# Anti-viral effects of aqueous extracts of *Aloe ferox* and *Withania somnifera* on herpes simplex virus type 1 in cell culture

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***ALOE FEROX* AND *WITHANIA SOMNIFERA* are among southern African plants commonly used for the treatment of sexually transmitted infections (STIs). Aqueous extracts from both species, together with aloin, isolated from *A. ferox,* were evaluated for antiviral activity against herpes simplex virus type 1 (HSV-1) *in vitro.* The aqueous extracts showed detectable activity at a concentration of 1000 μg/ml against the virus in monolayers of the Vero African green monkey cell cultures, whereas aloin showed significant activity at 62 μg/ml. HSV-1 is usually associated with mucocutaneous infections of the oropharynx but can also cause genital herpes. Herpes simplex virus type 2 (HSV-2) is the classical genital herpes pathogen, but with current sexual habits, can give rise to both oral and genito-anal infections. Our results indicate that the use of these two plant species for the treatment of STIs could have a scientific rationale.**

## Introduction

The indigenous people of the Eastern Cape province of South Africa have a long history of traditional plant usage for the treatment of various diseases and ailments.[1,2] This includes the use of plants for the treatment of sexually transmitted infections (STIs). STIs are common among young adults in the rural communities of the province. Herbalists take advantage of the biodiversity of plant species to treat these infections.[3]

In our previous survey of plants used for the treatment of STIs,[3] we found that the traditional healers and other knowledgeable rural dwellers of the study area listed *Aloe ferox* and *Withania somnifera* as two of the commonest species used for the treatment of genital herpes, a disease commonly caused by herpes simplex virus type 2 (HSV-2). Herpes simplex virus type 1 (HSV-1) has also been implicated in genital infections. *Aloe ferox* (Asphodelaceae) is widely used in traditional healing. According to several authors, the species is used for the treatment of leukaemia, as an anti-inflammation and anticancer agent and against neuroectodermal tumours.[1,4,5] *Withania somnifera* (Solanaceae) grows in the Eastern Cape. It is used for the treatment of arthritis, tuberculosis, cancer and STIs.[1,6,7]

The antimicrobial properties of these two plant species have been widely reported in the literature.[8–10] However, the reports have focused mainly on their antifungal and antibacterial properties, whereas information on their antiviral activity is scanty.[11] We examined the antiviral effects of *A. ferox* and *W. somnifera* on HSV-1 *in vitro* in this study.

## Materials and methods

See Appendix.

## Results and discussion

Vero cell monolayers treated with water extracts from *A. ferox* or *W. somnifera,* including the stock solution of aloin, exhibited altered morphology after one week. Their integrity, treated with concentrations from 3.9 μg/ml to 2000 μg/ml of these extracts, was maintained, although morphological changes and/or cell death, indicative of cytotoxic effects (CPE), were observed at a concentration of 2000 μg/ml. These observations were confirmed by the MTT assay (Table 1).

In the assay to assess the possible antiviral properties of the plants, aqueous extract from *A. ferox* exhibited partial activity against HSV-1 at a concentration of 1000 μg/ml when the virus was inoculated onto the cell cultures simultaneously with the plant extract. The infected cells showed 50–75% CPE 96 h after infection at 1000 μg/ml (see Table 2 in supplementary material online at www.sajs.co.za). Exposure to an aqueous extract from *W. somnifera,* on the other hand, delayed the appearance of CPE at 1000 μg/ml. The infected cells showed 25% CPE 96 h after infection (see Table 3 online). However, 100% CPE 96 h post-infection was noted at concentrations ranging from 7.8 μg/ml to 500 μg/ml. Water extract from *A. ferox* exhibited no activity against the replication of HSV-1 after viral adsorption had taken place at concentrations ranging from 7.8 μg/ml to 1000 μg/ml (see Table 4 online).

The extracts showed some activity, with no CPE, at a concentration of 1000 μg/ml. At this concentration, the extracts may contain compounds that are either true antivirals, but are present in quantities that are insufficient to inactivate all infectious viral particles, or they may be compounds that retard the replication and spread of the virus.[17,18] Aloin exhibited activity against HSV-1 at a concentration of 63 μg/ml, and the infected cells showed 25–50% CPE 120 h after infection (see Table 5 online). It is important to note that at a concentration of 63 μg/ml, aloin delayed the appearance of CPE, possibly due to interference in the replication cycle of the virus (see Table 6 online).

