Available online at www.sciencedirect.com

ScienceDirect

Journal of Acute Medicine 5 (2015) 62–68

www.e-jacme.com

Original Research

Antiviral activity of some plant oils against herpes simplex virus type 1 in Vero cell culture

Shahin Gavanji [a,*], Sayedeh Sana Sayedipour [b], Behrouz Larki [a], Azizollah Bakhtari [c]

[a] *Young Researchers and Elite Club, Khorasgan Branch, Islamic Azad University, Khorasgan, Isfahan, Iran*
[b] *Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran*
[c] *Department of Animal Science, Isfahan University of Technology, Isfahan, Iran*

Received 29 July 2014; revised 19 May 2015; accepted 9 July 2015
Available online 28 August 2015

Abstract

Background: Herpes simplex virus-1 (HSV-1) causes some of the most common viral infections in humans. Its genome is linear double-strand DNA. It is demonstrated that HSV is one of the causes of encephalitis, dermatitis, and genitourinary infections, and also the probable cause of cervical cancer. The aim of this study was to investigate the antiviral activity of different concentrations of medicinal plant essential oils on HSV type 1 in Vero cells *in vitro* condition.

Methods: In this study, in order to determine the antiherpetic effect of essential oils, the plaque reduction assay was carried out on Vero cells. For determination of cytotoxicity effect of essential oils, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was used. Analysis was performed using gas chromatography-mass chromatograph with a HP-5MS column.

Results: The cytotoxic effect of different concentrations of *Zataria multiflora* and *Eucalyptus caesia* showed no cytotoxic effect in 0.02% and 0.02%, respectively, and results indicated no cytotoxic effect in *Artemisia kermanensis, Satureja hotensis* L and *Rosmarinus officinalis* up to their concentrations of 0.04%. Anti- HSV activity of different oils (concentration $\leq$ 0.02) did not show any cytotoxic effect on Vero cells viability. Anti-HSV-1 activity of these oils proved that increasing concentration of oils would inhibit virus plaque formation.

Conclusion: It can be concluded that the oils studied in this research have significant inhibitory effect on HSV-1 and thus they could be used as an anti-HSV-1 agent in the context of herbal mouth wash.

Keywords: antiviral activity; essential oil; herpes simplex virus

1. Introduction

Herpes simplex virus (HSV) is a member of the herpesviridae family that can cause serious infection in humans. Its genome is linear double-strand DNA.[1] It is demonstrated that HSV is one of the causes of encephalitis, dermatitis, and genitourinary infections and also the probable cause of cervical cancer.[2,3] Two types of this virus are more prevalent: HSV-1, of which its common areas of infection are the face, lips, mouth cavity, and skin of the loin and above the loin area; and HSV-2, which affects the genital area and the skin of the area below the loin.[4,5] For treatment of the infection caused by herpes simplex, many different physical methods and drugs such as acyclovir, valacyclovir, famciclovir, penciclovir, and cidofovirare are introduced. The antiherpetic mechanism of these drugs is the inactivation of viral DNA polymerase enzyme.[6–8] Acyclovir is widely used as a routine treatment of this viral infection. This drug becomes active through viral thymidine kinase and inhibits viral DNA polymerase. Currently, because of an increase in viral mutations, production of tolerant enzymes, especially in people with immune

* Corresponding author. Islamic Azad University, Khorasgan Branch University Blvd, Arqavanieh, Jey Street, P.O. Box:81595-158, Isfahan, Iran.
E-mail addresses: shahin.gavanji@khuisf.ac.ir, shahin.gavanji@yahoo.com (S. Gavanji).

http://dx.doi.org/10.1016/j.jacme.2015.07.001

deficiency, is increasing.[9,10] The use of herbal drugs, because of their low side effects and low costs of production, attracts much attention. Herbal drugs are being used for treatment of different human diseases and their antiviral effects have been documented.[11,12] Researchers showed that natural compounds such as phenols, flavonoids, alkaloids, and therpens have antiherpetic properties.[11,13] *Zataria multiflora* Boiss is a member of the Labiatae family that is wildy grown in Iran, Afghanistan, and Pakistan. This plant has antimicrobial, antifungal, and antiparasitic activities, and has been recommended as a traditional medicine for treatment of infectious diseases.[14] The *Eucalyptus caesia* is another herbal plant, a species of the Myrtaceae family, which has anti-Herpes simplex virus activity and is widely used in various infectious diseases in folk medicine. *Eucalyptus* essential oil has been supplied antiseptic, antibacterial, and anti-inflammatory proprties.[14,15] *Rosmarinus officinalis* is a plant belonging to the Lamiaceae family with a strong antiseptic effect that is used in many pharmaceutical medicinal products.[16,17] *Satureja hotensis* is also a member of the Lamiaceae family. Several investigators have tested *S. hotensis* antimicrobial activity, and the results indicated effective antimicrobial activity of this medicinal plant so that it could be used as an alternative condidate for synthetic antibiotics-resistant bacteria.[18] *Artemisia kermanensis* is another plant used in traditional medicine for treatment of a variety of conditions, commonly viral infections, because plenty of research have been done on antiviral, antibacterial, and antifungal effects of this plant.[14,19] In the current study, the antiviral activity of different concentrations of some medicinal plant essential oils were examined on HSV-1 in Vero cells in *in vitro* condition.

