

## Chapter 6

### Evaluation of different extracts of selected south african plant species for antiviral activity

#### 6.1. Introduction

Viral infections remain a major threat to humans and animals and there is a crucial need for new antiviral agents. Infections caused by viruses prevail and the unavailability of effective antiviral agents for the majority of viral infections is a serious problem. Although vaccines are available to control some of these infections, no effective antiviral therapy for the treatment of the majority of viral diseases in animals currently exists. With the difficulty in development of new vaccines, it can equally be presumed that easy viral candidates for vaccine development have been exhausted. In the midst of the threat posed to food security both in the developed and developing world, maintenance and sustenance of our zoological habitat as well as recreational privileges accorded to man by the well-being of animals, demand effort to find novel, specific and less toxic antiviral agents to counter the existing dilemma.

Feline herpesvirus-1 (FHV-1) is the most common viral pathogen of domestic cats worldwide. In cats, 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 Willoughby, 1999; 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 worldwide. The clinical signs and pathological lesions associated with the infection have been described by Jenner (Luader *et al.*, 1954). 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). Dogs naturally infected with CDV have also been reported to serve as alternative animal models to study the pathogenesis of demyelination in various diseases, including multiple sclerosis (Baumgärtner and Alldinger, 2005; Vandeveld 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 causes 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 Capri pox 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).

Although several hundreds of plants that have potential as novel antiviral agents have been studied, there still exist innumerable potentially useful medicinal plants waiting to be evaluated with biological activity that may be associated with a single phytochemical, or a number of different plant constituents. The objective of this study was to assess the antiviral effect of different extracts of selected medicinal plants with ethnobotanical indications in South African folk medicine, for *in vitro* activity against canine distemper virus (CDV), canine parainfluenza virus-2 (CPIV-2), lumpy skin disease virus (LSDV) and feline herpes virus-1 (FHV-1). Therapeutic inhibition of virus infection involves a number of strategies and targets a variety of steps in the life cycle of the virus such as cell entry, virus replication or the assembly and release of virions. Four enveloped viruses were selected for this study based on the postulation that targeting the entry of enveloped viruses may be a strategic approach for therapeutic interference, given that the site of action of the substances that will inhibit the virus is likely to be extracellular and practically open to the inhibitor, since potent antiviral activity is dependent on the identification and inhibition of viral specific events of replication.

## **6.2. Materials and Methods**

### **6.2.1. Viral pathogens used in the study**

The animal viruses used in the study were obtained from the Department of Veterinary Tropical Diseases, University of Pretoria, and selected for the study based on the reasons described in section 3.11.1. Two DNA and RNA viruses were used in the study. The viruses were as follows: feline herpes virus-1 (FHV-1, dsDNA, enveloped), lumpy skin disease virus strain V248/93 (LSDV, dsDNA, enveloped), canine distemper virus (CDV, ssRNA, enveloped) and canine parainfluenza virus-2 (CPIV, ssRNA, enveloped). Viruses were propagated in monolayer cells and harvested as described in section 3.11.1. The effective titre (TCID<sub>50</sub>/mℓ) of each of the viruses was determined using the method of Reed and Muench (1938), prior to each assay.

### **6.2.2. Cell cultures**

The susceptible cell types compatible for the growth of the viruses were kindly provided by the Department of Veterinary Tropical Diseases, University of Pretoria. The cells used in this study were Crandell feline kidney cells (CRFK), Vero cells and bovine dermis (BD) cells, respectively. Cells were maintained in appropriate culture media in the study as described in section 3.11.1.

### **6.2.3. Determination of cytotoxic effect of extracts on cells**

The selected plants (Table 4.1) were extracted with solvents of varying polarity as described in section 3.3. The cytotoxic effect of different extracts of each plant was tested against each cell type using the MTT colorimetric assay described by Mosmann, 1983 (section 3.9). The cytotoxicity was expressed as 50% cytotoxic concentration (CC<sub>50</sub>) of substances to inhibit the growth of cells by 50%, when compared to untreated cells, calculated from the linear regression equation. Berberine chloride (Sigma) was used as a positive control; wells containing only cells without extract treatment were the negative control and a solvent control was also included.

#### 6.2.4. Virucidal assay

The virucidal assay described by Barnard *et al.* (1992) with slight modifications was used to evaluate the antiviral potential of extracts of the selected plants as described in section 3.11.2. The extent of cell damage caused by infective virus 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 (section 3.11.2.) Plant extracts exhibiting reduction of viral infectivity at concentrations of  $10^3$  and  $10^4$  dilution were considered to possess strong activity while those with  $10^2$  to 10 as moderate to weak activity respectively by microscopic evaluation of infected cells. For the MTT assay, antiviral activity was expressed as a selectivity index (SI), where SI index values of more than three indicate potential antiviral activity (section 3.11.2.).

