

# The Effect of Cumin Seed Extracts against Herpes Simplex Virus Type 1 in Vero Cell Culture

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## Abstract

**Background:** Cumin (*Cuminum cyminum* L. [family Apiaceae]) seed essential oil is reported to have antiseptic activity. Until now the antiviral properties of cumin seed extracts on viruses such as herpes simplex virus-1 (HSV-1) have not been studied. The objective of this study was to investigate the in vitro effects of aqueous, methanolic and hydroalcoholic extracts of cumin seed on HSV-1 growth in Vero cell line.

**Methods:** Antiviral activity of various concentrations aqueous, hydroalcoholic and methanolic extracts of cumin seed in Vero cells were studied using plaque reduction assays. The 50% cytotoxic concentration (CC<sub>50</sub>), 50% inhibitory concentration (IC<sub>50</sub>), and therapeutic index of the effective extracts were calculated.

**Results:** Methanolic extract of cumin seed showed a significant antiviral activity on HSV-1 in Vero cell line. Its CC<sub>50</sub> for Vero cells, IC<sub>50</sub> and the therapeutic index for HSV-1 were 0.45, 0.18 mg/mL and 2.5, respectively. Aqueous and hydroalcoholic extracts of cumin seeds showed no inhibitory effect on HSV-1.

**Conclusion:** The methanolic extract of cumin seed produces anti-HSV-1 effect. Probable interference of phenolic compounds with fusion of Vero cell membrane and HSV-1 envelope might be the mechanism of such inhibitory effect. Further studies are required to ascertain its in vivo antiviral properties and potential toxicity.

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**Keywords** • Cumin seed • herpes simplex virus type-1 • cell culture

## Introduction

Cumin (*Cuminum cyminum* L.) is the dried fruit of a small herbaceous plant of family Apiaceae. It was popular even during the Biblical times as an efficient food flavor for ceremonial feasts. From Latin America to North Africa and all over Asia cumin is the most popular spice used. Common spices of cumin have a long history of use in Eastern cultures as food flavors, perfumes and medicinal herbs. Cumin powder is generally used as a food additive for imparting flavor to foods.

*Cuminum cyminum* is a slender, branched herb with 13 to 15 cm height. Leaves are divided into long narrow segments

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similar to fennel, but much smaller, and are of a deep green color. The flowers are small rose or white color, in stalked umbels with only 4 to 6 rays. Seeds are oblong in shape, thicker in middle, compressed laterally about 5 mm long, resembling caraway seeds but lighter in color and bristly instead of smooth, almost straight, instead of being curved.<sup>1</sup>

The spice *Cuminum cyminum* (green cumin) is cultivated in Pakistan-India subcontinent, Iran, Egypt, Turkey, Morocco, China, Russia, Japan and Algeria. In Iranian folk medicine, dried fruits are commonly used in carminative, stomachic and stimulant, and astringent. It is also useful in diarrhea, dyspepsia, toothache and epilepsy.<sup>2</sup> The essential oil of the fruit is known to be an effective antiseptic besides other uses.<sup>1</sup>

Among important viral infections, herpes simplex virus type 1 (HSV-1) continue to be a common public health problem. It is usually associated with infections of the lips, mouth, and face. It is the most common herpes simplex virus and most people develop its primary infection in childhood. The virus often causes lesions inside the mouth, such as cold sores (fever blisters), or infection of the eye, especially the conjunctiva and cornea. It can also lead to infection of the lining of the brain, namely meningoencephalitis. It is transmitted by contact with infected saliva. By adulthood, up to 90 % of people will have antibodies to HSV-1. Unfortunately, those who carry the virus can spread the disease without even knowing it.<sup>3</sup>

People who have severe or prolonged infections, immune system problems, or frequent recurrences may need to take antiviral medications such as acyclovir (Zovirax), famciclovir (Famvir), and valacyclovir (Valtrex). Acyclovir has been widely used for the treatment of primary and recurrent HSV-1 infections. Some HSV mutants are resistant to this drug. Such a resistance constitutes a major problem in the treatment of HSV infections. Acyclovir however, only acts on actively-replicating HSV, and to be active, it needs to be phosphorylated by HSV thymidine kinase. However, the lack of thymidine kinase activity in some HSV mutants cause resistance to acyclovir.<sup>3-6</sup> Therefore, the search for other medicinal choices, including herbals, continues to be a popular alternative.

