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The Inhibitory Effect of Essential Oils on Herpes Simplex Virus Type-1 Replication *In Vitro*

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***Abstract*: The antiviral effect of 12 essential oils on herpes simplex virus type-1 (HSV-1) replication was examined *in vitro*. The replication ability of HSV-1 was suppressed by incubation of HSV-1 with 1% essential oils at 4 C for 24 hr. Especially, lemongrass completely inhibited the viral replication even at a concentration of 0.1%, and its antiviral activity was dependent on the concentrations of the essential oil. When Vero cells were treated with the essential oil before or after viral adsorption, no antiviral activity was found, which suggests that the antiviral activity of essential oils including lemongrass may be due to the direct interaction with virions.**

***Key words*: Essential oils, Lemongrass, HSV-1**

Herpes simplex virus-1 (HSV-1) causes some of the most common viral infections in humans, such as mucocutaneous herpes infections, herpetic keratitis, herpetic encephalitis, and neonatal herpes. Following primary infection, the particles of HSV-1 are carried by retrograde transport via sensory nerve endings to the ganglia, where the virions remain in a latent state until the development of reactivation by stimulation. It is conjectured that the recurrence of HSV-1 infection occurs by centrifugal spread of HSV-1 in axons from the ganglia, and by viral replication at the mucosal sites.

Acyclovir (ACV), a nucleoside analogue and selective anti-herpetic agent which has been widely used for therapy, inhibits the viral DNA replication through viral thymidine kinase, resulting in potent inhibition of viral DNA synthesis. However, ACV-resistant viruses have been increasingly isolated, particularly from immunocompromised hosts, such as patients with AIDS or malignancy, neonates, and recipients of bone marrow or organ transplantation (2, 6, 7, 14, 15, 17, 20). The prevalence of resistance to ACV during ACV therapy in immunocompromised patients is approximately 6% (4). HSV infections in those patients are more incident and severe than in normal hosts and they exhibit increased frequency of secondary herpes episodes (8). Thus, novel and safe anti-herpetic agents are needed.

Various kinds of essential oils have been used for the treatment of various human diseases such as respiratory infections, asthma, atopic dermatitis, allergic rhinitis, post-menopause syndrome, dysmenorrhoea, problems in pregnancy and delivery, psychosomatic disease, depression, panic disorder, jet lag and gastrointestinal diseases (3). It has been reported that essential oils show not only anti-bacterial and anti-fungal activities but also antiviral activity. The antiviral activity of tea tree and eucalyptus oils against HSV-1 and -2 has been demonstrated *in vitro* (19). The antiviral activity of Sandalwood oil, essential oil of *Santalum album* L., against HSV-1 and -2 has also been demonstrated *in vitro* (1). It has been reported that *Santolina insularis* essential oil directly inactivates the particles of HSV-1 and -2, thus preventing adsorption of virions to host cells and inhibiting cell-to-cell virus spread *in vitro* (5). However, there is little information on the anti-herpetic effect of essential oils.

The present study aimed at examining the effects of 12 kinds of essential oils on HSV-1 replication *in vitro* and analyzing the mode of action if essential oils had antiviral activity.

The essential oils used in the present study, *Cupressus sempervirens* (cypress), *Juniperus communis* (juniper),

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Abbreviations: ACV, acyclovir; FCS, fetal calf serum; HSV-1, herpes simplex virus type-1; MEM, minimum essential medium; PFU, plaque forming unit.

Table 1. Effects of various kinds of essential oils on HSV-1 replication

Concentration of essential oil (%)	1	0.1	0.05	0.005
	Percentage of plaque formation (%)			
Control	100±18.0		100±12.2	
Cypress	42.8±12.3	88±22.9	121±20.8	95±5.0
Juniper	119±4.1	71.4±7.1	85±5.0	105±13.2
Tropical basil	73.8±4.1	90.4±16.4	78.3±5.7	81.6±12.5
Tea tree	0.0±0.0	90.4±10.9	90±13.2	86.6±7.6
Peppermint	0.0±0.0	80.9±25.0	86.6±2.8	86.6±11.5
Marjoram	0.0±0.0	92.8±21.4	88.3±15.2	91.6±15.2
Eucaryptus	0.0±0.0	69±4.1	103±7.6	103±15.2
Ravansara	0.0±0.0	92.8±7.1	81.6±7.6	93.3±7.6
Lavender	0.0±0.0	80.9±10.9	86.6±16.0	88.3±12.5
Lemon	0.0±0.0	88±4.1	103±16.0	80±18.0
Rosemary	0.0±0.0	90.4±8.2	76.6±10.4	76.6±12.5
Lemongrass	0.0±0.0	0.0±0.0	21.6±2.8	56.6±10.4

HSV-1 was incubated for 24 hr at 4 C in the presence of each essential oil and in the absence of essential oil as control, and then Vero cells were infected with the mixture of HSV-1 and essential oils. After 4-day incubation, the viral titer was measured by plaque reduction method. The percentage of plaque number formed by the essential oil-incubated HSV-1 to that of control (the means ± SD) was calculated in triple independent experiments.

Melaleuca alternifolia (tea tree), *Ocimum basilicum album* (tropical basil), *Mentha piperita* (peppermint), *Origanum majorana* (marjoram), *Eucalyptus globulus* (eucalyptus), *Ravensara aromatica* (ravansara), *Lavandula latifolia* (lavender), *Citrus limonum* (lemon), *Rosmarinus officinalis* (rosemary) and *Cymbopogon citratus* (lemongrass), were supplied by Laboratoire Sanoflore, Ltd. (Lozeron, France). All essential oils were diluted in Eagle's minimum essential medium (MEM) supplemented with 5% fetal calf serum (FCS) (MEM/5%FCS) and dissolved in dimethyl sulfoxide (Wako Chemicals Co., Tokyo) at a final concentration of 0.2%.

