



**Characterisation of anti-  
viral compounds in  
Australian  
Bush Medicines**

**A report for the Rural Industries Research and  
Development Corporation**

by Associate Professor Robert Flower

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*Characterisation of antiviral compounds in Australian bush medicines*  
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# Foreword

Ethnobotany, the study of the interaction of indigenous peoples with plants, has been suggested as a useful guide for selecting plants that contain biologically active compounds. Only a limited number of the plants used in the medicine of the Australian Aboriginal peoples have been investigated in order to establish the biologically active constituents present and very few have been investigated for antiviral activity. In this study a number of known and novel antiviral compounds were isolated from Australian native plants traditionally used as a source of medicines

Viral diseases are becoming increasingly important with the emergence of resistance to established agents and development of new diseases. Antiviral activity has been detected in bush medicines include plants species from the genera *Dianella* and *Eremophila*. Potential benefits include commercialisation of new drugs and possibly demand for new crops to provide raw materials.

This report, a new addition to RIRDC's diverse range of over 400 research publications, forms part of New Plant Products R&D program, which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

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**Peter Core**  
Managing Director  
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# EXECUTIVE SUMMARY

## Objectives

The aim of this project was the isolation and identification of the chemical structure of antiviral compounds from Australian bush medicine plants and thus development of demand for cultivation of these plants.

## Background

Ethnobotany, the study of the interaction of indigenous peoples with plants, has been suggested as a useful guide for selecting plants that contain biologically active compounds. Only a limited number of the plants used in the medicine of the Australian Aboriginal peoples have been investigated in order to establish the biologically active constituents present and very few have been investigated for antiviral activity. In this study a number of known and novel antiviral compounds were isolated from Australian native plants traditionally used as a source of medicines.

## Research

A database of plants used as a source of medicines used in treatment of symptoms indicative of viral infection was assembled. Extracts from 40 different species were screened for antiviral activity against three different viruses. The most active extracts were *Pterocaulon sphacelatum* (Asteraceae) and *Dianella longifolia* var. *grandis* (Liliaceae). The extracts of *Euphorbia australis* (Euphorbiaceae) and *Scaevola spinescens* (Goodeniaceae) active against cytomegalovirus. Extracts of *Eremophila latrobei* subsp. *glabra* (Myoporaceae) and *Pittosporum phylliraeoides* var. *microcarpa* (Pittosporaceae) exhibited antiviral activity against Ross River Virus.

## Outcomes

*P. sphacelatum*, yielded the antiviral flavonoid chrysofenolol a 4'-hydroxy-3-methoxyflavone, one of a group of compounds known to be potent and specific inhibitors of replication of picornaviruses including the most frequent causative agent of the common cold.

Activity-guided fractionation of the root extract of *D. longifolia* resulted in the identification of chrysophanic acid (1,8-dihydroxy-3-methylanthraquinone) as the anti-polioviral component. Anthraquinones have not previously been found to inhibit non-enveloped viruses.

Chrysophanic acid inhibited an early stage in the poliovirus replication cycle and it may act as an inhibitor of proteases cleaving the picornaviral polyprotein.

*E. australis* yielded polyphenolic compounds responsible for the anti-HCMV activity.

### Implications

In this study, known and novel antiviral compounds were isolated from Australian native plants traditionally used by Aboriginal people as a source of medicines. The detection of pharmacologically active compounds in these extracts provided evidence that traditional Aboriginal medicines may be an important source of novel compounds. An anthraquinone was found to have antiviral activity to viruses from the same group as the virus which cause the common cold. The potential of this finding for use of this compound as a drug and cultivation of these plants as a source of this compound should be investigated.

# OBJECTIVES

As the number of agents available to treat viral diseases remains small, the aim of this project is the investigation of the concentrations of antiviral compounds in some *Dianella* spp, and other plants in which antiviral compounds have been detected, and thus development of demand for cultivation of these plants.