The antibacterial,<sup>7-12</sup> and antifungal,<sup>13,14</sup> effects of cumin, as a antimicrobial agent, have been shown previously. Moreover, it has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral agents for the treatment of human viral infections.<sup>15</sup> However,

the effects of cumin seed extract against viruses such as herpes simplex virus have not been studied systematically so far. The present study was conducted to examine the effects of aqueous, methanolic and hydroalcoholic extract of cumin seeds (*Cuminum cyminum* L.) on HSV-1 in Vero cell line.

## Materials and Methods

### *Cumin Seeds Provision*

Cumin seeds were kindly provided by Gol-Daru herbal company, Isfahan, Iran. The species of plant (*Cuminum cyminum* L.) with the herbarium number of GD-4592 were confirmed by an expert from the Department of Biology, Shiraz University, Shiraz, Iran.

### *Preparation of Cumin Seeds Extracts (CSEs)*

Cumin seeds were dried in the shade, and were ground to powder. One hundred grams of the powder was percolated with 1000 mL ethanol (70% v/v) in water. Hydroalcoholic extract evaporated over water bath, and then was dried over a rotary vacuum evaporator. The methanolic extract was prepared by maceration of the plant material with methanol for 3 days at room temperature. This procedure was repeated twice. The respective extracts were filtered and dried in the desiccator to yield a dense residue. The aqueous extract was prepared similarly, but in distilled water. Percentage yields (w/w) of hydroalcoholic, methanolic and aqueous extracts were found to be 12.2, 2.5 and 6.6, respectively.

### *Virus Stock*

Herpes simplex virus type 1 was isolated from the lip sores of a patient, and confirmed using neutralization test by guinea pig anti-HSV-1 serum (NIH, USA) and monoclonal (D and G) anti HSV-1 antibodies.<sup>16</sup>

### *Cumin Seeds Extracts Stocks Preparation*

*Cumin seeds extracts* stocks were prepared by dissolving 10 mg of each extract in one mL distilled water. It was then sterilized by filtration. For cytotoxicity and antiviral assays, the stock solutions of CSE were diluted in the maintenance medium of Dulbecco's modified Eagle's growth medium (DMEM, Sigma) supplemented with 2% fetal bovine serum (Gibco, Germany), 0.14% (v/v) sodium bicarbonate, 100 U/mL penicillin, 100 µg/mL streptomycin sulphate, and 0.25 µg/mL amphotericin B.

### *Cytotoxic Assays*

The cytotoxic effect of CSE was examined

in Vero cell line. Confluent Vero cells in 24-well plates (Nunc, Denmark) were prepared in DMEM, supplemented with 8% fetal calf serum, 0.14% (v/v) sodium bicarbonate, 100 U/mL penicillin, 100 µg/mL streptomycin sulphate, and 0.25 µg/mL amphotericin B (Gibco, Germany). Vero cell monolayers were rinsed twice with PBS and 3 mLs of the maintenance medium with different concentrations of CSE were added to each well. The plates were then incubated at 37°C with atmosphere of 5% CO<sub>2</sub> for seven days. Cells were observed microscopically every 24 hrs. The cytotoxicity effect of each extract was detected by trypan blue dye exclusion method and the 50% cytotoxic concentration (CC<sub>50</sub>) was determined by the Kärber method.<sup>16-18</sup>

#### Antiviral Assays

The inhibitory effect of CSE on HSV-1 was investigated using plaque reduction assay as described previously.<sup>18</sup> Phosphate buffered saline (PBS)- washed confluent Vero cells in 24-well plates were treated with the maintenance medium containing increasing concentrations of the extracts, and were incubated at 37°C for one hour prior to infection with the virus. After removing these treatments, monolayers were infected with 50 PFU/mL of HSV-1 for one hr. Subsequently, the monolayers were overlaid with 1% *carboxymethyl cellulose* (CMC) in the maintenance medium with the same increasing concentrations of CSE, and were incubated at 37°C with 5% CO<sub>2</sub> for four days. Controls for each series of experiment included uninfected cell monolayers, virus-infected untreated (figure 1) or treated cells with the different concentrations of extracts along with 1250 µg/mL acyclovir as an antiherpetic drug control, normal

