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SHORT COMMUNICATION

Activity of a Methanolic Extract of Zimbabwean *Crinum macowanii* against Exotic RNA Viruses *in vitro*

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The *in vitro* antiviral activity of a freeze-dried methanolic extract of the bulbs of Zimbabwean *Crinum macowanii* was determined in Vero cells infected with a number of exotic RNA viruses. At a concentration of 32 μg/mL, the crude methanolic extract reduced by 100% the viral cytopathic effect in Vero cells infected with yellow fever virus, and caused a 70% inhibition of the viral cytopathic effect in cells infected with Japanese encephalitis virus. No evidence of cytotoxicity was observed at this concentration. The freeze-dried extract inhibited the replication of the above mentioned viruses by 100% at a concentration of 10 μg/mL. No cytotoxicity was seen at this concentration.

Keywords: Crinum macowanii; yellow fever virus; Japanese encephalitis virus; Punta Toro virus; Venezuelan equine encephalitis virus.

INTRODUCTION

Extracts of *Crinum macowanii* (vlei lily, *dururu* (Shona), *umduze* (Ndebele)) have a number of applications in the traditional medicine of Zimbabwe. Infusions of the bulb of the plant are used to relieve backache, as an emetic, or to increase lactation in animal and human mothers. The powdered plant, mixed with porridge, is also used as a remedy for sexually transmitted diseases (Gelfand *et al.*, 1985). The antitumour activities of alkaloids of the Amaryllidacae have been extensively investigated, notably by the Pettit group (Pettit *et al.*, 1984, 1986). The isolation of trans-dihydronarciclassine, the principal antiviral and cytostatic constituent of the Chinese medicinal plant, *Zephyranthes candida*, was reported recently (Pettit *et al.*, 1990). The activity of selected amaryllis alkaloid constituents against exotic RNA viruses was described recently (Gabrielsen *et al.*, 1992a). Narciclasine, lycoricidine, pancratistatin, lycorine, pseudolycorine, and pretazettine were active *in vitro* against the flaviviruses: Japanese encephalitis virus (JE), yellow fever virus (YF), and dengue virus (type-4). Administration of pancratistatin at 4 mg/kg/day for 5 days protected mice against a lethal challenge with JE (Gabrielsen *et al.*, 1992a). These encouraging results prompted us to investigate the antiviral activity of a variety of amaryllis species. In this paper we report the initial observation of antiviral activity of a crude preparation of *Crinum macowanii*.

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MATERIALS AND METHODS

Plant materials and extraction. *Crinum macowanii* was collected in December 1990 in Harare, Zimbabwe. Fresh bulbs (2.5 kg) were air dried for 1 week and extracted (for 21 days) at room temperature (27 °C) with 9 L methanol. The filtered solution was concentrated to 300 mL. Freeze-drying of this extract afforded 40 g of a dark, hygroscopic resin.

Determination of antiviral activity. The *in vitro* antiviral and cytopathic effects of extracts were measured by observing inhibition of viral cytopathic effects (CPE) by using an MTT-assay (Gabrielsen *et al.*, 1992a, 1992b and references therein). Antiviral activity and cytotoxicity were reported as: (a) the concentration inhibiting 50% of CPE (IC_{50}); (b) cellular toxic concentration (TC_{50}), the drug concentration that reduced metabolic activity by 50%; and (c) the therapeutic index (TI), a measurement of the relative anticellular and antiviral effects of the drug. This index is the ratio of TC_{50} to IC_{50}, i.e. TC_{50}/IC_{50}. The results were determined in triplicate wells of a 96-well microtitre plate.

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Table 1. Antiviral activity of a freeze-dried extract of *C. macowanii* against exotic RNA viruses in vero cells

Virus	IC_{50} (μg/mL)	TC_{50} (μg/mL)	Therapeutic index
Yellow fever	4.33	29.33	6.76
Japanese encephalitis	5.12	29.60	5.79
Sandfly fever	—	30.30	—
Punta Toro	—	26.60	—
Venezuelan equine encephalitis	—	43.30	—

RESULTS AND DISCUSSION

The antiviral activity of the freeze-dried extract of *C. macowanii* was determined against a variety of exotic RNA viruses: the flaviviruses yellow fever (YF) and Japanese encephalitis (JE); the bunyaviruses Punta Toro (PT) and sandfly fever (SF); and the alphavirus, Venezuelan equine encephalitis (VE). The extract was highly active against the flaviviruses YF and JE. For these viruses, the CPE was reduced by 50% at an extract concentration of about 5 μg/mL (Table 1). At an extract concentration of 10 μg/mL, the viral CPE was eliminated in the case of JE and YF.

There was little antiviral activity at less than cytotoxic concentrations (about 30 μg/mL) for the bunyavirus, SF; the CPE was reduced by 34% at an extract concentration of 10 μg/mL, but higher doses of the extract did not afford a significant further reduction in CPE. Similarly, we observed a small antiviral effect against the bunyavirus, PT, where there was a reduction of viral CPE by 25% at 10 μg/mL. We observed no antiviral effects against the alphavirus VE at less than cytotoxic concentrations.

Gabrielsen *et al.* (1992a) reported that amaryllis alkaloids such as pancratistatin were highly active against the flaviviruses JE and YF but showed little activity short of toxic concentration against bunya- or alphavirus species. The results we obtained for the crude *C. macowanii* extract parallel their results and suggest that the antiviral activity of this extract may be due to similar alkaloidal components. Further exploitation of this lead will require the isolation, characterization, and evaluation of the active constituents of this extract.

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