2. Materials and methods

2.1. Plant materials

Fresh aerial parts of *Z. multiflora*, *E. caesia, A. kermanensis, S. hotensis* L, and *R. officinali*s were used. The herbs were collected from Lorestan and Chaharmahal provinces (Iran) in 2012. The herbs were then dried at room temperature for 3 days. The dried herb sample (500 g) were ground and subjected to hydro-distillation using a Clevenger-type apparatus. The oils were dried over anhydrous Na_2SO_4 and stored at 4°C in a sealed amber vial until use.

2.2. Oil analysis procedure

Analysis was performed using gas chromatography-mass chromatograph (GC-MC) with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 m). The carrier gas was helium at flow rate of 0.8 mL/min. The column temperature was kept at 50°C for 2 minutes and it was programmed to 200°C at a rate of 3°C/min and kept constant at 200°C for 10 minutes. The injection was performed in split mode with ratio of 50:1 at 250°C. The compounds were identified by comparison of *relative retention indices* with those reported in the literature and also by comparison of their mass spectra with published mass spectra.[20,21] The retention indices were determined for all the components according to the Van Den Dool method using n-alkanes as standards.[22]

2.3. In vitro analyses

2.3.1. Cells and viruses

African green monkey kidney cells (Vero; ATCC No. CCL81) were purchased from Pasteur Institute of Iran, Tehran, Iran, and they were grown in Eagle minimum essential medium (MEM) supplemented with 10% new- born calf serum, 100 U/mL penicillin (Gibco) and 100 μg/mL streptomycin (Gibco, USA). A virus stock of HSV type 1 was purchased from Pasteur Institute of Iran. Virus titers were determined by plaque assay in Vero cells and expressed as plaque-forming units per mL (PFU/mL). The viruses were stored at −70°C until use.

2.3.2. Cytotoxicity assay determination using MTT assay

In order to determine the cytotoxicity effect of essential oils, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was used. This method is based on mitochondrial succinate dehydrogenase activity, which converts the yellow dye of MTT to violet dye of formosan. The formosan can be solved in dimethyl sulfoxide (DMSO) and its optical density can be measured using enzyme-linked immunosorbent assay.[23] In each well of the plate, 180 μL of cell suspension was poured. Next, 20 μL of different concentrations of essential oil was added. The culture containing 5% DMSO was used as negative control. The plate was incubated for 48 hours in a CO_2 incubator at temperature of 37°C. Then, 20 μL MTT solution was added to any well and incubated for 2 hours. For dissolving formosan christa, 100 μL DMSO was added. The absorbance of MTT was measured at 560 nm. The vital percent of cells in negative control was considered to be 100 and obtained from the following equation. That obtained concentration from the essential oil, which reduced cells vitality to half, was observed as half maximal inhibitory concentration (IC_{50}).

$$\%\ \text{of cell survival} = \left[\frac{\text{Test compound } \mathbf{OD} - \text{Blank } \mathbf{OD}}{\text{Negative control } \mathbf{OD} - \text{Blank } \mathbf{OD}}\right] \times 100 \quad (1)$$

2.3.3. Antiviral activity

In order to determine the antiherpetic effect of the essential oils, the plaque reduction assay was performed on Vero cells. Plaque reduction is used for determination of the effect of compounds on the PFU) in comparison with standard. In any wells of plate, 400×10^3 μL Vero cells were cultivated in 1 mL Dulbecco modified Eagle medium (DMEM) culture containing 3% fetal bovine serum. To shape one cell layer, the plate was incubated for 24 hours. Next, 1 μL of viral suspension was added to each well. After allowing enough time for adsorption of virus by cells (1 hour at 37°C), the culture medium was replaced with 1 mL DMEM. After 10 minutes, this new

culture medium was removed and 2 mL DMEM containing methyl cellulose was added to each well. Twenty μL DMSO and 20 μL acyclovir were used as negative and positive controls, respectively. Plates were transferred to an incubator and after 48 hours, the plaques were colored according to virus titration steps. The numbers of plaques in each well were counted and the percent of inhibitory of any concentration was determined using the Eq. (2):

$$\% \text{ of inhibition} = \left[1 - \frac{(\text{number of plaque}) \text{ tested}}{\text{Number of control plaque}}\right] \times 100 \quad (2)$$