The activity of aloin and the partial activity of the aqueous extracts of the experimental plants could validate the use of *A. ferox* and *W. somnifera* by traditional healers, as these practitioners use water for extraction, and prescribe the extracts to their patients with no maximum concentration limit.

**Table 1**. Survival of the Vero African green monkey kidney cells in the presence of varying concentrations of aqueous extracts from *Aloe ferox, Withania somnifera* and aloin.

| Extract concentration (μg/ml) | Survival of Vero cells (%) | | |
|---|---|---|---|
| | *Aloe ferox* | *Withania somnifera* | Aloin |
| 2000 | 8 | 1 | 77 |
| 1000 | 107 | 84 | 67 |
| 500 | 125 | 96 | 74 |
| 250 | 130 | 100 | 111 |
| 125 | 106 | 93 | 110 |
| 63 | 110 | 91 | 100 |
| 31 | 108 | 103 | 96 |
| 16 | 98 | 103 | 96 |
| 7.8 | 101 | 93 | 102 |
| 3.9 | 101 | 105 | 115 |

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## Appendix

### Plant collection

Leaves of *A. ferox* and the roots of *W. somnifera* were collected from the Eastern Cape. Voucher specimens (Kambizi Med.2003/1, Kambizi Med.2003/2) were prepared, formally identified and deposited at the University of Fort Hare Botany Department. According to the traditional healers in the study area, only the roots of *W. somnifera* are used for the treatment of STIs.

### Preparation of the extracts

Air-dried plant materials (100 g each) were pulverized and extracted separately by shaking for 30 min in 800 ml of water. The extracts were filtered through Whatman No. 1 filter paper and evaporated to dryness under reduced pressure. Each dry extract was dissolved in nuclease-free water to a final concentration of 2000 $\mu$g/ml (w/v). A stock solution (2000 $\mu$g/ml) of pure dried aloin, similarly isolated from *A. ferox*, was also prepared. Solutions were stored at 4°C and used within 5 days.

Sterile stock solutions of the extracts were obtained by filtration through a 0.45-$\mu$m membrane (Ministart® filter unit, Sartorius, Göttingen). Dilutions of extracts and aloin, in serum-free Eagle's minimum essential medium (MEM) (Highveld Biological, Johannesburg), were tested for cytotoxicity at concentrations from 3.9 $\mu$g/ml to 2000 $\mu$g/ml. These dilutions were then tested for anti-viral activity.

### Cell cultures

Standard cell culture techniques[12] were used for all procedures using cell cultures. Monolayers of the Vero African green monkey cell line (ACACC No. 84113001, European Collection of Cell Cultures) were prepared by seeding 96-well microtitre trays with $10^5$ cells/ml. MEM supplemented with 5% heat-inactivated fetal calf serum (FCS) (Delta Bioproducts, Kempton Park, South Africa) and containing 100 U/ml penicillin and 100 $\mu$g/ml streptomycin was used for the propagation of the cells. Cell cultures were incubated at 37°C in 5% $CO_2$ in air in a humidified atmosphere. Maintenance medium was the same as the propagation medium, except that the former contained only 2% FCS.

### Virus stock

A stock suspension of HSV-1 with titres of $4.7 \times 10^6$ $TCID_{50}$/ml, was prepared from a clinical isolate of HSV-1 (Diagnostics Laboratory, Department of Medical Virology, National Health Laboratory Service, Pretoria). The virus was diluted in serum-free MEM and used at a final concentration of 100 $TCID_{50}$ per microtitre tray well.

