The antiviral activity of six South African plants traditionally used against infections in ethnoveterinary medicine

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Abstract

Viral infections remain a major threat to humans and animals and there is a crucial need for new antiviral agents especially with the development of resistant viruses. The hexane, dichloromethane, acetone and methanol extracts of six plant species selected for their traditional use against infections were tested for *in vitro* antiviral activity against canine distemper virus (CDV), canine Parainfluenza virus-2 (CPIV-2), feline herpesvirus-1 (FHV-1) and lumpy skin disease virus (LSDV). All extracts were tested for their cytotoxicity using a colorimetric tetrazolium-based (MTT) assay and were tested for antiviral efficacy at concentrations below CC_{50} values on the various cell types used in the study. The antiviral activity of extracts was tested using virucidal and attachment assays. In the virucidal assay, extracts were incubated with virus prior to infection. The most potent inhibition was observed with the acetone and methanol extracts of *Podocarpus henkelii* against CDV and LSDV, which inhibited replication of the viruses by >75% at 3 ug/ml with selectivity index (SI) values ranging between 12 and 45. Excellent activity was also found with the hexane extracts of *Plumbago zeylanica* and *Carissa edulis* against CDV, with the extracts reducing viral-induced CPE by 50% and 75% respectively. The

hexane extract of *Carissa edulis* had moderate activity against FHV-1 with EC_{50} <70 ug/ml and SI value <2. Only the acetone extract of *Podocarpus henkelii* moderately inhibited replication of LSD virus in the attachment assay, with low activity in other extracts. Of the four extracts with significant antiviral activity, two were prepared from *Podocarpus henkelii.* Therefore, future work will focus on isolating and characterizing the substance(s) responsible for bioactivity in extracts of this species.

Key words: South African medicinal plants, antiviral activity, CDV, LSDV, CPIV-2, FHV-I.

Abbreviations: Canine distemper virus (CDV), Lumpy skin disease virus (LSDV), Canine parainfluenza virus-2 (CPIV-2), Feline herpesvirus-1 (FHV-1)

Introduction

Unlike bacterial cells, which are free-living entities, viruses are obligate intracellular parasites, which contain little more than bundles of gene strands of either RNA or DNA, and may be surrounded by a lipid-containing envelope (Wagner and Hewlett, 1999). Every strain of virus has its own unique arrangement of surface molecules, which aids in its attachment to host cells. Following attachment, viruses utilise the host cell they invade to propagate new viruses. Hence their successes in nature have been attributed to differences in their genetic composition, means of transmission, efficient replication within host cells, and their ability to persist in the host (Wagner and Hewlett 1999). As such they cause various diseases in humans, animals and plants alike. Most alarming is the lack of effective treatment for many viral infections coupled with selection of resistant and cross-resistant mutants as well as the potential toxic effect of presently available therapeutics.

The use of herbal remedies has gained increased recognition globally within the last decades. The World Health Organisation (WHO) in 2001 reported that about 80% of the world's population, especially

those people in developing countries, rely on medicinal plants to treat various ailments. About 20 000 of the plant species used for these purposes have been documented by WHO (Gullece et al., 2006). Medicinal plants with strong antiviral activity to treat viral infections in humans and animals have been identified and those containing novel plant-derived antiviral agents with complementary and overlapping mechanisms of action have been studied (Venkateswaran et al., 1987; Hudson 1990; Thyagarajan et al., 1990; Chattopadhyay and Naik, 2007). Medicinal plants are progressively being explored as appropriate alternative sources for discovery of antiviral agents (Williams, 2001; Jassim and Naji, 2003; Camargo Filho et al. 2008; Lupini et al. 2009, Choi, et al., 2009) and more research is ongoing. Natural products, either as standardized plant extracts or pure compounds, comprise substances with diverse chemical structures, providing an unlimited pool of new drug leads (Vlietinck et al., 2006), possibly with less toxic effects. Additionally, studies evidencing the antiviral potential of plant extracts against viral strains resistant to conventional antiviral agents (Serkedjieva, 2003; Tolo, et al., 2006) highlight the need for exploring medicinal plants for natural antiviral components.