Antiviral activity of any sample was introduced as a selective indicator, which is the result of division of CC_{50} (concentration which has 50% inhibitory effect on cells) and IC_{50} (concentration which has 50% inhibitory effect on the virus). Finally, anti-HSV-1 activity of different concentrations, from 0.001% to 0.1%, had been tested.

3. Results

3.1. Cytotoxic effects on viability of Vero cells

The cytotoxic effect of different concentrations of *Z. multiflora* and *E. caesia* showed no cytotoxic effect in 0.02% and 0.02%, respectively, and results indicated no cytotoxic effect in *A. kermanensis, S. hotensis* L, and *R. officinalis* up to a concentration of 0.04%. In general, increasing the concentration of essential oils could significantly reduce the cell viability. CC_{50} values for *Z. multiflora*, *E. caesia, A. kermanensis, Satureja hotensis* L, and *R. officinalis* were calculated to be 0.166%, 0.287%, 0.254%, 0.245%, and 0.258%, respectively (Figure 1). In the control group, which was only treated with DMSO, the cellular survival was calculated as 100%.

3.2. Anti HSV-1 activity

Anti-HSV activity of different oils (concentrations 0.02 and below) did not show any cytotoxic effect on the viability of Vero cells. Anti-HSV-1 activity of these oils indicated that increasing concentrations of oils would inhibit virus plaque formation (Figure 2). IC_{50} values of *Z. multiflor*a, *E. caesia, A. kermanensis, S. hotensis* L, and *R. officinalis* oils were calculated 0.003%, 0.004%, 0.007%, 0.008%, and 0.006%, respectively (Table 1). Acyclovir was considered as positive control, and no inhibitory effect was observed on HSV-1 from its concentrations (0.001−0.01). It shows that the oils possess a better effect than acyclovir.

Also, the calculated selective indices (SI) for these oils were 55.44, 66.37, 38.81, 32.16, and 48.12, respectively (Table 1). This important criterion determines that SI≥4 should be considered suitable as an antiviral agent.[24]

3.3. GC-MS of the essential oils

The amounts of the essential oils were assessed by distillation with cold water based on the weight of dried sample. Results from *Z. multiflora* analysis showed that >34 chemical compounds are in existence (Table 2), of which 96.94% constitute the essential components. The main components of the essential oil are made by thymol (33.05%); other main compounds include carvacrol (25.88%), p-Cymene (11.34%) and α-Pinene (3.88%). Results from chemical analysis of rosemary essential oil (by GC) has identified 11 compounds (Table 3) that comprise 78.25% rosemary essential oil. Major components of the essential oil are α-Pinene (23.93%), camphene (8.7%), camphor (10.97%), verbenon (15.44%), p-Cymene (7.48%), and 3-octanone (5.63%). Results from *A. kermanensis* showed >50 chemical compounds (Table 4), which comprise 75.84% of its essential oil. The main