#### 6.2.5. 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 as described in section 3.12.3. Antiviral activity by CPE reduction and MTT assay was determined as previously described (section 3.11.2).

### 6.3. Results and Discussion

In this study, the hexane, dichloromethane, acetone and methanol extracts of the different plants were tested for their antiviral activity against all four viral pathogens used in this study. Results are presented as those extracts of the different plants at the lowest dilution that exhibited reduced CPE by microscopic examination of infected cells in the virucidal and attachment assays (Tables 6.1 and 2). 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.

In both the virucidal and attachment assays, the antiviral activity varied with the different extracts of the same plant as determined by virus-induced CPE by microscopic examination of infected cells (Tables 6.1 and 2). Some plant extracts exhibited moderate to good activity while others showed no evidence of reduced CPE. In the virucidal assay, the hexane extract of *Carissa edulis* inhibited by 75% FHV-1 and CDV induced CPE at  $10^3$  dilutions, while the DCM and methanol extracts at a dilution of  $10^1$  were able to inhibit viral-induced CPE by 25%. Despite the indication of reduced CPE of one or more of the tested viruses by these extracts, the acetone extract of this plant species was unable to inhibit viral-induced CPE of any of the viruses used in the study. A similar trend was also observed with the acetone extract of *Ekebergia capensis* and *Acokanthera schimperi* against all the tested pathogens (Table 1). While the DCM extract of *Ekebergia capensis* was able to inhibit viral-induced CPE of CPI-2 and CDV by 75% at  $10^3$  dilution of the extract, the hexane and methanol extracts inhibited viral-induced CPE of LSDV by 50% and 25% respectively at  $10^1$  dilution. With *Plumbago zeylanica*, all the extracts showed some degree of cell viability against either LSDV or CDV viruses, with the hexane extract exhibiting reduced CPE of CDV by 50% at  $10^3$  dilution. In contrast, only the acetone extract of *Schrebera alata* against LSDV and CDV and the methanol extract against LSDV induced CPE by 25% at  $10^2$  dilution while the hexane and DCM extracts could not. The acetone and methanol extracts of *Podocarpus henkelii* were able to inhibit viral-induced CPE of CDV and LSDV by 75% at  $10^3$  dilution while the DCM extract of this plant could not.

In the attachment assay were cells were exposed to viruses for various time intervals prior to addition of extracts. Variation was observed in the trend of inhibition of virus-induced CPE when compared to the virucidal assay. In this assay, only the DCM extract of *Carissa edulis* against CPI-2 exhibited reduced inhibition of viral infectivity by 25% as opposed to CDV in the virucidal assay, with the rest of the extracts being ineffective in reducing viral-induced CPE. The DCM and hexane extracts of *Ekebergia capensis* showed reduced LSDV and FHV-1 induced CPE by 50%, which was not the case in the virucidal assay (Table 6.2). The methanol extract of this plant exhibited no activity on all the tested pathogens in this assay. A similar variation was observed with extracts of *Acokanthera schimperi* in the attachment assay. While the hexane extract showed reduced FHV-1 induced CPE by 50% in the attachment assay, the effect was only observed against LSDV in the virucidal assay. Related trends were also observed with sensitivity of the viruses and activity of the different extracts of *Plumbago zeylanica*, *Schrebera alata* and *Podocarpus henkelii* (Table 6.2).

In the virucidal and attachment assays, SI values less than 1 represent weak, greater than 1 moderate, and greater than 3 good antiviral activities. The  $CC_{50}$ ,  $EC_{50}$  and SI values of those extracts that exhibited reduced CPE are presented in Tables 6.4 and 6.5. In general, good selectivity index values were obtained in the virucidal when compared to the attachment assay (Tables 6.3 and 6.4). The hexane extract of *Carissa edulis* in the virucidal assay exhibited weak activity against FHV-1 with  $EC_{50}$  of 73.17  $\mu\text{g}/\text{m}\ell$  and SI 1.22 while the same against CDV exhibited good activity with an  $EC_{50}$  12.37  $\mu\text{g}/\text{m}\ell$  and SI 6.14 (Table 6.3). However, this was not the case in the attachment assay. In the attachment assay, none of the extracts exhibited activity against the tested pathogens (Table 6.4). With *Ekebergia capensis*, weak activity was exhibited with the DCM extract against CPI-2 in both the virucidal and attachment assays while the hexane extract exhibited weak activity against FHV-1 in the attachment assay and not the virucidal assay (Tables 6.3 and 6.4). The hexane extract of *Plumbago zeylanica* on the other hand exhibited good activity against CDV with SI = 3.07 in the virucidal assay. Of all the plants used in this study the acetone extracts against CDV and the methanol extract of *Podocarpus henkelii* against LSDV exhibited good activity with SI values of 12.01 and 45.61 respectively in the virucidal assay (Table 6.3).