# INTRODUCTION

The research need was to identify plant species that may contain antiviral compounds that may be a source on new drugs and as a result of demand for these compounds, new crops. The strategy used to identify plants that may contain antiviral compounds was to identify those species utilised by the Australian Aboriginal peoples as traditional medicines. The plant species selected were those used for the treatment of symptoms indicative of viral illness.

Prior to the selection process, consideration was given to the types of human viral illnesses that may have been present in Australia prior to European settlement. Plant species used for the treatment of symptoms consistent with these types of illness were targeted. In order to select the plant species, the literature pertaining to the traditional medicinal use of plants by the Australian Aboriginal people was reviewed. A list of the selected plant species was composed, from which 40 different species could be collected in the field.

A database of plants used as a source of medicines used in treatment of symptoms indicative of viral infection was assembled. For 40 different plant species from the list, ethanolic extracts from were produced for antiviral testing. The extracts were screened for antiviral activity against three different viruses: one DNA virus, human cytomegalovirus (HCMV, *Herpesviridae*), and two RNA viruses, Ross River virus (RRV, *Togaviridae*) and poliovirus (*Picornaviridae*).

# METHODOLOGY

Buffalo green monkey kidney (BGM) and African green monkey kidney (Vero) cells were obtained from the Infectious Diseases Laboratory, Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia. Human embryonic lung (HEL) cells were obtained from Commonwealth Serum Laboratories, Parkville, Victoria, Australia. Cells were grown in Dulbecco's modified eagle medium (DMEM) with sodium bicarbonate, 3.7 g/L, glucose, 4.5 g/L, HEPES buffer, 15 mM, (CSL, Parkville, Victoria, Australia), glutamine, 2 mM, gentamicin, 16 µg/ml, penicillin, 12 µg/ml, and heat inactivated foetal calf serum (5% v/v for BGM and Vero cells; for HEL cells 10% v/v was used to grow the cells, and 2% v/v was used to maintain the cells in antiviral assays). Viruses were obtained from the Infectious Diseases Laboratory, Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia. Poliovirus type 3 (Sabin), and Coxsackievirus B4 (clinical isolate) were propagated in BGM cells; Ross River virus (RRV, strain T48) and herpes simplex virus type 1 (HSV-1, clinical isolate) were propagated in Vero cells; human rhinovirus type 2 (HRV-2) was propagated in low passage number HEL cells. All viruses were propagated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>, in air, with the exception of HRV-2 which was grown at 33°C.

Activity-guided fractionation of the various extract was using assays of inhibition of virus-induced cytopathic effects. For the various extracts activity-guided fractionation and chemical techniques were used to determine the structure. For example, from the roots of *Dianella longifolia* var. *grandis* activity guided fractionation led to the identification of the anthraquinone chrysophanic acid as the antiviral component of the extract. The chemical structure was confirmed by nuclear magnetic resonance (NMR) spectrometry. <sup>1</sup>H NMR (Varian Inova, 600MHz): (CCl<sub>4</sub>/acetone-d<sub>6</sub>, 9/1). δ (ppm) 2.45 [3H, s, ArCH<sub>3</sub>], 7.00 [1H, m, C2(H)], 7.17 [1H, dd, C7(H)], 7.52 [1H, m, C4(H)], 7.61 [1H, t, C6(H)], 7.69 [1H, dd, C5(H)], 11.83 [1H, s, ArOH], 11.93 [1H, s, ArOH]. The spectral assignment was confirmed with one bond (gHSQC) and long range (gHMBC) heteronuclear coupling experiments. The <sup>1</sup>H-NMR profile and behaviour on thin layer chromatography was identical to that of an authentic sample of chrysophanic acid. The commercial material was used for all further antiviral testing described below.

# RESULTS

Extracts from six plant species were found to have activity against one of the three viruses tested. The most active extracts were *Pterocaulon sphacelatum* (Asteraceae) and *Dianella longifolia* var. *grandis* (Liliaceae). The extracts of *Euphorbia australis* (Euphorbiaceae) and *Scaevola spinescens* (Goodeniaceae) active against HCMV. Extracts of *Eremophila latrobei* subsp. *glabra* (Myoporaceae) and *Pittosporum phylliraeoides* var. *microcarpa* (Pittosporaceae) exhibited antiviral activity against RRV.