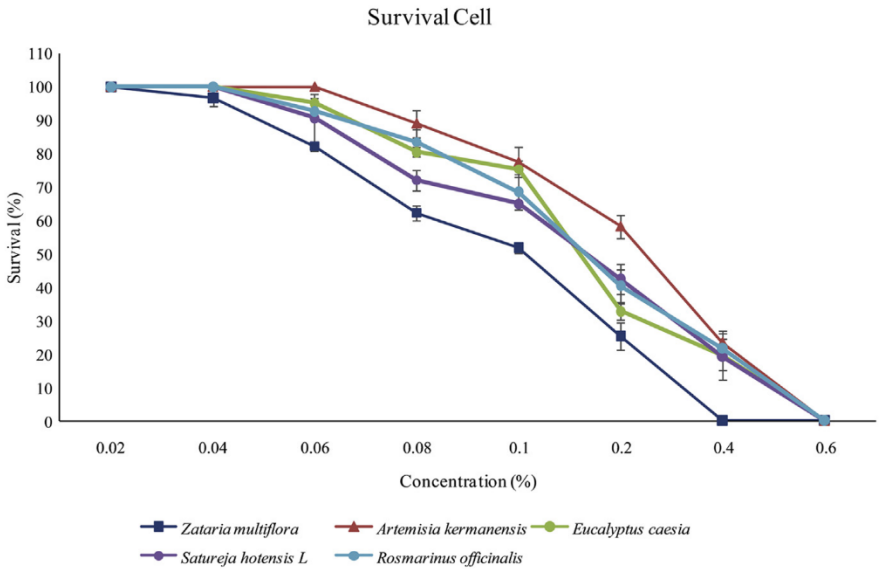

Figure 1. Cytotoxic activity of different concentrations of herbal medicine essential oils. Each bar represents the mean ± standard deviation of three independent experiments.

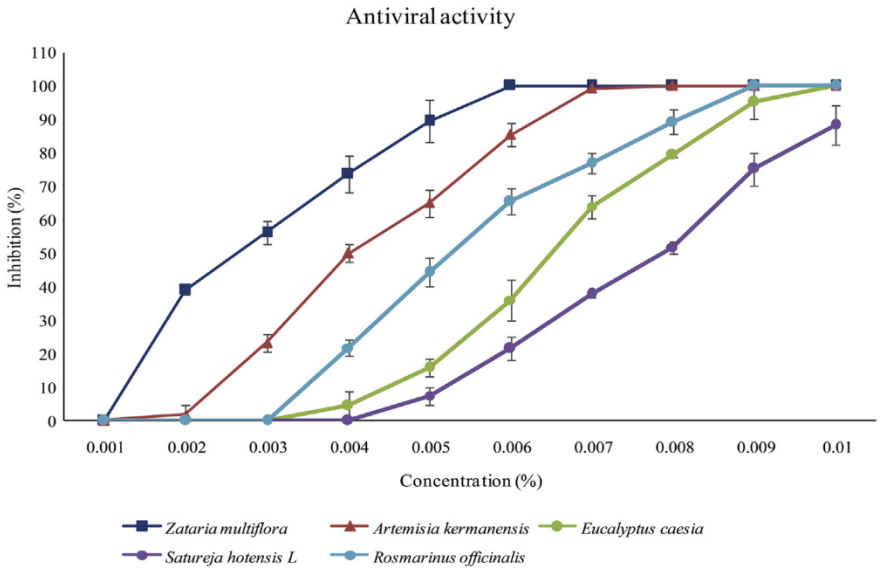

Figure 2. Antiherpes simplex virus-1 activity of different concentrations of herbal medicine essential oils. Each value is the result of mean ± standard deviation of three independent experiments.

compounds of the essential oil include p-Menth-1, 5-dien-8-ol (4.38%), camphor (14.36%), and β-thujone (6.23%). *S. hotensis* L analysis resulted in 20 compounds of which 94.25% constitute the essential components (Table 5). The major components of the essential oil are made by carvacrol (32.38%) and other major compounds include γ-terpinene (31.96%), thymol (9.96%), p-Cymene (6.62%), and α-Terpinene (4.31%). Another plant is *E. caesia,* which possess 27 compounds (Table 6) of which 96.94% constitute the essential components. The major components of the essential oil are made by 1,8-CINEOL (40.18%) and other major compounds are p-Cymene (14.11%), γ-Terpinene (12.43%), α-Pinene (7.7%), and Terpinene-4-ol (5.62%).

4. Discussion

In the current study, acyclovir was used as positive control, and no inhibitory effect was observed in its tested concentrations (0.001−0.01) on HSV-1. The tested oils were more effective compared with acyclovir, which might be due to the existence of bacterial resistance or low concentrations of the drug. However, due to the existence of bacterial resistance in some HSV viruses, discovering new drugs for treatment of herpes infections is important. Currently, herbal plants as a source with a high potential for discovering new drugs (from medicinal plant) for treatment of herpes infections, are being studied for the creation of new applicable antiviral drugs. Herbal essences and their oils have been increasingly used in traditional medicine and they possess many compositions such as phenolic, flavonoid, alkaloid, saponin, esteroide, glycoside, and tannin, which can be used for treatment of infectious diseases. Therefore, they can be used as alternative drugs.[25] In 2008, anti-HSV-2 activity of *T. vulgaris* oil has investigated. This oil inhibits the growth of virus in cell culture with IC_{50} of 0.0007% and its calculated selectivity index (SI) was 10.[26] In this study, we examined the effect of *Z. multiflora* oil on HSV-1 IC_{50}. The comparison between our results with the work of Koch et al[26] in 2008 shows that *Z. multiflora* oil is more effective than *T. vulgaris* oil. The antiviral effect of oils of various plants is related to their active components; oils are generally mixtures of terpenic components, which can be obtained from different parts of the plants.[19] The natural phenolic compounds of oils are believed to possess the ideal antimicrobial activity. Therefore, the higher concentration of phenolic compounds in the oils leads to higher antimicrobial properties. Previously, a number of phenolic compounds such as eugenol and carvacrol had been isolated from *Z. multiflora* oil. Also, it has been shown that reaction among different

Table 1
Assessment of CC_{50}, IC_{50}, and selectivity index in five different herbal essential oils.