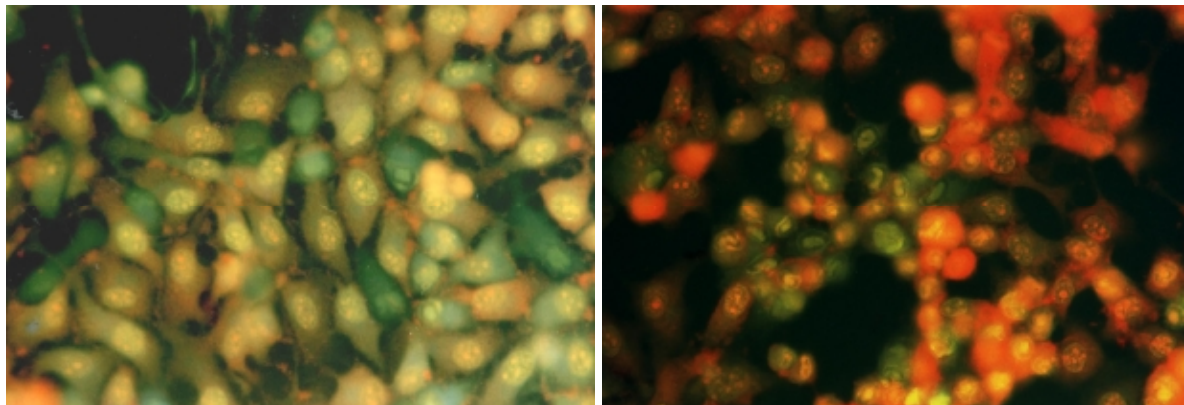
cell monolayers and monolayers without exposure to the extracts and inoculated with the virus. The virus plaques formed on cells were fixed with methanol for 10 min and stained with 0.5% crystal violet solution. The inhibitory concentration 50 (IC<sub>50</sub>) was calculated for CSE by the Kärber method.<sup>16-18</sup>

#### Statistical Analysis

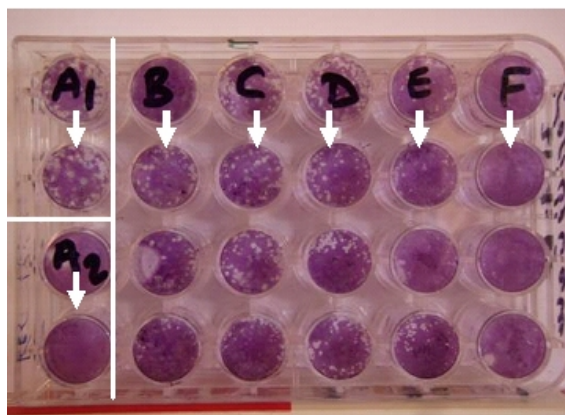
The mean of viral plaque numbers from two different experiments were compared using one way analysis of variance (ANOVA) by SPSS version 11.5. Dunnett test was also used as the post-hoc test. A *P*-value of less than 0.05 was considered statistically significant.

## Results

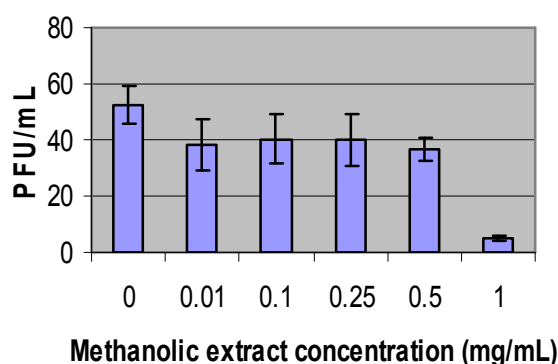
The results showed that compared to control wells with no CSE, different concentrations of aqueous and hydroalcoholic extracts of cummin seeds could not reduce viral plaques significantly (data not shown), however methanolic extracts of cummin seeds had a significant inhibitory effect against HSV-1. Results of cytotoxicity assays showed that the CC<sub>50</sub> of aqueous, hydroalcoholic and methanolic extracts of cummin seeds were 0.18, 0.8, and 0.45 mg/mL respectively. Moreover, IC<sub>50</sub> and the therapeutic index (CC<sub>50</sub>/IC<sub>50</sub>) of methanolic CSE against HSV-1 were, 0.18 mg/mL and 2.5, respectively. When applied one hour before Vero cell infection with HSV-1, methanolic CSE at the concentration of 0.5, 0.25, 0.1 and 0.01 mg/mL caused a significant (*P*<0.05) reduction in viral plaques compared to the control samples (figures 2,3) Lower methanolic CSE concentrations did not have such an effect, and no plaques were observed in acyclovir control wells.



**Figure 1:** Left: uninfected normal Vero cells (X400). The cytoplasm and the round nuclei with one or two nucleolus are observable. Right: HSV-1 infected Vero cells showing *cytopathic effect* (CPE) with rounding and clumping in individual cells which were gradually progressing to small plaques (×400).