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-toxic 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  $\mu\text{g}/\text{m}\ell$  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).

Table 6.1. 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

Plants	Extracts	Virus	Time score (h)			Log concentration
			1	2	3	
<i>Carissa edulis</i>	Hexane	FHV-1, CDV	+++	+++	+++	10 <sup>3</sup>
	DCM	CDV	+	+	+	10 <sup>1</sup>
	Methanol	LSDV	+	+	+	10 <sup>1</sup>
<i>Ekebergia capensis</i>	Hexane	LSDV	++	++	++	10 <sup>2</sup>
	DCM	CPI-2, CDV	+++	+++	+++	10 <sup>3</sup>
	Methanol	LSDV	+	+	+	10 <sup>1</sup>
<i>Acokanthera schimperi</i>	Hexane	LSDV	+	+	+	10 <sup>1</sup>
	DCM	CDV	+	+	+	10 <sup>1</sup>
<i>Plumbago zeylanica</i>	Hexane	CDV	++	++	++	10 <sup>3</sup>
	DCM	LSDV	+	+	+	10 <sup>1</sup>
	Acetone	LSDV, CDV	+	+	+	10 <sup>1</sup>
	Methanol	LSDV	+	+	+	10 <sup>1</sup>
<i>Schrebera alata</i>	Acetone	LSDV, CDV	+	+	+	10 <sup>2</sup>
	Methanol	LSDV	+	+	+	10 <sup>2</sup>
<i>Podocarpus henkelii</i>	Hexane	LSDV	+	+	+	10 <sup>2</sup>
	Acetone	CDV	+++	+++	+++	10 <sup>3</sup>
	Methanol	LSDV	++	+++	+++	10 <sup>3</sup>

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, CPI-2 = Canine Para influenza virus-2, FHV-1 = Feline herpes virus, + + + = 75% inhibition, + + = 50% inhibition, + = 25% inhibition, h = duration in hours of incubation of virus with extracts prior to inoculation on to cells

Table 6.2. 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

Plants	Extracts	Virus	Time score (h)			Log concentration
			1	2	3	
<i>Carissa edulis</i>	DCM	CPI-2	+	+	+	10 <sup>1</sup>
<i>Ekebergia capensis</i>	DCM	CPI-2, LSDV	++	++	++	10 <sup>3</sup>
	Hexane	FHV-1	++	++	++	10 <sup>3</sup>
<i>Acokanthera schimperi</i>	Methanol	CPI-2, LSDV	++	++	++	10 <sup>1</sup> / 10 <sup>3</sup>
	Hexane	FHV-1	++	++	++	10 <sup>3</sup>
<i>Plumbago zeylanica</i>	Acetone	LSDV	++	++	++	10 <sup>3</sup>
	DCM	LSDV	+	+	+	10 <sup>2</sup>
<i>Schrebera alata</i>	Acetone	LSDV	++	++	++	10 <sup>1</sup>
	Methanol	CDV	++	++	++	10 <sup>1</sup>
<i>Podocarpus henkelii</i>	Acetone	LSDV	+++	+++	+++	10 <sup>3</sup>

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, CPI-2 = Canine parainfluenza virus-2, FHV-1 = Feline herpes virus, +++ = 75% inhibition, ++ = 50% inhibition, + = 25% inhibition, h = duration of exposure of cells to virus prior to addition of extract

