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Phytochemical Screening and Antiviral Activity of some Medicinal Plants from the Island Soqotra

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Methanol and hot-aqueous extracts of 25 different plant species, used in Yemeni traditional medicine and growing, partly as endemic plants, on the island Soqotra have been investigated for their antiviral activity. In addition, the phytochemical identification of the main chemical constituents was performed. The extracts were assayed in two *in vitro* viral systems, which used influenza virus type A/MDCK cells and herpes simplex virus type 1/Vero cells, at non-cytotoxic concentrations. The herpes simplex virus type 1 showed more sensitivity than the influenza virus type A against the extracts investigated. The methanol extracts of *Boswellia ameero*, *Boswellia elongata*, *Buxus hildebrandtii*, *Cissus hamaderohensis*, *Cleome socotrana*, *Dracaena cinnabari*, *Exacum affine*, *Jatropha unicostata* and *Kalanchoe farinacea* showed anti-influenza virus type A activity with 50% inhibition (IC_{50}) concentrations ranging from 0.7 to 12.5 μg/mL. In addition, 17 plants of the 25 investigated exhibited anti-HSV-1 activity. The antiviral activity of some active extracts was also observed on a molecular level. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: medicinal herbs; Yemen; Soqotra; antiviral; influenza virus; HSV.

INTRODUCTION

Traditional practice still plays a fundamental role in several areas of Yemen, especially on the island Soqotra. The considerable variety in topography, as well as the pronounced local differences in climate reflect the uniqueness of the vegetation and the outstanding floral diversity of this island.

Within the past decade therapeutic options for viral infections have improved significantly. However, simultaneously antiviral treatment is associated with the emergence of resistant viruses. The further disposal of resistant strains is one reason for therapeutic failure (Gadreau *et al.*, 1998; Emery, 1998; Safrin *et al.*, 1999). Furthermore, many of the licensed drugs are toxic as well as being expensive (Pillay *et al.*, 2002), thus the search for potential sources for the development of new drugs is very important. Based on broad experience in traditional herbal medicine, several studies in different areas were performed to detect active natural products in higher plants. In these studies different viruses were included, e.g. herpes simplex virus (HSV), feline immunodeficiency virus, coxsackie virus, influenza virus, parainfluenza virus, respiratory syncytial virus (Abad *et al.*, 2000; Rajbhandari *et al.*, 2001; Schmitt *et al.*, 2001; Cos *et al.*, 2002; Li *et al.*, 2002; Ooi *et al.*, 2004; Li *et al.*, 2004; Vijayan *et al.*, 2004). The assumption that scarce research had been done on Yemeni plants and the possibility that these plants may have potential as antiviral agents, enhanced their study. Recently, the antimicrobial activity of some of these plants was reported (Mothana and Lindequist, 2005). The present study deals with the antiviral activity of 47 methanol and hot-water extracts of 25 medicinal plants belonging to 19 different families, which are used traditionally in the treatment of infectious diseases such as skin and respiratory tract infections and other diseases. The extracts have been examined in two *in vitro* viral systems, influenza virus type A/MDCK cells and herpes simplex virus type 1/Vero cells, at non cytotoxic concentrations. Moreover, the present investigation was carried out to identify the chemical constituents of the methanol extracts of the tested plants.

MATERIAL AND METHODS

Plant materials. The plants were collected by Dr Ramzi Mothana from different locations on the island Soqotra (Yemen) at the beginning of spring and in the winter of 2002 and identified at the Botany Department, Faculty of Science, Sana'a University. Voucher specimens were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

Extraction of plant material. The air dried and powdered plant materials (10 g of each) were extracted

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successively under shaking with chloroform 3–5 times at room temperature, followed by 90% methanol at 50 °C for 3–5 times and finally with water at 70 °C. The extracts obtained were filtered and evaporated under vacuum or by using a freeze dryer to give the crude dried extract. In the evaluation for antiviral activity the methanol and the hot aqueous extracts were included, whereas the chloroform extracts were not tested because of their high toxicity.

Cells and viruses. MDCK (Madin-Darby canine kidney) and Vero cells (African green monkey kidney cells) were passaged in Eagle's minimal essential medium (MEM) supplemented with 5% FCS (Gibco, Paisley, UK). Herpes simplex virus (HSV-1 strain KOS) in Vero cells was propagated and titrated. Human influenza virus A/WSN/33 (H1N1) London was multiplicated in the allantoic cavity of embryonated hen eggs. After virus titration in cells, the virus was stored at −70 °C until use.