*P. sphacelatum*, yielded the antiviral flavonoid chrysosplenol C (3, 7, 3' trimethoxy, 5, 6, 4' trihydroxyflavone). This compound is a 4'-hydroxy-3-methoxyflavone, one of a group of compounds known to be potent and specific inhibitors of picornaviral replication. These compounds inhibit the replication of rhinoviruses, the most frequent causative agent of the common cold.

Activity-guided fractionation of the root extract of *D. longifolia* resulted in the identification of chrysophanic acid (1,8-dihydroxy-3-methylanthraquinone) as the anti-polioviral component. Anthraquinones have not previously been found to inhibit non-enveloped viruses.

Chrysophanic acid inhibited an early stage in the poliovirus replication cycle and it may act as an inhibitor of proteases cleaving the picornaviral polyprotein or as a capsid-binding agent. The compound did not have significant antiviral activity against four other viruses tested: coxsackievirus B4, human rhinovirus 2 (*Picornaviridae*), and the enveloped viruses Ross River virus (*Togaviridae*) and herpes simplex virus type 1 (*Herpesviridae*).

Four anthraquinones structurally-related to chrysophanic acid, rhein, 1,8-dihydroxyanthraquinone, emodin and aloe-emodin were also tested for activity against poliovirus. The results suggested that the hydrophobicity of chrysophanic acid, and the methyl group attached to C-3 on the molecule were important for its anti-polioviral activity.

Partial activity-guided fractionation of an extract of whole plants of *E. australis*, active against HCMV, was undertaken. Tannins or other polyphenolic compounds in the extract, of little commercial interest, were responsible for the anti-HCMV activity.

# DISCUSSION OF RESULTS

## *Chrysosplenol C from P. sphacelatum*

Chrysosplenol C (3,7,3'-trimethoxy, 5,6,4'-trihydroxyflavone) was identified as the major antipicornaviral component of an extract of the green aerial parts of *P. sphacelatum*. The antiviral activity of this compound was not novel. Chrysosplenol C belongs to a group of compounds known as the 4'-hydroxy-3-methoxyflavones which are potent and specific inhibitors of picornaviral replication, including the replication of polioviruses, coxsackieviruses, echoviruses and human rhinoviruses. Human rhinoviruses are the most frequent causative agent of the common cold, and other picornaviruses including some of the coxsackieviruses and echoviruses also cause respiratory infections. It was therefore of interest that this compound was isolated from *P. sphacelatum*, a species used by Australian Aboriginal peoples for the treatment of colds and other respiratory tract infections.

Chrysosplenol C, isolated from a plant in the genus *Chrysosplenium* (Saxifragaceae) has been shown to inhibit the replication of human rhinovirus type 2 in vitro. These authors concluded that the *Chrysosplenium* spp., which contain large amounts of chrysosplenol C, may be useful medicinal herbs against the common cold caused by rhinovirus infection.

Independently, 2 other groups discovered the antipicornaviral activity of the 4'-hydroxy-3-methoxyflavones. Investigators from the Roche pharmaceutical company reported the in vitro anti-rhinovirus activity of 3,7,3'-trimethoxy, 5,4'-dihydroxyflavone (called Ro 09-0179) which was isolated from a Chinese medicinal herb. Ro 09-0179 differs from chrysosplenol C only at position 6 in the molecule. Other compounds with a 4'-hydroxy-3-methoxyflavone structure were also reported as the active components of African medicinal plants which exhibited activity against picornaviruses. These compounds included the 3-methylethers of quercetin and kaempferol and the 3, 3'-dimethylether of quercetin. The compounds exhibited potent activity against human picornaviruses in vitro at doses non-toxic to cells.

The potent activity of 4'-hydroxy-3-methoxyflavones in vitro has led to extensive study of their structure-activity relationships, mechanism of action and spectrum of antiviral activity.

