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Antiviral Activity Against Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) of Ethnobotanically Selected Ethiopian Medicinal Plants

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Ethiopian medicinal plants used for the treatment of a variety of ailments including infectious diseases were screened for activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). Seventy-one polar and nonpolar extracts derived from 21 plants belonging to 14 families were tested for inhibition of viral replication using HIV-1 (III_B) and HIV-2 (ROD) strains. Selective inhibition of viral growth was assessed by the simultaneous determination of the *in vitro* cytotoxicity of each of the extracts against MT-4 cells. Six extracts made from the root bark of *Bersama abyssinica* Fresen, the leaves of *Combretum paniculatum* Vent., and *Dodonaea angustifolia* L.f., and the stem bark of *Ximenia americana* L. displayed antiviral activity at concentrations that were nontoxic to MT-4 cells. The highest selective inhibition of HIV-1 replication was observed with the acetone fraction of *C. paniculatum* and the methanol fraction of *D. angustifolia* which showed selectivity indices (ratio of 50% cytotoxic concentration to 50% effective antiviral concentration) of 6.4 and 4.9, and afforded cell protection of viral induced cytopathic effect of 100% and 99%, respectively, when compared with control samples. The greatest degree of antiviral activity against HIV-2 was achieved with the acetone extract of *C. paniculatum* (EC_{50}: 3 μg/mL), which also showed the highest selectivity index (32). The 50% cytotoxic concentration ranged from 0.5 μg/mL for the hexane extract of *D. angustifolia* L.f., the most cytotoxic of the extracts tested, to >250 μg/mL for some extracts such as the methanol fraction of *Alcea rosea* L., the least toxic tested. Only the polar extracts that were obtained by extraction with hydroalcohol, methanol or acetone exhibited inhibition of viral growth at subtoxic concentrations. The results obtained in this study enable the selection of extracts which show some specificity of action and support the further investigation of these extracts for their potential as new lead antiretroviral compounds. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: HIV-1; HIV-2; antiviral activity; *Bersama abyssinica*; *Combretum paniculatum*; *Dodonaea angustifolia*; *Ximenia americana*.

INTRODUCTION

According to the most recent figures released by WHO (1999), over 33 million people world-wide are living with the acquired immunodeficiency syndrome (AIDS) or are infected with the causative agent, the human immunodeficiency virus (HIV). The most affected is sub-Saharan Africa where in some countries, up to one in four of the adult population has contracted the disease. In Ethiopia, it is estimated that AIDS has claimed the lives of 1 million people since the beginning of the epidemic and a further 2.6 million adults and children were estimated to live with HIV/AIDS by the end of 1997 (UNAIDS/WHO, 1998). For people living with HIV/AIDS today the disease is still fatal, although some have access to life-prolonging drugs. These expensive antiretroviral drugs are still far beyond the means of most developing countries. Arguably, the development of safe, effective and low-cost anti-HIV drugs is among the top global priorities of drug development, since the disease is not yet curable and mortality is high. It has been stated that the replicative cycle of HIV involves ten steps that could be considered as targets for the rational design of antiretroviral drugs (De Clercq, 1995). Thus, most research efforts in this area have been directed towards the synthetic design of compounds, particularly nucleotide analogues, to interrupt HIV infection.

In the past decade considerable attention has been given to screening of plant extracts for possible anti-HIV activity (Lednicer and Sander, 1991; Weislow *et al.*, 1989). Such endeavours have been undertaken with the

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aim of isolating bioactive compounds as an alternative source to chemical synthesis. Screening of plant extracts for antiretroviral activity has given interesting results in that most plant-derived anti-HIV compounds inhibit the replication of the virus by interfering with one or more of the ten steps of HIV replicative cycle (Vlietinck *et al.*, 1998; Matthée *et al.*, 1999). Anti-HIV active compounds of plant origin do not fall into a certain class of compounds but rather possess diverse chemical structures (Vlietinck *et al.*, 1997). The therapeutic efficacy of a handful of these compounds, such as the benzylisoquinoline alkaloid, papaverine (Bassetti *et al.*, 1989), and the saponin, glycyrrhizin (Hattori *et al.*, 1989), has been studied in AIDS patients. It is evident therefore that plants can be useful sources or leads for the discovery of novel anti-HIV compounds.

In the present study, 71 extracts prepared from 21 plant species used in Ethiopian traditional medicine for the treatment of various ailments were tested for their inhibitory effects on the *in vitro* replication of HIV-1 and HIV-2. The assay used in this investigation allowed simultaneous estimation of the cytotoxicity of these extracts in MT-4 cells and the evaluation of their anti-HIV potential.

MATERIALS AND METHODS

Plant material. Selection of plants and the parts of plants used in this study were based on the information obtained from actively practising traditional healers and also from published traditional uses of the plants in curing HIV-related symptoms such as skin infections, cough, diarrhoea and fever (Table 1).