Cytotoxicity assay. The effect of the plant extracts on the proliferation of MDCK und Vero cells was determined in 96-well tissue culture plates (8 mm diameter, Falcon Plastic, NJ) and incubated at 37 °C in a humidified atmosphere supplied with 5% CO_2. Confluent monolayers were incubated with twofold serial dilutions of extracts (100, 50, 25, 12.5 µg/mL) in medium for 72 h. The 50% cell-inhibitory concentration (CC_{50}) was determined by dye uptake assay using medium as a control.

Antiviral assays. Antiviral activity was determined using the dye uptake assay (Nowotny *et al.*, 1997; Rajbhandari *et al.*, 2001). MDCK and Vero cells were seeded in 96-well tissue culture plates at an initial cell concentration of 60 000 cells/well and incubated for 24 h in a humidified 5% CO_2 atmosphere and preincubated with 100 µL of serial dilutions of plant extracts in medium (100, 50, 25, 12.5, 6.25, 3.1, 1.5 and 0.7 µg/mL) in triplicate for 30 min at 37 °C. Subsequently the cells were infected with 30 $TCID_{50}$ of the virus suspension and further incubated for 72 h at 37 °C. The virus suspension and dilution medium without samples were added, respectively, to the cell cultures to serve as the virus control and cell control. The supernatant was replaced by 200 µL neutral red solution (0.005%) and the cells were incubated for 3 h at 37 °C. The dye incorporated by viable cells was eluted with 100 µL ethanol/water/glacial acetic acid solution (50:50:1) under shaking for 15 min. The absorbance was measured at 540 nm and the percentage protection was calculated by the following formula (Kodama *et al.*, 1996):

$$(OD_T)_V - (OD_C)_V/(OD_C)_M - (OD_C)_V \times 100(\%).$$

where $(OD_T)_V$, $(OD_C)_V$ and $(OD_C)_M$ correspond to absorbances in virus infected cells with test compounds, virus infected cells without test compounds and the mock infected control respectively.

Phytochemical screening. The screening of the chemical constituents was carried out with the methanol extracts using chemical methods and TLC according to the methodology suggested by Farnsworth (1966) and Marini-Bettolo *et al.* (1981).

RESULTS AND DISCUSSION

A total of 47 extracts derived from 25 plant species collected from the island Soqotra (Yemen) were screened for antiviral activity against influenza virus A and herpes simplex virus type 1. Table 1 shows the herbal names, families and parts used. The antiviral activity was evaluated at concentrations that were non-toxic for the cell system. These results are summarized in Table 2. Antiviral activity against herpes simplex virus was observed for 17 methanol and three aqueous extracts. Seven methanol and nine aqueous extracts showed antiviral activity against influenza virus. The most marked effects against herpes simplex virus were estimated for the methanol extracts of *Boswellia elongata*, *Buxus hildebrandtii* and *Euryops arabicus* with IC_{50} values of 0.35 µg/mL. For influenza virus A the highest activities were observed for aqueous extracts of both *Boswellia* species, *Fagonia luntii* and *Jatropha unicostata* with IC_{50} values ranging between 0.7 and 3.1 µg/mL and for methanol extracts of *Boswellia elongate*, *Dracaena cinnabari*, *Exacum affine* and *Kalanchoe farinacea* with IC_{50} values between 0.7 and 3.1 µg/mL.

The antiviral activities of extracts derived from *Boswellia ameero* and *Jatropha unicostata* against influenza virus were also confirmed at the level of nucleic acid by PCR experiments. Reduction of nucleic acid synthesis in comparison with the control was observed. The strong activities of *Boswellia elongata* and *Buxus hildebrandtii* against herpes simplex virus were also found in experiments with tenfold higher virus doses. It was interesting to note that the aqueous and methanol extracts of *Boswellia elongata* and *B. ameero* also exhibited strong antibacterial activity (Mothana Lindequist, 2005). The estimated antiviral activities are in accordance with their use in traditional ethnomedicine. *Boswellia* species, which were highly active against influenza virus in our experiments, are used to cure colds and bronchitis. *Cassia socotrana*, *Cissus subaphylla* and *Jatropha unicostata*, active against herpes simplex virus, are used for skin diseases and wounds. These screening results establish a base for studies to determine the compounds responsible for antiviral activity.