A range of natural and synthetic flavone compounds for cytotoxicity and antiviral activity against poliovirus and human rhinovirus have been tested by others. Chrysosplenol C contains the structural requirements for a potent inhibitor of picornaviral replication. It has a 4'-hydroxyl group, a methoxy group at position 3, and a polysubstituted A- ring including a hydroxyl substituent at position 5. Substitution of the 3' position of the B-ring results in increased cytotoxicity with little effect on antiviral potency. Chrysosplenol C, therefore, is more cytotoxic than some of the 3-O-methylkaempferol derivatives, which lack substitution at the 3' position.

A 50% cytotoxic dose for chrysosplenol C of 20 µg/mL (in HeLa cells) has been reported with a minimum effective dose against rhinovirus of 1.25 µg/mL. The non-cytotoxic dose for chrysosplenol C extracted from *P. sphacelatum* in this study was 3.91 µg/mL (10.86 µM). This was not inconsistent with the higher value (20 µg/mL) reported, because these authors were measuring the 50% cytotoxic dose, rather than the non-cytotoxic dose. The compound was found to have more potent activity against poliovirus (EC<sub>50</sub> 0.27 µg/mL) than reported for rhinovirus by Tsuchiya et al. (1985). Rhinovirus is usually less sensitive than poliovirus to the inhibitory effects of 4'-hydroxy-3-methoxyflavones.

Studies undertaken to elucidate the mechanism of action of the 4'-hydroxy-3-methoxyflavones have shown that they inhibit the replication of the RNA genome of the picornaviruses. These compounds have been shown to preferentially block plus-strand viral RNA synthesis compared to minus-strand synthesis. Evidence suggests that they may interfere with binding of viral replication complexes to vesicular membranes in the cell.

In traditional Australian Aboriginal medicine *P. sphacelatum* has been used as an inhalation therapy for the treatment of colds and respiratory infections. The plant has been used to make a steam inhalation, but

crushed fresh leaves of the plant have also been inserted into the nasal cavity, or dried and inhaled like a snuff. Chrysosplenol C is non-volatile (mpt 218-220°), and as such is unlikely to have been effective in a steam inhalation therapy. Other components of the plant, such as the aromatic essential oils, may as an inhalation provide relief from symptoms of the common cold. Aromatic oils mildly irritate the mucous membranes of the respiratory tract and throat, increasing secretion of mucous and saliva, which can relieve sore throat and nasal congestion.

When crushed plant material was inserted into the nose, the inhalation of the essential oils may have contributed to a therapeutic effect. It is interesting to speculate, however, that there may also have been some beneficial effect from the release of the antiviral flavonoid into nasal secretions resulting in inhibition of picornaviral replication in the cells lining the nasal cavity. Replication of viruses that cause common cold, including rhinoviruses and coxsackieviruses, occurs in the epithelial cells that line the upper respiratory tract. Many antiviral compounds trialed clinically for the treatment of common cold are administered intranasally to achieve high concentrations at the site of virus replication.

### ***Chrysophanic acid from *Dianella longifolia****

Chrysophanic acid (chrysophanol) is a naturally occurring anthraquinone that has been isolated from a number of natural sources including plants, lichens and microbes. In this study chrysophanic acid was identified as the active component of an extract of the medicinal plant *Dianella longifolia*, which had previously been found to inhibit poliovirus. Although anthraquinones structurally related to chrysophanic acid have previously been shown to inhibit enveloped viruses by both virucidal and non-virucidal mechanisms, this is the first report of activity of this type of compound against a non-enveloped virus. Chrysophanic acid was found to inhibit poliovirus in both CPE inhibition and yield reduction assays at concentrations well below those that caused toxicity to actively growing cells. The maximum non-toxic concentration of the compound in the 3 cell lines tested was found to be 12.5 µg/ml (49.1 µM). This result was not inconsistent with previously reported toxicity data for the compound.