### Cytotoxicity assay

The extracts were tested for cytotoxicity by exposing monolayers of the Vero cell cultures to dilutions of the sterile extracts. Doubling dilutions of the extracts, in serum-free MEM, from a concentration of 3.9 $\mu$g/ml to 2000 $\mu$g/ml, were used for testing on 24-hour-old monolayers of Vero cells. The cells were monitored visually, by light microscopy, daily for seven days and on the seventh day tested for cytotoxicity using a tetrazolium salt reduction (MTT) assay,[13] based on the method of Hussain *et al.*[14] Monolayers of cells exposed to serum-free MEM alone were used as a control. The same procedure was repeated for aloin isolated from *A. ferox.*[15] Due to the inadequate quantity of aloin isolated from our plant extract, the aloin used in this experiment was from a commercial source (Sigma Chemical Co., St Louis, MO).

### Antiviral assay

Dilutions of the plant extract and aloin were tested for antiviral activity at the final concentrations of 7.8, 16, 31, 63, 125, 250, 500 and 1000 $\mu$g/ml. The 24-hour-old monolayers of Vero cells in 96-well microtitre trays were starved in serum-free MEM for 1 h in a humidified $CO_2$ atmosphere (5% $CO_2$/95% filtered air) at 37°C. After starvation, the serum-free MEM was withdrawn and 100 $\mu$l (100 $TCID_{50}$) of virus was added to the wells and allowed to adsorb to the cell cultures for 1 h at 37°C in 5% $CO_2$ in air in a humidified atmosphere. After adsorption for 1 h, the unbound virus was withdrawn, and the cells rinsed once with serum-free MEM, after which 200 $\mu$l of the appropriate dilution of extracts in serum-free MEM was added to each of six wells. The cell cultures were incubated at 37°C in a humidified $CO_2$ atmosphere (5% $CO_2$/95% filtered air). As positive control, cells infected with virus were maintained in serum-free MEM, and cells mock-infected with 100 $\mu$l serum-free MEM and maintained in serum-free MEM, serving as negative controls. Cells were examined daily for seven days, by light microscopy, for the appearance of any cytopathic effect (CPE). The absence of CPE at a specific concentration of the extracts or aloin was considered to be indicative of antiviral activity.

**This article is accompanied by supplementary material online at www.sajs.co.za**

## Supplementary material to:

Kambizi L., Goosen B.M., Taylor M.B. and Afolayan A.J. (2007). Anti-viral effects of aqueous extracts of *Aloe ferox* and *Withania somnifera* on herpes simplex virus type 1 in cell culture. *S. Afr. J. Sci.* **103**, 359–360.

**Table 2**. Dose–response relationship of the inhibition of adsorption and subsequent replication of 100$TCID_{50}$ herpes simplex virus type 1 on Vero African green monkey kidney cells when virus was inoculated simultaneously with various concentrations of aqueous extracts from *Aloe ferox*.

| Extract concentration (μg/ml) | Cytopathic effect (%) | | | | | |
|---|---|---|---|---|---|---|
| Hours post-infection: | 24 h | 48 h | 72 h | 96 h | 120 h | 144 h |
| 1000 | 0 | 2/6 10* | 50–75 | 50–75 | 75–100 | 75–100 |
| 500 | 0 | 3/6 10 | 75–100 | 100 | 100 | 100 |
| 250 | 0 | 3/6 10 | 75–100 | 100 | 100 | 100 |
| 125 | 0 | 5/6 10 | 75–100 | 100 | 100 | 100 |
| 63 | 0 | 5/6 10 | 75–100 | 100 | 100 | 100 |
| 31 | 0 | 10 | 75–100 | 100 | 100 | 100 |
| 16 | 0 | 10 | 75–100 | 100 | 100 | 100 |
| 8 | 0 | 10 | 75–100 | 100 | 100 | 100 |
| Positive control | 0 | 4/6 1 | 75–100 | 100 | 100 | 100 |
| Negative control | 0 | 0 | 0 | 0 | 0 | 0 |

*In 2 of 6 wells 10% CPE was observed.