Feline herpesvirus-1 (FHV-1) is the most common viral pathogen of domestic cats worldwide. It causes infections of the eye characterised by conjunctivitis, and profuse ocular and nasal discharges. In severe cases, disease progression leads to keratitis and ulceration of the cornea as well as severe upper respiratory tract involvement (Gaskell and Dawson, 1994; Andrew, 2001; Maggs, 2005). In contrast, canine distemper virus (CDV) infection affects predominantly canines, which serve as the natural host of the virus (Deem et al., 2000). The virus causes highly contagious, systemic disease in dogs world-wide. Despite the fact that infection of dogs may result in an array of clinical forms, immunosuppression and demyelinating leukoencephalitis characterize the main outcome in this species (Krakowka et al., 1985; Appel, 1987). Dogs naturally infected with CDV may also serve as alternative animal models to study the pathogenesis of demyelinisation in various diseases, including multiple sclerosis (Baumgärtner and Alldinger, 2005; Vandevelde and Zurbriggen, 2005; Beineke et al., 2009). Canine

parainfluenza virus-2 (CPIV-2) is another pathogen that affects dogs. It is closely related to simian virus 5 (SV5), human SV5 related isolates, porcine, ovine and feline parainfluenza viruses and to a lesser extent, the mumps virus (Randall, et al., 1987, Ajiki et al., 1982). The virus is one of several pathogens that cause kennel cough in dogs. Natural infection with CPIV-2 in dogs is self-limiting and restricted to the upper respiratory tract although some authors have reported the isolation of the virus from organs other than the respiratory tract (Evermann et al., 1980; Macartney et al., 1985).

Lumpy skin disease virus (LSDV) affects cattle and is caused by a capripox virus. The disease is infectious, eruptive and occasionally fatal, affecting cattle of all ages and breeds. It is characterised by fever, skin nodules, necrotic plaques in mucosae and lymphadenopathy. During outbreaks, morbidity may be as high as 100% and mortality up to 40%. Severe economic losses during outbreaks are associated with emaciation, damage to hides, infertility in males and females, mastitis and reduced milk production (Barnard et al., 1994).

Viral infections can be controlled either through prophylactic or therapeutic intervention. Although vaccines are available to control these infections, no effective antiviral therapy for the treatment of these diseases currently exists. The availability of vaccines to the majority of the people living in rural areas, particularly in developing countries, is limited owing to economic constraints. The objective of the present study was to assess the antiviral effect of different extracts of selected medicinal plants with ethnobotanical indications in South African folk medicine against canine distemper virus (CDV), canine parainfluenza virus-2 (CPIV-2), lumpy skin disease virus (LSDV) and feline herpes virus-1 (FHV-1). The plants were selected on the basis of their traditional indications in treating various infections in humans and animals.

2. Materials and Methods

2.1. Plant collection and preparation of extracts

Leaves of six plants were collected from the Lowveld National Botanical Garden (NBG) in Nelspruit, Mpumalanga province, South Africa, in the month of April. Voucher specimens are present in the herbarium at the Lowveld NBG, or in the National Herbarium in the Pretoria National Botanical Garden (Table 1). The leaf material was air-dried at room temperature and milled into fine powder using a Macsalab mill (Model 200 LAB) Eriseo, Bramley. The traditional indications of the plants selected for the studies are represented in Table 1. Separate aliquots of ground plant material were extracted (10:1 solvent to dry plant material) using hexane, dichloromethane, acetone and methanol. Each extract was dried under low temperature before being reconstituted to 100 mg/ml in DMSO. Serial 10-fold dilutions in cell culture medium were prepared to final concentrations of 1, 0.1, 0.01, and 0.001 mg/ml to determine their cytotoxic concentrations.

2.2. Virus culture

The following viruses were used in the study: feline herpesvirus–1 (FHV-1, dsDNA), canine distemper virus (CDV, ssRNA), canine parainfluenza virus-2 (CPIV, ssRNA) and lumpy skin disease virus strain V248/93 (LSDV, dsDNA). The susceptible cell types compatible for the growth of the viruses were Crandell feline kidney cells (CRFK), Vero cells (for the canine viruses) and bovine dermis cells, respectively. Viruses and host cells were kindly provided by Prof E.H. Venter of the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria. The viruses, 0.5 ml, were inoculated onto confluent cell monolayers appropriate for the growth of each of the viruses in 75 cm^3 culture flasks. Cells were maintained in Minimum Essential Medium (MEM, Highveld Biological) containing 5% fetal calf serum (FCS, Highveld Biological) supplemented with gentamicin (Virbac) and

incubated at 37°C in an atmosphere of 5% CO_2 and observed daily for evidence of CPE. The flasks were subsequently frozen at -70°C and thawed to release cell-associated virus. The $TCID_{50}$ values of each of the viruses used in this study were determined using the method of Reed and Muench (1938).