The antipoliovirus activity of this compound was also of interest because *D. longifolia* has been used as a traditional medicine for the treatment of the common cold by Aboriginal people in Southern Australia. Some viruses of the family Picornaviridae, to which poliovirus belongs, are important causative agents of the common cold. Rhinoviruses are the most frequent cause of winter colds, and some enteroviruses including coxsackieviruses and echoviruses are important causes of summer colds. In this study, however, chrysophanic acid did not exhibit in vitro antiviral activity against the two other picornaviruses tested: the rhinovirus HRV-2 and the enterovirus CVB4. This may indicate that the activity of the compound is limited to poliovirus. More extensive testing against a range of picornaviruses is required in order to establish the compound's spectrum of activity. It would be of interest to test the compound against coxsackievirus A21 or A24, which are considered close relatives of the polioviruses, as well as being potential causative agents of the common cold. Chrysophanic acid did not demonstrate any significant activity against the two enveloped viruses tested (HSV-1 and RRV). This result is consistent with previous findings that the compound lacks significant virucidal or other antiviral activity against enveloped viruses.

Investigation of the mechanism by which chrysophanic produces its antipoliovirus effect, suggested that the compound does not cause irreversible disruption of viral particles (virucidal effect). Pre-incubation of a poliovirus stock with chrysophanic acid at a concentration of 10 µg/ml, 500 times higher than the EC50 for the compound, produced no decrease in the infectious virus titer of the stock.

Cells were pre-incubated with chrysophanic acid for 24 hours prior to infection with poliovirus in order to test if the compound made the cells less susceptible to poliovirus infection. An antiviral state may be induced by compounds which have effects such as alteration of cell surface receptors or induction of host cell defence mechanisms. When BGM cells were pre-incubated with chrysophanic acid, inhibition of poliovirus-induced CPE occurred even though the cells were washed to remove the compound prior to the addition of virus. The EC50, however, was significantly higher (0.48 µg/ml compared with 0.02 µg/ml when the compound was present during virus replication but not pre-incubated with cells). If the compound exerted an effect on cells which made them less susceptible to poliovirus infection, pre-incubation with the cells prior to addition of virus would be expected to potentiate this type of effect. The lower level of activity of the compound in the pre-incubation assay suggests that chrysophanic acid does not inhibit

poliovirus by inducing an antiviral state in cells. The residual activity after washing cells to remove the chrysophanic acid may be explained by the hydrophobic nature of the compound. The poor aqueous solubility of the compound may mean that it has a tendency to accumulate in cells, and not be completely removed by washing of cells with aqueous-based solutions. This has been observed for other hydrophobic compounds that inhibit picornaviruses.

A further experiment was carried out to determine the point at which chrysophanic acted in a single cycle of poliovirus growth. Chrysophanic acid was found to inhibit poliovirus when added during and immediately after a 1 h viral adsorption period, but when the compound was added 1h after the adsorption period, it was no longer active. This suggested that the compound acted at an early stage in the poliovirus replication cycle. The greatest reduction in virus yield occurred when the compound was present during the adsorption period. This suggests that the compound may bind to poliovirus particles to either prevent attachment to cellular receptors and/or an early step in virus replication such as uncoating. This pattern of inhibition is consistent with other compounds that act as picornavirus capsid-binding agents such as 4',6-dichloroflavan, chalcones; rhodanine, arildone, the WIN compounds.

These compounds of diverse chemical structure all possess a high degree of hydrophobicity, and act by binding to a hydrophobic pocket in the viral capsid protein VP1. This pocket lies beneath the canyon formed at the junction of VP1 and VP3 (8). Some of the capsid-binding such as 4',6-dichloroflavan and rhodanine are flat molecules in addition to being hydrophobic properties also shared by chrysophanic acid.

Testing of four other structurally related anthraquinones was performed in order to obtain some information about the structure-activity relationship of the anti-poliovirus activity of chrysophanic acid. The results suggested that the methyl group attached to the C-3 position on the molecule is important for activity against poliovirus. Aloe-emodin (in which the C-3 methyl group is substituted with a hydroxyl function) and 1,8-dihydroxyanthraquinone (which lacks a C-3 methyl group) were substantially less active (approximately 25-fold) than chrysophanic acid in the inhibition of CPE assay. In rhein, substitution of the C-3 position with a bulky, polar carboxylic acid function resulted in complete loss of anti-poliovirus activity. Emodin retains the C-3 methyl function, but has an extra hydroxyl group attached to C-6. It was the most active of the four extra anthraquinones tested, but was still significantly (approximately 12-fold) less active than chrysophanic acid. This suggested that increasing the polarity of the molecule results in a reduction of activity, which is consistent with the hydrophobicity of chrysophanic acid being important for its anti-poliovirus activity. Derivatives of chrysophanic acid with a longer non-polar chain attached to C-3 may have more potent anti-poliovirus activity.