**Table 3**. Dose–response relationship of the inhibition of replication of 100$TCID_{50}$ herpes simplex virus type 1 on Vero African green monkey kidney cells after adsorption of the virus and subsequent incubation of the infected cell cultures in various concentrations of aqueous extracts from *Aloe ferox*.

| Extract concentration (μg/ml) | Cytopathic effect (%) | | | |
|---|---|---|---|---|
| Hours post-infection: | 24 h | 48 h | 72 h | 96 h |
| 1000 | Toxic/0* | Toxic/0 | Toxic/100 | Toxic/100 |
| 500 | Toxic/0 | Toxic/0 | Toxic/100 | Toxic/100 |
| 250 | 0 | 3/6 10 | 50–100 | 100 |
| 125 | 0 | 2/6 10 | 50–100 | 100 |
| 63 | 0 | 3/6 10 | 50–100 | 100 |
| 31 | 0 | 3/6 10 | 50–100 | 100 |
| 16 | 0 | 4/6 10 | 50–100 | 100 |
| 7.8 | 0 | 3/6 10 | 50–100 | 100 |
| Positive control | 0 | 4/6 10 | 50–100 | 100 |
| Negative control | 0 | 0 | 0 | 0 |

*Toxicity was noted in the cell cultures by visual examination: 0% CPE was observed.

**Table 4**. Dose–response pattern of the inhibition of adsorption and subsequent replication of 100 $TCID_{50}$ herpes simplex virus type 1 on Vero African green monkey kidney cells when virus was inoculated simultaneously with various concentrations of water extract from *Withania somnifera*.

| Extract concentration (μg/ml) | Cytopathic effect (%) | | | | |
|---|---|---|---|---|---|
| Hours post-infection: | 24 h | 48 h | 72 h | 96 h | 120 h |
| 1000 | 0 | 10 | 10 | 25 | 100 |
| 500 | 0 | 10 | 25–50 | 100 | 100 |
| 250 | 0 | 10 | 50–75 | 100 | 100 |
| 125 | 0 | 10 | 50–75 | 100 | 100 |
| 63 | 0 | 10 | 75–100 | 100 | 100 |
| 31 | 0 | 10 | 75–100 | 100 | 100 |
| 16 | 0 | 10 | 75–100 | 100 | 100 |
| 7.8 | 0 | 10 | 75–100 | 100 | 100 |
| Positive control | 0 | 10 | 75–100 | 100 | 100 |
| Negative control | 0 | 0 | 0 | 0 | 0 |

**Table 5**. Dose–response pattern of the inhibition of adsorption and subsequent replication of 100 $TCID_{50}$ herpes simplex virus type 1 on Vero African green monkey kidney cells when virus was inoculated simultaneously with various concentrations of aloin.

| Extract concentration (μg/ml) | Cytopathic effect (%) | | | | |
|---|---|---|---|---|---|
| Hours post-infection: | 24 h | 48 h | 72 h | 96 h | 120 h |
| 500 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 |
| 250 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 |
| 125 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 |
| 63 | 0 | 0 | 10–25 | 10–25 | 25–50 |
| 31 | 0 | 10–25 | 50 | 100 | 100 |
| 16 | <10 | 75 | 100 | 100 | 100 |
| 7.8 | <10 | 75 | 100 | 100 | 100 |
| 3.9 | <10 | 75 | 100 | 100 | 100 |
| Positive control | <10 | 75 | 100 | 100 | 100 |
| Negative control | 0 | 0 | 0 | 0 | 0 |

**Table 6**. Dose–response pattern of the inhibition of replication of 100 $TCID_{50}$ herpes simplex virus type 1 on Vero African green monkey kidney cells after adsorption of the virus and subsequent incubation of the infected cell cultures in various concentrations of aloin.

| Extract concentration (μg/ml) | Cytopathic effect (%) | | | | | |
|---|---|---|---|---|---|---|
| Hours post-infection: | 24 h | 48 h | 72 h | 96 h | 120 h | 144 h |
| 500 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 |
| 250 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 |
| 125 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 | Toxic/0 |
| 63 | 0 | 0 | 0 | 0 | 10 | 25 |
| 31 | 0 | 0<10 | 25 | 50–75 | 50–75 | 50–75 |
| 16 | <10 | 10 | 50–100 | 50–100 | 50–100 | 50–100 |
| 7.8 | <10 | 10 | 50–100 | 75–100 | 75–100 | 75–100 |
| 3.9 | <10 | 10–25 | 50–100 | 75–100 | 75–100 | 75–100 |
| Positive control | <10 | 10–25 | 100 | 100 | 100 | 100 |
| Negative control | 0 | 0 | 0 | 0 | 0 | 0 |