2.3. Cytotoxicity assay

To determine the cytotoxic concentration of the extracts of the selected plants, cells were seeded at a density of 2.5 x 10^3 cells/ml in 96-well flat-bottomed cell culture microtitre plates. After 24 h of incubation at 37°C in a humidified CO_2 atmosphere (5% CO_2), cells were exposed to various concentrations of the extracts (see section 2.1) in quadruplicate for each concentration for 120 h. Viability of cells was determined using the tetrazolium-based colorimetric assay (MTT assay) described by Mosmann (1983). The medium on the cells was removed, replaced with fresh medium, and 30 ul of MTT was added before the cells were incubated for a further 4h. The medium containing MTT was then removed, 50 ul DMSO was added to each well and the plates were gently rocked to dissolve the formazan crystals. The cytotoxic effect of extracts on cells was indicated by a clear appearance or lack of purple formazan colour in the wells. The optical density (OD) was measured at a wavelength of 570 nm and a reference wavelength of 630 nm using a Versamax microplate reader. The cytotoxicity was expressed as the concentration of extract that inhibited the growth of cells by 50% (CC_{50}), calculated from a linear regression equation. Monolayers of Vero cells, bovine dermis (BD) cells or Crandell feline kidney (CRFK) cells without extract treatment served as negative controls.

2.4. Virucidal assay

The virucidal activity of plant extracts was evaluated using the method described by Barnard et al. (1992) with slight modifications. Non-cytotoxic concentrations of extracts were serially diluted 10-fold in

MEM containing 5% FCS and an equal volume of virus (20 µl) at an infective titre of 10^2 $TCID_{50}$/ml was added to each concentration and incubated at 37°C for various time intervals ranging from 1 to 3 h. Growth medium on confluent cell monolayers grown in 96-well plates was removed and 200 ul of the extract-virus mixture at each concentration were added to the cells in quadruplicate and incubated until CPE was observed, which was mostly between 1 and 5 days. The negative control comprised of non-infected and untreated cells while positive controls consisted of infected and untreated cells. The extent of cell damage was determined by the presence of CPE when compared to infected untreated and uninfected untreated controls by microscopic examination as well as the MTT colorimetric assay. The degree of cell destruction by microscopic examination was scored using reduction indexes described by Vanden Berghe et al. (1993). Plant extracts reducing viral infectivity at dilutions of 10^4 and 10^3 have strong activity while those only inhibiting growth at dilutions of 10^2 to 10 have moderate to weak activity.

The relative safety of the extracts was determined by calculating the selectivity index (SI). This indicates the concentration ratio of killing viruses to killing cells. It is calculated by dividing the cytotoxic concentration (CC_{50}) by the effective antiviral concentration (EC_{50}), in the same units. Selectivity index values of more than three indicate potentially safe antiviral activity of the test extract (Chattopadhyay et al., 2009). The EC_{50} was calculated as follows:

$EC_{50} = [(OD_t)_v - (OD_c)_v] / [(OD_c)_{mock} - (OD_c)_v] * 100$

Where $(OD_t)_v$ is the OD of the cells treated with virus and substance, $(OD_c)_v$ is the OD of the cells treated with virus (virus control), and $(OD_c)_{mock}$ is the OD of the mock infected cells (cell control).

2.5. Attachment assay

The ability of the viruses to attach to the host cell was tested using the method of Barnard et al. (1993) with slight modifications. Cells appropriate for the growth of each virus were seeded in 96-well flat-

bottomed microtitre plates and incubated at 37°C in a 5% CO_2 incubator to attain an 80% confluent monolayer. Medium on the cells was removed and equal volumes of virus (50 µl) at an infective titre of 10^2 $TCID_{50}$/ml was added to cells and incubated at different time intervals from 1-3 h. Thereafter, cells were washed with phosphate buffered saline (PBS) to remove the unattached virus. Ten-fold serially diluted extracts at non-cytotoxic concentrations were added onto cells and incubated again at 37°C in an atmosphere of 5% CO_2 and observed daily for evidence of CPE. The ability of the extract to prevent subsequent replication of the virus in the host cell was scored microscopically and by the MTT assay. Negative controls comprised non-infected and untreated cells while positive controls consisted of infected and untreated cells. Antiviral activity by CPE reduction and MTT assay was determined as previously described.