Essence	CC_{50} (%)	R^2(%)	R^2adj (%)	IC50 (%)	R^2(%)	R^2adj(%)	CC50/IC50(SI)
Zataria multiflora	0.166	86.90	86.20	0.003	94.50	94.20	55.44
Artemisia kermanensis	0.287	96.20	95.90	0.004	96.40	96.30	66.37
Eucalyptus caesia	0.254	87.60	87.00	0.007	96.60	96.50	38.81
Satureja hotensis L	0.245	89.50	88.90	0.008	97.80	97.70	32.16
Rosmarinus officinalis	0.258	90.30	89.80	0.006	97.00	96.80	46.12

CC_{50} = concentration which has 50% inhibitory effect on cells; IC_{50} = half maximal inhibitory concentration; SI = selectivity index.

Table 2
Compositions of *Zataria multiflora* Boiss.

No.	Composition	%	RI
1	α-Thujene	0.34	931
2	α-Pinene	3.88	937
3	Camphene	0.18	951
4	Verbenene	0.02	956
5	Sabinene	0.02	974
6	β-Pinene	0.68	979
7	β-Myrcene	0.68	993
8	α-Phellandrene	0.11	1007
9	Δ-3-Carene	0.04	1012
10	α-Terpinene	1.32	1016
11	p-Cymene	11.34	1025
12	Limonene	0.67	1032
13	1,8-Cineole	0.55	1030
14	γ-Terpinene	4.73	1057
15	*trans*-Sabinene hydrate	0.27	1087
16	Linalool	1.46	1098
17	Borneol	0.37	1162
18	Terpinen-4-ol	0.82	1186
19	α-Terpineol	0.67	1191
20	Carvacrol methyl ether	0.77	1239
21	Carvol	0.77	1239
22	*trans*-Anethole	2.46	1281
23	Thymol	33.05	1285
24	Carvacrol	25.88	1297
25	Thymyl acetate	1.03	1311
26	Carvacryl acetate	0.69	1371
27	β-Caryophyllene	1.83	1412
28	Aromadendrene	0.84	1437
29	α-Humulene	0.09	1443
30	Germacrene-D	0.13	1473
31	Ledene	0.77	1491
32	*cis*-α-Bisabolene	0.09	1537
33	(+) Spathulenol	0.24	1579
34	Caryophyllene oxide	0.15	1589
	Total	**96.94**	

RI = Retention Index.

compounds of oil plays an important role in antimicrobial potential of the plant. Eugenol and carvacrol have synergic antimicrobial activity.[27] Schnitzler et al.[15] studied the antiviral activity of *E. caesia* oils against HSV-1 and -2. The oil inhibited the growth of viruses with IC_{50} values equal to 0.009 μg/mL and 0.008 μg/mL, respectively. Results indicated

Table 3
Compositions of *Rosemarinus officinalis*.

No.	Composition	%	RI
1	α-Pinene	23.93	942.318
2	Camphen	8.7	955.436
3	Vernenen	1.3	959.826
4	3-Octanone	5.63	991.679
5	p-Cymene	7.48	1026.95
6	Limonene	2.99	1031.3
7	p-Cymenene	1.13	1089.33
8	Camphor	10.97	1144.39
9	Naphtalene	0.32	1178.44
10	p-Cymen-8-ol	0.36	1182.72
11	Verbenon	15.44	1208.57
	Total	**78.25**	

RI = Retention Index.

Table 4
Compositions of *Artemisia kermanensis*.

