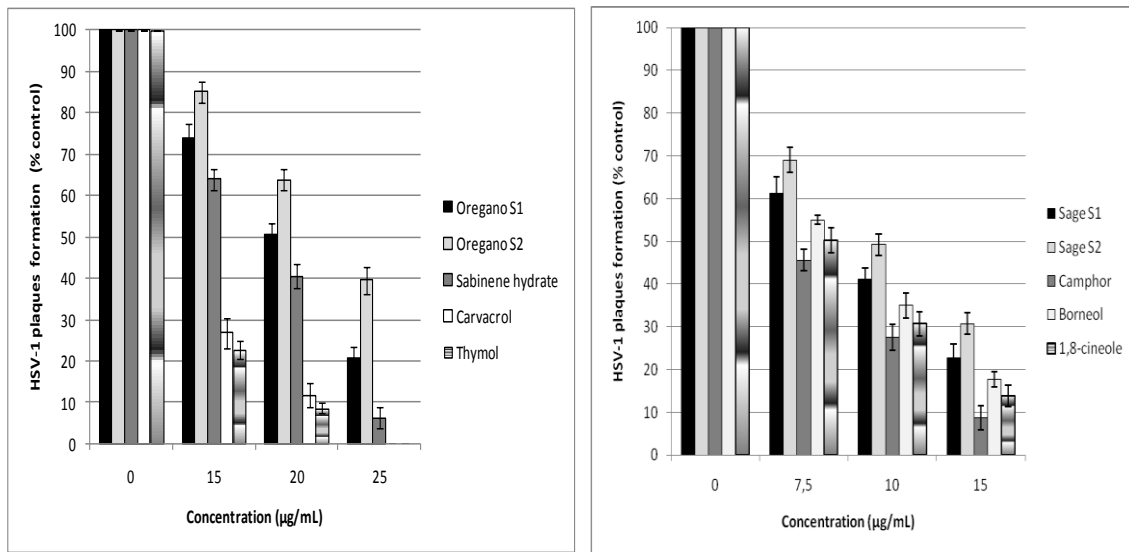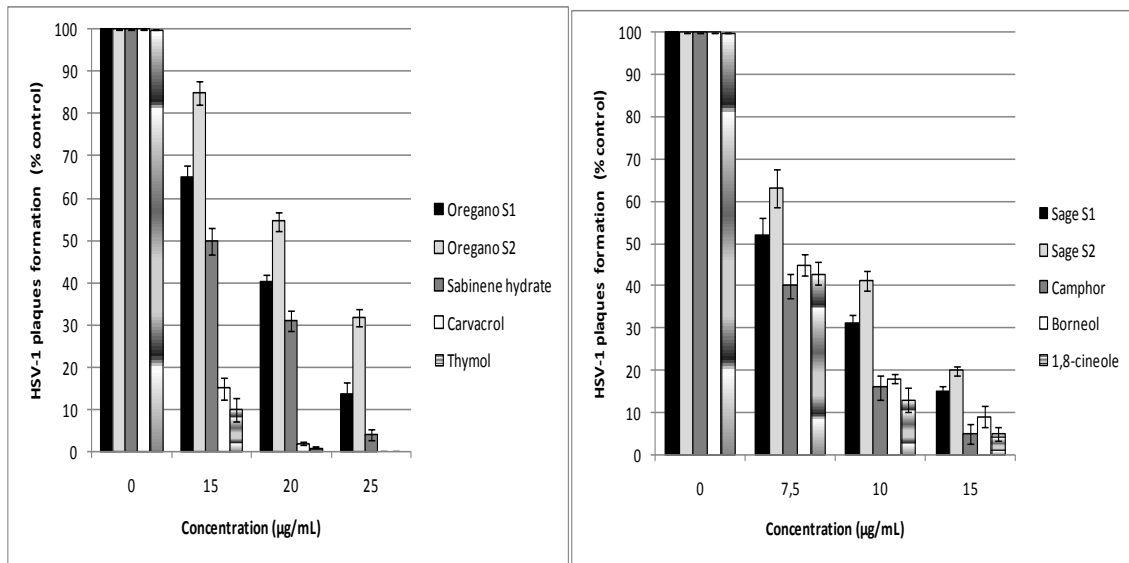
Figure 1

(A)

(B)

Figure 2

(A)   (B)

Figure 3

(A)

(B)