3. Results and Discussion

In this study, the hexane, dichloromethane, acetone and methanol extracts of six selected plants were tested for their antiviral activity against a range of viral pathogens. Results are presented as those extracts of the different plants that reduced CPE after microscopic examination in infected cells (Tables 2 and 3). Prior to antiviral activity testing, the toxic effect of the different extracts was evaluated to ensure the extracts did not exert deleterious effects on cell viability. The CC_{50}, EC_{50} and SI values of those extracts that exhibited reduced CPE are presented in Tables 4 and 5.

In the virucidal and attachment assays, no differences were observed in virus-induced CPE by microscopic examinations when the extracts were incubated with the virus or virus-infected cells for various time intervals. Extracts exhibiting reduced viral infectivity at concentrations of 10^3 and 10^4 dilutions indicate strong activity. In the virucidal assay, viruses were treated with extracts before infection of cells. Vanden Berghe et al. (1993) suggested that the antiviral activity of a crude plant

extract should be detectable in at least two subsequent dilutions of the maximum non-cytotoxic concentration so as to be able to differentiate between virus-induced CPE and that due to the toxic effect of extracts. In addition, Cos et al. (2006) defined quality standards for primary evaluation for activity screening of natural products. The authors suggested a stringent endpoint of EC_{50} values <100 ug/ml as a standard for antiviral efficacy of natural products, such as plant extracts. Apart from EC_{50} values, SI values of more than three are considered to be indicative of potential antiviral activity (Chattopadhyay et al., 2009).

Reduction in virus-induced CPE ranged from moderate to good antiviral activity against tested pathogens in one or more extracts of the plants tested when evaluated microscopically (Tables 2 and 3). In the virucidal assay, the EC_{50} and SI values of extracts of the different plants exhibiting activity ranged between 3.36 and 73.17 ug/ml and 0.88 to 45.61 respectively. The hexane extract of *Carissa edulis* showed moderate to excellent activity against FHV-1 and CDV, with SI values of 1.22 and 6.14 respectively. Other authors have reported antiviral activity of an aqueous extract prepared from the roots of *Carissa edulis* with remarkable anti-herpes simplex virus (HSV) activity *in vitro* and *in vivo* for both wild type and resistant strains of HSV (Tolo et al., 2006). Although a different plant part and extract was used in that study, the activity observed with the hexane leaf extract against FHV-1 in this study supports earlier reports of the presence of substances with anti-herpes virus activity in *Carissa edulis.* Some degree of antiviral activity was also observed with the dichloromethane extract of *Ekebergia capensis* against CPIV-2 and CDV with SI values < 1, suggesting a less potent effect of the extracts against test pathogens. Nawawi et al. (1999) tested the aqueous and methanolic leaf extracts of *Plumbago zeylanica* for anti-HSV-1 activity in the plaque reduction assay. Although different methods were used in that study, the methanol extracts of *P. zeylanica* at 91 ug/ml did not inhibit HSV-1 plaque formation in Vero cells. The report is consistent with our finding where the methanol extract of this plant exhibited no inhibitory effect on FHV-1. Interestingly, the hexane extract exhibited good activity against

CDV with an excellent SI value of 3.07. Of all the extracts tested in the virucidal assay, the acetone and methanol extracts of *Podocarpus henkellii* against CDV and LSDV presented the best antiviral activity with SI values > 10 (Table 4).