No.	Composition	%	RI
1	Artemisiatriene	0.41	926
2	α-Pinene	0.54	934
3	Camphene	0.93	949
4	Verbenene	1.88	954
5	Benzaldehyde	0.11	960
6	β-Pinene	0.08	977
7	p-menthatriene	0.57	993
8	yomogi alcohol	2.67	1001
9	α-Terpinene	0.2	1016
10	PARA CYMENE	1.88	1024
11	1,8-Cineole	1.82	1030
12	Artemisia Ketone	0.11	1032
13	trans-Carane	0.13	1050
14	γ-Terpinene	0.41	1056
15	Artemesia alcohol	1.48	1082
16	Styrene	0.82	1087
17	α-Thujone	13.83	1108
18	β-Thujone	6.23	1117
19	trans-Pinocarveol	1.39	1138
20	Camphor	4.13	1142
21	Camphore	10.23	1144
22	p-Menth-1,5-dien-8-ol	2.04	1147
23	1-Menthene	0.49	1156
24	Pinocarvone	1.37	1160
25	Borneol	1.97	1164
26	p-Mentha-1,5-dien-8-ol	4.38	1166
27	Terpinene-4-ol	1.01	1175
28	Naphthalene	0.73	1178
29	p-Cymen-3-ol	1.26	1182
30	α-Terpineol	0.72	1188
31	Verbenone	1.53	1206
32	Norbornane	0.36	1215
33	Cuminic aldehyde	1.1	1235
34	(+)-Carvone	0.48	1239
35	Carvotanacetone	0.28	1243
36	CIS-MYRTANOL	0.15	1247
37	Carvenone	0.12	1253
38	Chrysanthenyl acetate	1	1256
39	Cinnamic aldehyde-E	0.16	1264
40	Bornyl acetate	2.3	1280
41	Thymol	1.29	1286
42	Carvacrol	1.78	1297
43	α-Copaene	0.23	1368
44	Methyl cinnamate	0.15	1375.7
45	(Z)-Jasmone	0.22	1393.1
46	Methyleugenol	0.15	1399.3
47	trans-Caryophyllene	0.3	1395.6
48	α-Curcumen	0.15	1475.4
49	Spathulenol	0.25	1569
50	Caryophyllene	0.07	1644.5
	Total	**75.84**	

RI = Retention Index.

that *E. caesia* oils possess more effective anti-HSV-1 activity in lower concentrations. In 2009, Mancini et al.[28] investigated the inhibitory effect of *R. officinalis* extracts against HSV-1 in different concentrations (100 μg/mL and 300 μg/mL). Results showed that *R. officinalis* extract (concentration of 300 μg/mL) has a good inhibitory effect on HSV-1.[28] Results of our study proved that *R. officinalis* possess strong anti-herpetic activity. In this research, anti HSV-1 activity of different

Table 5
Compositions of *Satureja hotensis* L.

No.	Composition	%	RI
1	α-Thujene	0.88	931
2	α-Pinene	1.32	937
3	Camphene	0.14	951
4	Sabinene	0.07	974
5	β-Pinene	0.57	979
6	β-Myrcene	1.45	993
7	α-Phellandrene	0.39	1007
8	δ-3-Carene	0.1	1012
9	α-Terpinene	4.31	1016
10	p-Cymene	6.62	1025
11	Limonene	1.63	1032
12	1,8-Cineole	0.25	1030
13	β-Ocimene Z	0.15	1038
14	γ-Terpinene	31.96	1057
15	α-Thujone	2.17	1087
16	Borneol	0.3	1062
17	α-Terpineol	0.22	1086
18	Thymol	9.96	1285
19	Carvacrol	32.38	1285
20	β-Caryophyllene	0.26	1412
	Total	**94.25**	

RI = Retention Index.

concentrations of *R. officinalis* essential oils were examined and results showed more effective inhibitory effect of the oil against HSV-1, in lower concentrations, compared with *R. officinalis* extracts. Research in 2007 showed that *Artemisia arborescens* has effective antiherpetic activity with IC_{50} 4.1 μg/mL and 2.4 μg/mL for HSV-1 and -2, respectively.[29] In our study, we examined the antiviral activity of *A. kermanensis* against HSV-1; this oil and those used in the study by Saddi et al.[29] were both included in the Artemisia family. Comparison between anti-HSV-1 activity of *A. kermanensis* and *A. arborescens* showed that *A. kermanensis* oil has more antiherpetic activity, and 0.004% of *A. kermanensis* could inhibit >50% of HSV-1 titer. It seems that phenolic compounds existing in the thyme oil can cause some abnormalities in the structure and function of proteins in the membranes of Vero cells or HSV-1 envelope, which consequently inhibit the binding and penetration of the virus into cells. Natural plant compounds have various antiherpetic mechanisms of action that depend on active compounds of the plant. *Chamaecyparis obtusa* (a medicinal plant) could cause interference in immediate-early gene expression, which leads to interference in HSV gene expression.[30] It is reported that another medicinal plant known as *Tripterygium hypoglaucum*, which is used in folk medicine, has antiherpetic properties through inhibition of early and late gene expression.[31] The phenolic compounds are extracted from aromatic plants, seeds, stem bark, roots, and spices, and have been used as natural antiviral agents. Eugenol (4-allyl-1-hydroxy-2-methoxybenzene), the most studied compound, is mainly found in oil of cloves and in essential oils of cinnamon, and basil. This compound presents inhibitory effects on lipid peroxidation and an effective antivirus replication of either RNA or DNA virus. This agent has a mechanism of action that disables the viral lipidic envelope.[32−34]

Table 6
Compositions of *Eucalyptus caesia*.