HSV-1 Amakata strain which had been clinically isolated from a patient with herpetic ocular disease was used in the experiments (10, 12, 13). The virus was propagated, titrated on monolayered Vero cell (African green monkey kidney cells) cultures in MEM/5%FCS and stored at −80 C until the experiment.

Essential oils were tested for antiviral activity against HSV-1 by a plaque reduction method using monolayered Vero cell cultures. At first, 150 to 300 plaque forming units (PFU) of HSV-1 was incubated with each essential oil at concentrations of 0.1 to 0.005% at 4 C for 24 hr. The mixtures of essential oils and HSV-1 were added to the confluent monolayered Vero cell cultures in 24-well microplates (Falcon). After a 1 hr adsorption period at 37 C, the cells were washed to remove unadsorbed viruses, and the medium was replaced with 2% methylcellulose in MEM/5%FCS. After 4-day incubation in a 5% CO_2 atmosphere at 37 C, the cells in the microplates were fixed with 2% glutaraldehyde, and stained with 1% methylene blue before the plaques formed were counted. The results are presented as a percentage of virus control from three independent experiments.

The essential oils used in this study completely inhibited the growth of HSV-1 *in vitro* at a concentration of 1% except cypress, juniper and tropical basil (Table 1). Especially, lemongrass completely inhibited the growth of HSV-1 at a concentration of 0.1%, about 80% at a concentration of 0.05%, and about 50% at a concentration of 0.005%.

Next, in order to determine the mode of antiviral activity, the confluent monolayered Vero cell cultures were incubated with each essential oil at a concentration of 0.1% in a 5% CO_2 atmosphere at 37 C. After 24-hr incubation, the medium was removed, and the cells were washed. The cells were then infected with 100 to 500 PFU of HSV-1. The unadsorbed viruses were removed after 1 hr by cell lavages, and then the cells were incubated for 4 days. After that, plaques formed were counted. The results did not show any antiviral activity of essential oils (Table 2).

Finally, the confluent monolayered Vero cell cultures were infected with 100 to 500 PFU of HSV-1 at 37 C. After a 1 hr adsorption period, unadsorbed viruses were removed by cell washing. The medium was replaced with 2% methylcellulose in MEM/5%FCS and each essential oil at a concentration of 0.1%. After 4-day incubation, plaques formed were counted. The results showed no antiviral activity of essential oils (Table 3).

The antibacterial and antifungal activity of lemongrass oil, of which the major components were geranial and neral, has been reported (9, 11, 18, 21, 22). We showed the antibacterial activity of lemongrass against

Table 2. Effect of essential oils on HSV-1 replication before viral adsorption

	Percentage of plaque formation (%)
Control	100±14.6
Cypress	89.8±13.8
Juniper	90.6±12.6
Tropical basil	92.3±18.3
Tea tree	115.2±11.4
Peppermint	89.8±15.6
Marjoram	110.1±13.1
Eucaryptus	94.5±15.3
Ravansara	77.9±12.3
Lavender	92.3±13.4
Lemon	96.5±14.0
Rosemary	94.0±8.3
Lemongrass	87.9±18.0

Cells were pre-treated with 0.1% essential oil for 24 hr at 37 C before viral adsorption, and were then infected with HSV-1. After 4-day incubation, the viral titer was measured. The results are presented as a percentage of the plaque number in essential oil-treated cells to that in non-treated control (the means±SD) in six replicate samples.

Table 3. Effect of essential oils on HSV-1 replication after viral adsorption

	Percentage of plaque formation (%)
Control	100±12.2
Cypress	93.4±9.8
Juniper	89.8±12.5
Tropical basil	92.0±12.7
Tea tree	98.5±11.2
Peppermint	97.8±3.6
Marjoram	92.0±7.4
Eucaryptus	99.2±7.4
Ravansara	96.3±9.6
Lavender	97.1±11.8
Lemon	89.1±11.5
Rosemary	91.3±9.9
Lemongrass	96.3±14.1

Cells were infected with HSV-1, and then treated with 0.1% essential oil at 37 C after viral adsorption. After 4-day incubation, the viral titer was measured. The results are presented as a percentage of plaque number in essential oil-treated cells to that in non-treated control (the means ± SD) in six replicate samples.

Helicobacter pylori in vitro and *in vivo* (16), however, the antiviral activity of the lemongrass has been not reported. The results in the present study demonstrated that lemongrass possessed the strongest antiviral activity of the essential oils used in the study, and that its activity was dependent on the concentrations used *in vitro*.

It is well known that tea tree oil has strong anti-bacterial, antiviral and anti-fungal activity. The antiviral activity of tea tree against HSV-1, and -2 has been reported (19), however the results in the present study demonstrated that because tea tree possessed antiviral activity against HSV-1 at a concentration of 1%, but not at a concentration of 0.1%, lemongrass showed the stronger antiviral activity than tea tree.

In order to determine the mode of antiviral activity, Vero cells were treated with essential oils before or after HSV-1 adsorption, and antiviral activity was not detected, which suggests that the anti-herpetic activity of essential oils may be due to the direct interaction with the virions, binding to viral envelopes and glycoproteins.

In general, antiviral activity is classified into viral particle inactivation, adsorption inhibition and growth inhibition. It is suggested from the results of the present study that the essential oil inactivates directly the viral particles.

We concluded that lemongrass oil may be the most effective essential oil against HSV-1 infection. The antiviral activity of lemongrass in animal models infected with HSV-1 could help clarify the exact role of lemongrass in HSV-1 infection. The topical use of essential oils, especially lemongrass, for the treatment of recurrent HSV-1 infections may be useful for recurrent ocular and dermal infection with HSV-1.

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