In the attachment assay, where virus was incubated with cells for different time intervals prior to addition of extracts, the activity of the dichloromethane extract of *Ekebergia capensis* was similar to that obtained in the virucidal assay against CPIV-2, suggesting a potent intracellular and extracellular activity against the virus (Tables 4 and 5). Also, the hexane extract of this plant exhibited some degree of intracellular activity against FHV-I with SI value < 1, which was not observed in the virucidal assay. Similar observations were also recorded for other plant extracts against the different pathogens (Tables 4 and 5). A degree of antiviral activity was observed with the hexane extract of *Acokanthera schimperi* against FHV-1, the acetone extract of *Plumbago zeylanica* against LSDV and the acetone extract of *Podocarpus henkelii* against LSDV with SI values ranging between 0.55 and 1.25 and EC_{50} values from 30.93 to 95.69 ug/ml. Crude plant extracts contain a diversity of constituents that may exert their antiviral effect either singularly or in concert with each other. The fact that some degree of inhibition was observed with these extracts may suggest that the extracts contain an active component in low concentrations that may be responsible for the observed activity. These substances may, if present at higher concentrations, be capable of inactivating the virus as well as prevent its replication in the host system.

4. Conclusion

Extracts of plants with antiviral activity were more potent in the virucidal than the attachment assay. Of the twenty-four extracts tested in the virucidal assay, four extracts had significant antiviral activity, two of which were different extracts of *Podocarpus henkelii* against two unrelated viruses. Therefore, future

work will focus on isolation and characterization of substances responsible for bioactivity in *Podocarpus henkelii*.

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Table 1
Plant species used in this study and traditional indications.

Plant species	Family	Voucher specimen number	Plant part	Indication	Reference
Acokanthera schimperi (A.DC) Benth. var. *rotundata* Codd.	Apocynaceae	NBG 584177	Leaf	Headache, epilepsy, amnesia, eye disease, syphilis, rheumatism	Abebe and Ayehu (1993)
Carissa edulis (Forssk.) Vahl.	Apocynaceae	PBG841631	_	Schistosomiasis	Ndamba et al. (1994)
Ekebergia capensis Sparrm	Meliaceae	NBG1322	Roots	Gastritis, hyperacidity, coughing	Pujol (1990)
Podocarpus henkelii Stapf ex Dallim. & Jacks.	Podocarpaceae	PBG818945	Bark and Sap	Chest pain, gall-sickness (animals)	Watt and Breyer-Brandwijk (1962) and Hutchings et al. (1996)
Plumbago zeylanica L.	Plumbaginaceae	NBG307004	Roots	Pneumonia	Van der Merwe et al. (2001)
Schrebera alata (Hochst.) Welw.	Oleaceae	PBG584579	_	_	_

Table 2
Virucidal activity of extracts of selected plants against test organisms following incubation of virus with extracts for 1–3 h prior to inoculation onto confluent host monolayer cells.

Plant species	Extract	Virus	Percent inhibition of cytopathic effect (h)			Log concentrations tested
			1	2	3	
Carissa edulis	Hexane	FHV-1 and CDV	75	75	75	10^{-3}
	DCM	CDV	25	25	25	10^{-1}
	Methanol	LSDV	25	25	25	10^{-1}
Ekebergia capensis	Hexane	LSDV	50	50	50	10^{-2}
	DCM	CPIV-2 and CDV	75	75	75	10^{-3}
	Methanol	LSDV	25	25	25	10^{-1}
Acokanthera schimperi	Hexane	LSDV	25	25	25	10^{-1}
	DCM	CDV	25	25	25	10^{-1}
Plumbago zeylanica	Hexane	CDV	50	50	50	10^{-3}
	DCM	LSDV	25	25	25	10^{-1}
	Acetone	LSDV and CDV	25	25	25	10^{-1}
	Methanol	LSDV	25	25	25	10^{-1}
Schrebera alata	Acetone	CDV and LSDV	25	25	25	10^{-2}
	Methanol	LSDV	25	25	25	10^{-2}
Podocarpus henkelii	Hexane	LSDV	25	25	25	10^{-2}
	Acetone	CDV	75	75	75	10^{-3}
	Methanol	LSDV	75	75	75	10^{-3}

Plant extracts that show evidence of virucidal activity are represented. Log concentrations indicate the dilution at which the extract exhibited reduced viral-induced CPE. LSDV, lumpy skin disease virus; CDV, canine distemper virus; CPIV-2, canine parainfluenza virus-2; FHV-1, feline herpesvirus-1.

Table 3
Antiviral activity of extracts of selected plants against test organisms following addition of virus onto monolayer cells and incubation for 1–3 h prior to addition of extracts.