No.	Composition	%	RI
1	α-Thujan	0.3	929
2	α-Pinene	7.7	937
3	Campene	0.09	951
4	Sabinene	0.08	975
5	β-Pinene	0.7	979
6	β-Myrcene	0.63	992
7	α-Terpinene	0.2	1018
8	p-Cymene	14.11	1029
9	1,8-Cineol	40.18	1034
10	γ-Terpinene	12.43	1059
11	α-Terpinenol	1.74	1087
12	Linalool	0.13	1099
13	Fenchyl alcohol	0.07	1112
14	trans-Pinocarveol	0.62	1136
15	Methofuran	0.16	1159
16	Borneol	0.13	1162
17	Terpinene-4-ol	5.62	1174
18	p-Cymen-8-ol	0.72	1181
19	Menthol	1.07	1184
20	α-Terpineol	1.53	1187
21	*trans*-Carveol	0.77	1214
22	*cis*-Carveol	0.41	1226
23	Carvone	0.25	1239
24	Geraniol	0.52	1249
25	Thymol	0.51	1280
26	Carvacrol ethyl ether	0.52	1288
27	Carvacrol	0.41	1295
	Total	**91.6**	

RI = Retention Index.

5. Conclusion

Considering the findings of this study, it can be concluded that the oils studied in this research have significant inhibitory effect on HSV-1 and thus they could be used as an anti-HSV-1 in the context of herbal mouthwash. However, to ensure medicinal properties and potential of toxicity of the essential oils, intraoral examination on laboratory animals seems to be necessary.

Conflicts of interest

All authors declare no conflicts of interest.

References

1. Reuven NB, Staire1 AE, Myers RS, Weller SK. The herpes simplex virus type 1 alkaline nuclease and single-stranded DNA binding protein mediate strand exchange in vitro. *J Virol*. 2003;77:7425−7433.
2. Greenberg MS. Ulcerative vesicular and bullous lesions. In: Lynch AM, Brightman VJ, Greenberg MS, eds. *Burket's Oral Medicine Diagnosis and Treatment*. 9th ed. vol. 2. Philadelphia: JB Lippincott Co; 1994:11−50.
3. Elad S, Zadik Y, Hewson I, et al. A systematic review of viral infections associated with oral involvement in cancer patients: a spotlight on Herpesviridea. *Support Care Cancer*. 2010;18:993−1006.
4. Gupta R, Warren T, Wald A. Genital herpes. *Lancet*. 2007;370:2127−2137.
5. Johnston C, Koelle DM, Wald A. HSV-2: in pursuit of a vaccine. *J Clin Invest*. 2011;121:4600−4609.