In the virucidal and attachment assays, no differences were observed in virus-induced CPE by microscopic examinations when the extracts were incubated for various time intervals. However, the trend and severity of virus-induced CPE by microscopic examination depending on the plant extracts varies between the two assays. This variation may possibly be related to the subjective nature of scoring associated with microscopic determination of CPE. In the virucidal assay, viruses were treated with extracts before infection of cells. Although some extracts were unable to completely inactivate the viruses, virus-induced CPE was markedly reduced following inoculation onto cell culture. 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 in both the virucidal and attachment assays. 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  $\mu\text{g}/\text{m}\ell$  and 0.88 to 45.61  $\mu\text{g}/\text{m}\ell$  respectively. The hexane extract of *Carissa edulis* had moderate to good 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*. *Carissa edulis* has been found to contain chemical constituents such as steroids, terpenes, tannins, flavonoids and cardiac glycosides (Ibrahim, 1997), benzenoids, lignans, phenylpropanoids, sesquiterpenes and coumarins (Achenbach *et al.*, 1983; Bentley *et al.*, 1984). Monoterpenes have been shown to exhibit low cytotoxicity and relatively strong anti-herpes simplex virus-1 action. The mechanism by which the monoterpene isoborneol inhibits antiviral activity involves the interactions of its hydroxyl groups with virus envelope lipids. Isoborneol may also inhibit virus replication and the glycosylation of viral proteins with resultant loss in HSV-1 infectivity (Armaka *et al.*, 1999). Furthermore, potent anti-HSV-1 activities have also been reported for monoterpenes such as cineol and borneol, a stereoisomer of isoborneol. An analysis of diterpenes has shown that putranjivain A, isolated from *Euphorbia jolkini* (Euphorbiaceae), may interfere with HSV-2 ability to attach and penetrate cells and may also affect late stages in the replication of the virus (Khan *et al.*, 2005). Other groups of triterpenes have also been shown to inhibit infected cell polypeptide production in late stages of infection, reduce viral DNA synthesis or influence HSV-1 DNA synthesis and exhibit inhibitory effects on viral plaque formation (Khan *et al.*, 2005). Antiviral activity has also been reported for flavonoids (Li *et al.*, 2002), while sesquiterpenes have been demonstrated to exhibit moderate virucidal activity against a number of enveloped viruses including HSV-1, HCMV, measles virus and influenza A virus (Hayashi *et al.*, 1996). Recent studies based on the evaluation of the antiviral activity of various synthetic coumarins have revealed that some of them serve as potent non-nucleoside RT-inhibitors, inhibitors of HIV-integrase or HIV-protease (Kostova *et al.*, 2006).

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. The triterpenoid class of compounds isolated from *Ekebergia capensis* has been found to possess anti-HSV-1 activity (Ryu *et al.*, 1993). 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, the methanol extracts of *Plumbago zeylanica* at 91 µg/ml in that study did not inhibit HSV-1 plaque formation in Vero cells. The report is consistent with our findings where the methanol extract of this plant species exhibited no inhibitory effect on FHV-1. Interestingly, the hexane extract exhibited promising activity against CDV with a good SI value of 3.07. Earlier reports from investigations of the chemical constituents of *Plumbago zeylanica* revealed the presence of two plumbagic acid glucosides, 3'-O-β-glucopyranosyl plumbagic acid and 3'-O-β-glucopyranosyl plumbagic acid methylester along with five naphthoquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone), and five coumarins (seselin, 5-methoxyseselin, suberosin, xanthyletin and xanthoxyletin) in root extracts of this species (Lin *et al.*, 2003). Some naphthoquinones like rhinacanthin-C and rhinacanthin-D isolated from *Rhinacanthus nasutus* have been shown to possess antiviral activity against cytomegalovirus (CMV) with EC<sub>50</sub> values of 0.02 and 0.22 µg/ml respectively (Sendl *et al.*, 1996). Of all the extracts tested in the virucidal assay, the acetone and methanol extracts of *Podocarpus henkelii* against CDV and LSDV presented the best antiviral activity with SI value > 10 (Table 6.3). Biflavones of the amentoflavone and hinokiflavone groups, terpenoids and nor- and bisnorditerpenoid dilactones are major taxonomic markers in the family Podocarpaceae. Amentoflavone isolated from the ethanol extract of *Selaginella sinensis* showed potent antiviral activity against respiratory syncytial virus (RSV), with an IC<sub>50</sub> of 5.5 µg/ml (Ma *et al.* 2002). Although the constituent responsible for the observed activity in the different extracts of these plants is not clear, the possibility that similar classes of compounds present in the extracts may be responsible for the observed activity cannot be ruled out.

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 less potent ability of the extract to inactivate the virus prior to inoculation on cell monolayers (Tables 6.3 and 6.4). Also, the hexane extract of this plant had some degree of activity against FHV-1 with SI value < 1, which was not observed in the virucidal assay. The observed activity may be related to interference with the replication cycle of the virus in the attachment assay. Similar observations were also recorded for other plant extracts against the different pathogens (Tables 6.3 and 6.4). A degree of antiviral activity was also 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<sub>50</sub> values from 30.93 to 95.69.