Plant species	Extract	Virus	Percent inhibition of cytopathic effect (h)			Log concentrations tested
			1	2	3	
Carissa edulis	DCM	CPIV-2	25	25	25	10^{-1}
Ekebergia capensis	DCM	CPIV-2 and LSDV	50	50	50	10^{-3}
	Hexane	FHV-1	50	50	50	10^{-3}
Acokanthera schimperi	Methanol	CPIV-2 and LSDV	50	50	50	$10^{-1}/10^{-3}$
	Hexane		50	50	50	10^{-3}
Plumbago zeylanica	Acetone	LSDV	50	50	50	10^{-3}
	DCM	LSDV	25	25	25	10^{-2}
Schrebera alata	Acetone	LSDV	50	50	50	10^{-1}
	Methanol	CDV	50	50	50	10^{-1}
Podocarpus henkelii	Acetone	LSDV	75	75	75	10^{-3}

Plant extracts that show evidence of antiviral activity are represented. Log concentrations indicate the dilution at which the extract exhibited reduced viral-induced CPE. LSDV, lumpy skin disease virus; CDV, canine distemper virus; CPIV-2, canine parainfluenza virus-2; FHV-1, feline herpesvirus-1.

Table 4
Selectivity index (SI) values indicating virucidal activity of extracts of selected plants following incubation of virus with extracts prior to inoculation onto confluent host monolayer cells.

Plant species	Extract	Virus	EC_{50}[a]	CC_{50}[b]	SI[c]
Carissa edulis	Hexane	FHV-1 and CDV	73.17/12.37	89.41/76.00	1.22/6.14
	DCM	CDV	–	0.37	–
	Methanol	LSDV	–	111.66	–
Ekebergia capensis	Hexane	LSDV		14	
	DCM	CPIV-2 and CDV	30.93/30.93	27.27/27.27	0.88/0.88
	Methanol	LSDV	–	13.13	–
Acokanthera schimperi	Hexane	LSDV	–	36.58	–
	DCM	CDV	–	0.37	–
Plumbago zeylanica	Hexane	CDV	11.73	36.05	3.07
	DCM	LSDV	–	6.66	–
	Acetone	LSDV and CDV	–	32.21/32.21	–
	Methanol	LSDV	–	243.26	–
Schrebera alata	Acetone	CDV and LSDV	–	30.39/30.93	–
	Methanol	LSDV	–	243.68	–
Podocarpus henkelii	Hexane	LSDV	–	14.52	–
	Acetone	CDV	3.76	45.17	12.01
	Methanol	LSDV	3.36	153.24	45.61

LSDV, lumpy skin disease virus; CDV, canine distemper virus; CPIV-2, canine parainfluenza virus-2; FHV-1, feline herpesvirus-1; –, extracts that exhibited no reduced CPE.

[a] Concentration of the sample (μg/ml) required to inhibit virus-induced CPE by 50%.

[b] Concentration (μg/ml) producing 50% cytotoxic effect.

[c] CC_{50}/EC_{50}.

Table 5
Selectivity index (SI) values indicating antiviral activity of extracts of selected plants against test organisms following addition of equal volume of virus onto monolayer cells and incubation prior to addition of extracts.

Plant species	Extract	Virus	EC_{50}[a]	CC_{50}[b]	SI[c]
Carissa edulis	DCM	CPIV-2	–	0.37	–
Ekebergia capensis	DCM	CPIV-2 and LSDV	30.93/–	27.27/2.48	<1/–
	Hexane	FHV-1	78.21	43.11	0.55
Acokanthera schimperi	Methanol	CPIV-2 and LSDV	–	<1/51.66	–
	Hexane	FHV-1	51.89	30.29	0.58
Plumbago zeylanica	Acetone	LSDV	54.93	13.53	0.25
	DCM	LSDV	–	6.66	–
Schrebera alata	Acetone	LSDV	29.87	>1000	–
	Methanol	CDV	–	25.49	–
Podocarpus henkelii	Acetone	LSDV	95.69	107.39	1.12

LSDV, lumpy skin disease virus; CDV, canine distemper virus; CPIV-2, canine parainfluenza virus-2; FHV-1, feline herpesvirus-1; –, extracts that exhibited no reduced CPE.

[a] Concentration of the sample (μg/ml) required to inhibit virus-induced CPE by 50%.

[b] Concentration (μg/ml) producing 50% cytotoxic effect.

[c] CC_{50}/EC_{50}.