6. Bacon TH, Levin MJ, Leary JJ, Sarisky RT, Sutton D. Herpes simplex virus resistance to acyclovir and penciclovir after two decades of antiviral therapy. *Clin Microbiol Rev*. 2003;16:114−128.
7. Gilbert C, Bestman-Smith J, Boivin G. Resistance of herpesviruses to antiviral drugs: clinical impacts and rnolecular mechanisms. *Drug Resist Update*. 2002;5:88−114.
8. Betz UAK, Fischer R, Kleymann G, Hendrix M, Rübsamen-Waigmann H. Potent in vivo antiviral activity of the herpes simplex virus primase-helicase inhibitor BAY 57-1293. *Antimicrob Agents Chemother*. 2002;46:1766−1772.
9. De Clercq E. Antiviral drugs in current clinical use. *J Clin Virol*. 2004;30:115−133.
10. Wozniak MA, Mee AP, Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. *J Pathol*. 2009;217:131−138.
11. Pesola JM, Coen DM. In vivo fitness and virulence of a drug-resistant herpes simplex virus 1 mutant. *J Gen Virol*. 2007;88:1410−1414.
12. Tolo FM, Rukunga GM, Muli FW, et al. Anti-viral activity of the extracts of a Kenyan medicinal plant *Carissa edulis* against herpes simplex virus. *J Ethnopharmacol*. 2006;8:92−99.
13. Xiang YF, Pei Y, Wang YF. Current status of natural products from plants as anti-herpes simplex virus 1 agents. *Virol Sin*. 2008;23:305−314.
14. Mehrsorosh H, Gavanji S, Larki B, et al. Essential oil composition and antimicrobial screening of some Iranian herbal plants on *Pectobacterium carotovorum*. *Global NEST J*. 2014;16:240−250.
15. Schnitzler P, Schön K, Reichling J. Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. *Pharmazie*. 2001;56:343−347.
16. Calabrese V. Biochemical studies of a natural antioxidant isolated from rosemary and its application in cosmetic dermatology. *Int J Tissue React*. 2000;22:5−13.
17. Petiwala SM, Berhe S, Li G, et al. Rosemary (*Rosmarinus officinalis*) Extract modulates CHOP/GADD153 to promote androgen receptor degradation and decreases xenograft tumor growth. *PLoS One*. 2014;9:e89772.
18. Sefidkon F, Sadeghzadeh L, Teimouri M, Asgari F, Ahmadi SH. Antimicrobial effects of the essential oils of two Satureja species in two harvesting time. *J Med Aromatic Plants*. 2007;2:174−182.
19. Daferera DJ, Ziogas BN, Polissiou MG. GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicilliumdigitatum. *J Agric Food Chem*. 2000;48:2576−2581.
20. Adams RP. Identification of essential oil components by gas chromatography-quadropole mass spectroscopy. *J Am Soc Mass Spectrom*. 2005;16:1902−1903.
21. Sparkman OD. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. *J Am Soc Mass Spectrom*. 1997;8:671−672.
22. Van Den Dool H, Kratz PD. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatog*. 1963;11:463−471.
23. Buttke TM, McCubrey JA, Owen TC. Use of an aqueous soluble tetrazolium/formazan assay to measure viability and proliferation of lymphokine-dependent cell lines. *J Immunol Methods*. 1993;157:233−240.
24. Tsuchiya Y, Shimizu M, Hiyama Y. Antiviral activity of natural occurring flavonoids in vitro. *Chem Pharm Bull*. 1985;33:3881−3886.
25. Gavanji S, Larki B, Mohammadi E, Bakhtari A. Antimicrobial and cytotoxic evaluation of some herbal essential oils in comparison with common antibiotics in bioassay condition. *Integ Med Res*. 2014;3:142−152.
26. Koch C, Reichling J, Schneele J, Schnitzler P. Inhibitory effect of essential oils against herpes simplex virus type 2. *Phytomedicine*. 2008;15:71−78.
27. Didry N, Dubreuil L, Pinkas M. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. *Pharm Acta Helv*. 1994;69:25−28.
28. Mancini DAP, Torres RP, Pinto JR, Mancini-Filho J. Inhibition of DNA virus: Herpes-1 (HSV-1) in cellular culture replication, through an antioxidant treatment extracted from rosemary spice. *Braz J Pharm Sci*. 2009;45:127.
29. Saddi M, Sanna A, Cottiglia F, et al. Antiherpesvirus activity of Artemisia arborescens essential oil and inhibition of lateral diffusion in Vero cells. *Ann Clin Microbiol Antimicrob*. 2007;26:6−10.
30. Ren Z, Zhang CH, Wang LJ. In vitro anti-viral activity of the total alkaloids from *Tripterygium hypoglaucum* against herpes simplex virus type1. *Virol Sin*. 2010;25:107−114.
31. Hirabayashi K, Itwata S, Matsumoto H, et al. Antiviral activities of glycyrrhizin and its modified compounds against human immunodeficiency virus type 1 (HIV-1) and herpes simplex virus type 1 (HSV-1) in vitro. *Chem Pharm Bull*. 1991;39:112−115.
32. Benencia F, Courregs MC. Antiviral activity of sandalwood oil against herpes simplex − 1 and −2. *Phytomedicine*. 1999;6:119−123.
33. De Logu A, Loy G, Pellerano ML, Bonsignore L, Schivo ML. Inactivation of HSV-1 and HSV-2 and prevention of cell-to-cell virus spread by *Santolina insularis* essential oil. *Antiviral Res*. 2000;48:177−185.
34. Minami M, Kita M, Nakaya T, Yamamoto T, Kuriyama H, Imanishi J. The inhibitory effect of essential oils on herpes simplex virus type-1 replication in vitro. *Microbiol Immunol*. 2003;47:681−684.