The phytochemical screening (Table 1) revealed the presence of triterpenoids and phytosterols in *Boswellia sp.*; steroidal alkaloids, flavonoids and triterpenoids in *Buxus hildebrandtii*; phenolic acids and tannins in *Exacum affine*; flavonoids, terpenoids and fatty acids in *Jatropha unicostata* and sterols, fatty acids and tannins in *Kalanchoe farinacea*. It is already known that such compounds possess antiviral activities (Poehland *et al.*, 1987; Eugster *et al.*, 1997; Liu *et al.*, 1999; Cheng *et al.*, 2002; Du *et al.*, 2003; Wei *et al.*, 2004). The studies on Nepalese medicinal plants demonstrated that highly condensed tannins were responsible for the effect of the extract from *Bergenia ligulata* against the influenza virus (Rajbhandari *et al.*, 2003). Thus, the chemical constituents found in this study could also be responsible for the observed antiviral activity of the plant extracts investigated. The antiviral activity could also be caused by an immunomodulating action (Melchardt *et al.*, 1994; Bodinet and Freudenstein, 1999). In a previous study it was demonstrated that oral treatment of

Table 1. List of plants screened for antiviral activity and the results of phytochemical screening

Medicinal plant	Voucher specimen no.	Family	Part tested	Chemical constituents
Boswellia ameero Balf. f.[a]	SP-M106	Burseraceae	B	Triterpenoids and phytosterols
Boswellia elongata Balf. f.[a]	SP-M102	Burseraceae	B	Triterpenoids and phytosterols
Buxus hildebrandtii Baill.	SP-M100	Buxaceae	L	Steroidal alkaloids, flavonoids and triterpenoids
Carphalea obovata (Balf. f.) Verdcourt	SP-D213	Rubiaceae	L	Anthraquinones, flavonoids and terpenoids
Cassia socotrana Serrato[a]	SP-A010	Caesalpiniaceae	L, T	Anthraquinones and flavonoids
Cissus hamaderohensis Radclife-Smith[a]	SP-N020	Vitaceae	L	Flavonoids, coumarins and phytosterols
Cissus subaphylla (I.B. Balf. f.) Planch.[a]	SP-N025	Vitaceae	L	Flavonoids, coumarins and phytosterols
Cleome socotrana Balf. f.[a]	SP-N030	Capparaceae	L, T	Glucosinolates, flavonoids and triterpenoids
Commiphora parvifolia Engl.[a]	SP-M108	Burseraceae	B	Triterpenoids
Cystostemon socotranus Balf. f.[a]	SP-M118	Boraginaceae	L, S	Alkaloids
Dendrosicyos socotrana Balf. f.[a]	SP-A015	Cucurbitaceae	L, S	Triterpenoids
Dorstenia socotrana A.G. Miller[a]	SP-M122	Moraceae	S, L	Coumarins, flavonoids, chalcones and steroids
Dracaena cinnabari Balf. f.[a]	SP-D225	Agavaceae	L, F	Isoflavonoids
Euryops arabicus Steud.ex Jaub.& Spach	SP-D203	Compositae	L, F	Terpenoids (sesquiterpenoid lactons) and flavonoids
Exacum affine Balf. f.[a]	SP-M112	Gentianaceae	L, F	Iridoids, phenolic acids and tannins
Fagonia luntii Baker	SP-C005	Zygophyllaceae	L, S	Saponins and triterpenoids
Gnidia socotrana Gilg	SP-D207	Thymelaeaceae	L, T	Sesquiterpenoids, steroids and flavonoids
Jatropha unicostata Balf. f.[a]	SP-N035	Euphorbiaceae	B	Flavonoids, terpenoids and fatty acids
Kalanchoe farinacea Balf. f.[a]	SP-D201	Crassulaceae	L, F	Fatty acids, sterols and tannins
Pulicaria stephanocarpa Balf. f.[a]	SP-C006	Compositae	L, F	Terpenoids and flavonoids
Punica protopunica Balf. f.[a]	SP-D223	Punicaceae	T, L	Alkaloids and tannins
Trichocalyx obovatus Balf. f.[a]	SP-D201	Acanthaceae	L, F	Flavonoids and terpenoids
Withania adunensis Vierh.[a]	SP-M110	Solanaceae	L, T	Steroids (withanolids) and flavonoids
Withania riebeckii Schweinf.ex Balf. f.[a]	SP-M116	Solanaceae	L, T	Steroids (withanolids) and flavonoids
Zygophyllum quatarense M.N. Hadidi	SP-C007	Zygophyllaceae	L, S	Saponins, triterpenoids and flavonoids

[a] Endemic plant, B, bark; F, flower; L, leaves; R, resin; S, stems; T, fruits.