The picornaviral proteases (2A and 3C) are cysteine proteases (a cysteine residue acts as the nucleophile in the active site of the enzyme) which are structurally related to the trypsin-like serine proteases. The poliovirus and rhinovirus 2A protease is inhibited by two compounds (elastatinal and methoxy-Ala-Ala-Pro-Val-chloromethylketone) that are specific inhibitors of the mammalian elastases, a group of serine proteases. As a result, it has been suggested that the poliovirus and rhinovirus 2A proteases have a substrate-binding pocket very similar to that of elastases.

While anthraquinones have not previously been reported to inhibit picornaviral proteases, it is interesting that human elastases called human leukocyte elastase (HLE) and cathepsin G, are inhibited by anthraquinone derivatives. These human proteases were tested with a series of anthraquinone derivatives, 4 of which were chrysophanic acid, emodin, aloe-emodin, and rhein. The relative potency of these compounds against the HLE enzyme was comparable with the pattern seen for these same compounds against poliovirus in the present study, and suggests that the 2A protease of poliovirus could be a target for these compounds. Testing with other anthraquinone derivatives against HLE by suggested that the important substituents for activity were a hydrophobic substituent at C3 or C2 of the anthraquinone (probably forming stabilising hydrophobic interactions with the enzyme), and a free hydroxyl group at C1 (presumably involved in hydrogen-bonding with the enzyme). These authors also found that the presence of a hydroxyl substituent at C6 reduced activity against the enzyme.

In conclusion, the anthraquinone chrysophanic acid has been found to exhibit *in vitro* antiviral activity against poliovirus. The compound acts at an early stage of the poliovirus replication cycle, but does not

have a virucidal effect on poliovirus particles. Although the compound was not active against two other picornaviruses tested (HRV-2 and CVB4), more extensive studies with other picornaviruses, including coxsackievirus A21, are being undertaken in order to determine the compound's spectrum of activity.

Ethnobotany, the study of the interaction of indigenous peoples with plants, has been suggested as a useful guide for selecting plants that contain biologically active compounds. Only a limited number of the plants used in the medicine of the Australian Aboriginal peoples have been investigated in order to establish the biologically active constituents present and very few have been investigated for antiviral activity. In this study a number of known and novel antiviral compounds were isolated from Australian native plants traditionally used as a source of medicines.

# OUTCOMES

## COMPARED WITH OBJECTIVES

### **A database was assembled, plants collected and screened for antiviral activity and antiviral compounds identified**

Results of the antiviral screening of bush medicines and description of the compound isolated from *Pterocaulon sphacelatum* have been accepted for publication in the *Journal of Ethnopharmacology*. A manuscript describing the compound and the mechanism of antiviral activity for *Dianella longifolia* is attached.

#### **Publication**

**Semple S.J., Reynolds G.D., O'Leary M.C. and Flower R.L.P.** (1998) Screening of Australian medicinal plants for antiviral activity. *J. Ethnopharmacology* **60**: 163-172.

## IN RELATION TO OBJECTIVES

### ***Fractionate extracts with anti-viral activity***

Activity-guided fractionation of the extract of *Pterocaulon sphacelatum* has been completed. Solvent-solvent partitioning, flash silica chromatography and recrystallisation lead to the purification of the major active component of the extract. Two other compounds were also isolated from the plant using recrystallisation, flash chromatography, and high performance liquid chromatography (HPLC). Activity-guided fractionation of the extract of *Dianella longifolia* has been completed. Solvent-solvent partitioning, flash silica chromatography HPLC and recrystallisation lead to the identification of the major active component of the extract. Two other compounds were also isolated from the plant.