Table 6.3. Selectivity index (SI) values (ug/ml) indicating virucidal activity of extracts of selected plants following incubation of virus with extracts prior to inoculation onto confluent host monolayer cells

Plants	Extracts	Virus	EC <sub>50</sub> <sup>a</sup>	CC <sub>50</sub> <sup>b</sup>	SI <sup>c</sup>
<i>Carissa edulis</i>	Hexane	FHV-1, CDV	73.17/12.37	89.41 / 76.00	1.22 / 6.14
	DCM	CDV	–	0.001	–
	Methanol	LSDV	–	111.66	–
<i>Ekebergia capensis</i>	Hexane	LSDV	–	14	–
	DCM	CPI-2, CDV	30.93/ 30.93	27.27/27.27	0.88 / 0.88
	Methanol	LSDV	–	13.13	–
<i>Acokanthera schimperi</i>	Hexane	LSDV	–	36.58	–
	DCM	CDV	–	0.001	–
<i>Plumbago zeylanica</i>	Hexane	CDV	11.73	36.05	3.07
	DCM	LSDV	–	6.66	–
	Acetone	LSDV, CDV	–	14 /32.21	–
	Methanol	LSDV	–	243.26	–
<i>Schrebera alata</i>	Acetone	LSDV, CDV	–	>1000 / 30.93	–
	Methanol	LSDV	–	243.68	–
<i>Podocarpus henkelii</i>	Hexane	LSDV	–	14.52	–
	Acetone	CDV	3.76	45.17	12.01
	Methanol	LSDV	3.36	153.24	45.61

<sup>a</sup> EC<sub>50</sub> = concentration of the sample required to inhibit virus-induced CPE by 50%, <sup>b</sup> CC<sub>50</sub> = concentration producing 50% cytotoxic effect <sup>c</sup>SI = CC<sub>50</sub> / EC<sub>50</sub>, LSDV = Lumpy skin disease virus, CDV = Canine distemper virus, CPI-2 = Canine parainfluenza virus-2, FHV-1 = Feline herpes virus, - = extracts that exhibited no reduced CPE

Table 6.4. Selectivity index (SI) values (ug/ml) 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

Plants	Extracts	Virus	EC <sub>50</sub> <sup>a</sup>	CC <sub>50</sub> <sup>b</sup>	SI <sup>c</sup>
<i>Carissa edulis</i>	DCM	CPI-2	–	0.001	–
<i>Ekebergia capensis</i>	DCM	CPI-2, LSDV	30.93 / –	27.27 / 2.48	< 1 / –
	Hexane	FHV-1	78.21	43.11	0.55
<i>Acokanthera schimperi</i>	Methanol	CPI-2, LSDV	–	0.001 / 51.66	–
	Hexane	FHV-1	51.89	30.29	0.58
<i>Plumbago zeylanica</i>	Acetone	LSDV	54.93	13.53	0.25
	DCM	LSDV	–	6.66	–
<i>Schrebera alata</i>	Acetone	LSDV	29.87	>1000	–
	Methanol	CDV	–	25.49	–
<i>Podocarpus henkelii</i>	Acetone	LSDV	95.69	107.39	1.12

<sup>a</sup> EC<sub>50</sub> = concentration of the sample required to inhibit virus-induced CPE by 50%, <sup>b</sup> CC<sub>50</sub> = concentration producing 50% cytotoxic effect <sup>c</sup>SI = CC<sub>50</sub> / EC<sub>50</sub>, LSDV = Lumpy skin disease virus, CDV = Canine distemper virus, CPI-2 = Canine parainfluenza virus-2, FHV-1 = Feline herpes virus, - = extracts that exhibited no reduced CPE

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(s) 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 preventing its replication in the host system.

#### **6.4. Conclusion**

Extracts of plants with antiviral activity were more potent in the virucidal than the attachment assay. Of the extracts tested in the virucidal assay, four extracts showed significant antiviral activity, two of which were different extracts of *Podocarpus henkelii* against two unrelated viruses. Although a different extractant was used than that used by traditional healers, the presence of antiviral compounds in *Podocarpus henkelii* against two unrelated viruses may justify on a chemotaxonomic basis the traditional use of related species *Podocarpus latifolius* and *Podocarpus falcatus* in the traditional treatment of canine distemper infection in dogs. In the next chapter, the extracts of the different plant species will be evaluated for their antifungal activity.