**Figure 2:** Reduction of HSV-1 plaques by methanolic extract of CSE. Methanolic extract of CSE at the concentrations of 0.01, 0.1, 0.25, 0.5 and 1 mg/mL, in B,C, D, E and F vertical rows respectively, caused reduction in viral plaques compared to the control A1 wells. A2 wells are uninfected controls well with no plaque.



**Figure 3:** Growth of HSV-1 (PFU/ml) in the absence and presence of various concentrations of cumin seeds methanolic extract. PFUs were significantly ( $P < 0.05$ ) reduced by 0.01-1 mg/mL of the extract. The data are from two experiments in quadruplicate.

## Discussion

Plant extracts and their essential oils have a wide range of activities, including inhibitory action on pathogens, effects on physiopathologies (e.g. anti-inflammatory and anti-diarrheal properties) and activity in different body systems, e.g. endocrine and immune system.<sup>19,20</sup>

In spite of advances in medical sciences and pharmacology, traditional medicine and therapeutic plants continue to exist in many countries. Medicinal plants have been used in medicine since about 3000 years B.C., and continue to be popular all over the world.<sup>21</sup>

In the present study, cytotoxicity assays was first done to determine the cytotoxic concentrations of different CSEs against Vero cell line before examining the anti-HSV properties of CSEs. *Cumin seeds extracts* was toxic to Vero cell line at concentrations  $>1$  mg/mL for different CSEs, and therefore, lower concentrations were

chosen for antiviral assays. The present study indicated significant antiviral activity of methanolic CSE against HSV-1 when it was applied one hour before Vero cell infection with HSV-1, however, aqueous and hydroalcoholic CSEs showed no significant inhibitory effect on HSV-1 in Vero cell line.

The antimicrobial activity of essential oils of cumin seeds was reported by other investigators. Ozcan (2003) reported that essential oils of cumin potentially might be used as antibacterial agents to prevent the spoilage of food products.<sup>12</sup> Sun et al. (2007) described that cumin oils had a potential effect as antifungal preservatives for the control of storage diseases of various crops.<sup>13</sup> Iacobellis et al. (2005) also suggested using cumin oils to control bacterial diseases.<sup>7</sup> Ani et al. (2006) reported the inhibitory effect of cumin extract on the growth of some food-borne pathogenic and spoilage bacteria.<sup>22</sup> The antibacterial effect of cumin extract was also shown by Shahidi (2004) and Damasius et al. (2007).<sup>23,24</sup> It was demonstrated by Ani et al. (2006) that cumin contained a number of polyphenolic compounds including gallic acid, protocatechuic acid, caffeic acid, ellagic acid, ferulic acid and also flavonols such as quercetin and kaempferol, and the antioxidant and antibacterial effects of cumin extract was attributed to its component.<sup>22</sup> Singh et al. (2004) reported that the phenolic compounds in spices like cumin significantly contribute to the flavor, taste, and the medicinal properties.<sup>25</sup> Ninfali et al. (2005) reported that, among the species they studied, cumin had the most significant antioxidant capacity.<sup>26</sup> Chemical analysis of the cumin seed essential oil have shown that the principal active compounds of these oils are principally carvacrol, thymol, citral, eugenol, 1–8 cineole, limonene, pinene, linalool and their precursors.<sup>27</sup> As Fung et al. (1977) described the effect of phenolic antioxidants on microbial growth and toxin production could be the result of the ability of phenolic compounds to alter microbial cell permeability, leading to the loss of macromolecules from the cell interior. They could also interact with membrane proteins, causing a deformation in its structure and functionality.<sup>28</sup>

The exact mechanism of CSE antiviral activity has not been studied yet. It might be due to the interaction of some components of CSE including phenolics with Vero cell membrane and/or HSV-1 envelope. These polyphenolic compounds are soluble in methanol,<sup>22</sup> and may justify the significant effect of methanolic CSE compared to other CSEs in this study. Deformation in structure and function of Vero cell

membrane proteins and HSV-1 envelope may be the mechanism by which methanolic CSE exerts inhibitory effects on HSV-1. It might also be due to its phenolic compounds, which have protein denaturizing activity.

These findings can form the basis for further studies to isolate active compounds, elucidate their structures, and evaluate them against a wider range of microorganisms with the goal to find new therapeutic principles.

## Conclusion

The findings of the present study suggest that the methanolic extract of cumin seeds have inhibitory effect on HSV-1 probably by interfering of its phenolic compounds with the fusion of Vero cell membrane and HSV-1 envelope. Given the effects of methanolic CSE in the present study, it might be considered as a candidate for anti-HSV-1 herbal preparation. However in vivo studies are required to fully elucidate its medicinal properties and potential toxicities.

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**Conflict of Interest:** None declared

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