#### **Publications**

**Semple S.J., Reynolds G.D., and Flower R.L.P.** (1999) Antiviral activity of *Pterocaulon sphacelatum*. *J. Ethnopharmacology*, in press.

**Semple S.J., Reynolds G.D., and Flower R.L.P.** (1999) Antiviral activity of *Dianella longifolia*. Manuscript submitted for publication.

Partial activity-guided fractionation of an extract of whole plants of *E. australis*, active against HCMV, was also undertaken.

### ***Identify and examine spectrum of activity of anti-viral compound(s) - anti-picornaviral compounds***

Structural elucidation techniques including nuclear magnetic resonance (NMR), mass spectrometry (ms) and ultraviolet spectroscopy were used to identify the anti-picornavirus compound from *Pterocaulon sphacelatum* as Chrysosplenol C (5,6,4'-trihydroxy-3,7,3'-trimethoxyflavone), not previously isolated from the plant genus *Pterocaulon*. The other compounds from the extract of this plant were identified as simple coumarin compounds, which did not demonstrate anti-viral activity.

The antiviral compound in *Dianella longifolia* was identified using structural elucidation techniques including nuclear magnetic resonance (NMR), mass spectrometry (ms) and ultraviolet spectroscopy. The active compound was Chrysophanic acid (1,8-dihydroxy-3-methylanthraquinone). The compound has not been isolated previously from this plant species and inhibited poliovirus at concentration of 20 pg/ml, well below the concentration inhibiting the growth of cells.

Two additional compounds previously isolated from *Dianella laevis* (now a synonym for *D. longifolia*), the naphthalene derivatives called dianellin and dianellidin have been isolated from the extract of *D. longifolia* and identified by nuclear magnetic resonance (NMR). However they demonstrated no activity against poliovirus.

Chrysophanic acid inhibited an early stage in the poliovirus replication cycle, but did not have an irreversible virucidal effect on poliovirus particles. Four structurally-related anthraquinones, rhein, 1,8-dihydroxyanthraquinone, emodin and aloe-emodin were also tested for activity against poliovirus. None of the four compounds was as active as chrysophanic acid against the virus. The results suggested that the hydrophobicity of chrysophanic acid, and the methyl group attached to C-3 on the molecule are important for its anti-polioviral activity

The anti-picornaviral compound from *Pterocaulon sphacelatum*, Chrysosplenol C, belongs to a group of compounds known as the 4'-hydroxy-3-methoxyflavones which are known potent inhibitors of picornaviral RNA synthesis at non-cytotoxic concentrations. These compounds are specific inhibitors of picornaviruses, including rhinovirus, a major cause of the common cold. They do not inhibit other RNA or DNA viruses.

The antiviral spectrum of Chrysophanic acid from *Dianella longifolia* was investigated. The compound did not have significant antiviral activity against four other viruses tested: coxsackievirus B4, human rhinovirus 2 (*Picornaviridae*), and the enveloped viruses Ross River virus (*Togaviridae*) and herpes simplex virus type 1 (*Herpesviridae*)

***Identify and examine spectrum of activity of anti-viral compound(s) - anti-CMV compounds.***

Partial activity-guided fractionation of an extract of whole plants of *E. australis*, active against HCMV, was undertaken. The active fraction of the extract was found to act at a stage prior to virus entry into cells. The removal of tannins from one of the active fractions resulted in the loss of anti-HCMV activity. This suggested that tannins or other polyphenolic compounds in the extract were responsible for the anti-HCMV activity.

# IMPLICATIONS AND RECOMMENDATIONS

In this study known and novel antiviral compounds were isolated from Australian native plants traditionally used by Aboriginal people as a source of medicines, the first implication of this study was that traditional Aboriginal medicines may be an important source of novel compounds. An anthroquinone was found to have antiviral activity to viruses from the same group as the virus which causes the common cold. The potential of this finding for use of this compound as a drug and cultivation of these plants as a source of this compound should be investigated.