Table 2. Antiviral activities of the investigated plant extracts

Medicinal plant	Extract	Anti-influenza virus-A		Anti-HSV-1	
		IC_{50} (μg/mL)[a]	CC_{50} (μg/mL)[b]	IC_{50} (μg/mL)[a]	CC_{50} (μg/mL)[b]
Boswellia ameero	Methanol	12.5	–	1.5	–
	Water	3.1	–	–	–
Boswellia elongata	Methanol	3.1	–	0.35	–
	Water	3.1	–	–	–
Buxus hildebrandtii	Methanol	–	75	0.35	25
	Water	–	–	–	–
Carphalea obovata	Methanol	–	–	–	–
	Water	–	–	–	–
Cassia socotrana	Methanol	–	25	1.5	–
	Water	6.3	–	–	–
Cissus hamaderohensis	Methanol	–	–	25	–
Cissus subaphylla	Methanol	–	–	0.7	–
	Water	–	–	–	–
Cleome socotrana	Methanol	–	–	–	–
	Water	4.3	–	–	–
Commiphora parvifolia	Methanol	–	–	13.0	–
	Water	–	–	–	–
Cystostemon socotranus	Methanol	–	–	–	–
	Water	–	–	–	–
Dendrosicyos socotrana	Methanol	–	25	–	25
	Water	–	–	–	25
Dorstenia socotrana	Methanol	–	–	–	–
	Water	–	–	32.1	–
Dracaena cinnabari	Methanol	1.5	–	12.5	–
	Water	–	–	–	–
Euryops arabicus	Methanol	–	–	0.35	–
	Water	–	–	–	–
Exacum affine	Methanol	0.7	–	12.5	–
	Water	3.1	–	–	–
Fagonia luntii	Methanol	–	–	3.1	–
	Water	3.1	–	–	–
Gnidia socotrana	Methanol	–	–	–	12.5
	Water	12.5	–	–	–
Jatropha unicostata	Methanol	2.5	–	1.5	–
	Water	1.5	–	–	–
Kalanchoe farinacea	Methanol	3.1	–	3.1	–
Pulicaria stephanocarpa	Methanol	–	–	1.5	–
	Water	–	–	50	–
Punica protopunica	Methanol	–	–	–	–
	Water	75.7	–	5.8	–
Trichocalyx obovatus	Methanol	12.5	50	25	50
Withania adunensis	Methanol	–	12.5	6.25	12.5
	Water	–	–	–	–
Withania riebeckii	Methanol	–	12.5	–	25
	Water	–	–	–	–
Zygophyllum quatarense	Methanol	–	–	6.25	–
	Water	–	–	–	–
Amantadine HCl		16.8			
Acyclovir				0.7	

[a] IC_{50} = 50% inhibitory concentration.
[b] CC_{50} = 50% cell toxicity concentration.
– No measurable effect.
The values are the mean of four experiments.

mice with an extract mixture from *Echinacea* species, *Baptisia tinctoria* and *Thuja occidentalis* protected the animals against influenza A virus infection (Bodinet *et al.*, 2002). One may suppose that the therapeutic effect in the ethnomedical praxis is partially due to this effect. Therefore, other pharmacological activities should be studied in future.

In conclusion, the present results demonstrate that *Boswellia* species, *B. hildebrandii*, *E. affine*, *J. unicosta* and *K. farinacea* seem to be potential sources for developing antiviral drugs. The findings may offer an explanation for the traditional use of these plant treatments against viral infections. Triterpenoids, flavonoids, tannins and sterols being constituents of many plants with biological activity, may be responsible for the antiviral activity obtained. Further investigations, isolation and identification of the antiviral active components are in progress.

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