Alcea rosea L. (syn. *Althaea rosea* (L.) Cav.), *Calpurnia aurea* (Ait.) Bentham, *Justitia schimperiana* (Hochst. ex Nees) T. Andres. (syn. *Adhatoda schimperiana* (Hochst. ex Nees), *Salvia leucantha* Cav., *Solanecio gigas* (Vatke) C. Jeff. (syn. *Senecio gigas* Vatke), *Stephania abyssinica* (Quart. Dill. ex Rich.) Walp. and *Verbena officinalis* L. were collected in October 1996 in and around Addis Ababa. *Ajuga integrifolia* Ham.-Buch. (syn. *A. remota* Benth.), *Artemisia abyssinica* Schtz.-Bip ex Richard, *Artemisia afra* Jacq. ex Willd., *Coriandrum sativum* L. and *Melilotus elegans* Salzm. ex Ser. (syn. *M. abyssinica* Baker) were purchased in an open market in Addis Ababa in January 1997. All plant parts of *Bersama abyssinica* Fresen, *Dovyalis abyssinica* (Rich.) Warburg, *Securidaca longepedunculata* Fresen, *Withania somnifera* (L.) Dunal in DC. and *Ximenia americana* L. used in the present investigation were supplied by Dr Dawit Abebe of the Department of Traditional Medicine, Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa. Dr Kaleab Asres and Mr Melaku Wondafrash collected *Clerodendron discolour* (Kolotzsch) Vatke, *Combretum paniculatum* Vent. and *Dodonaea angustifolia* L.f. (*D. viscosa* (L.) Jacq.) in October 1997 from and around Jimma, a city some 330 km southwest of Addis Ababa. Leaves of *Vernonia galamensis* (Cass.) Less. were obtained from Wondo Genet Medicinal Plant Research Station, some 265 km south of Addis Ababa. The plants were identified at the herbarium of the Department of Traditional Medicine, EHNRI or at the National Herbarium, Department of Biology, Addis Ababa University (AAU), Addis Ababa, Ethiopia. Herbarium voucher specimens were preserved at the Department of Pharmacognosy, School of Pharmacy, AAU.

Preparation of extracts. The hydroalcohol extracts were prepared by exhaustive percolation at room temperature of the powdered plant material in 80% methanol followed by removal of the solvent under reduced pressure. The organic solvent fractions were obtained by successive hot extraction of the powdered plant material using a soxhlet apparatus. Thus, the powdered plant material was first extracted exhaustively with petroleum ether (40 °–60 °C) and the organic solvent evaporated to dryness at a reduced pressure. The marc was allowed to dry in open air and was further extracted sequentially with dichloromethane, acetone and methanol.

Extraction of alkaloids. Powdered leaves of *S. abyssinica* (200 g) were macerated with 80% methanol for 72 h. The filtered extract was concentrated to dryness under reduced pressure and extracted into 2% H_2SO_4. The acidic aqueous solution was made alkaline with 26% NH_4OH (pH 9) and extracted with $CHCl_3$ (3×100 mL). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated to dryness yielding a dark brown syrup 1.28 g, (0.64%). Roots (200 g) and twigs (200 g) were extracted by the same procedure to yield 1.64 g (0.82%) and 0.50 g (0.25%), respectively. Each of the extracts was placed in a vacuum oven at 40 °C before it was used for anti-HIV testing.

Cell cultures. The human T-lymphocytic MT-4 cells were grown in RPMI 1640 DM (Dutch modification) medium (Life Technologies, Merelbeke, Belgium), supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 0.1% sodium bicarbonate and 20 μg/mL gentamicin (complete medium). The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Virus. HIV-1 (III_B) and HIV-2 (ROD) were obtained from the culture supernatants of HIV-1 and HIV-2 infected MT-4 cell lines, respectively. The virus titres of the supernatants were determined in MT-4 cells (Reed and Muench, 1938). The virus stocks were stored at −70 °C until used.

Anti-HIV assay. The cytotoxicity and antiviral activity assays were based on evaluating cell death caused by plant extract toxicity and inhibition of viral cytopathic effect, respectively, as previously described by Pauwels *et al.* (1988). Stock solutions ($10 \times$ final concentration) of plant extracts were added in 25 μL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial five-fold dilutions of plant extracts were made directly in flat bottom 96-well plastic microtitre trays using a Biomek 2000 robot (Beckman Instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each extract.

HIV-1 or HIV-2 stock (50 μL) at 100–300 $ICID_{50}$ (cell culture infectious dose) or culture medium was added to either the infected or mock-infected part of the microtitre tray. Mock-infected cells were used to evaluate the effect of plant extracts on infected cells and to determine the

Table 1. Vernacular names and traditional uses of Ethiopian plants screened for anti-HIV activity

Family, Botanical name	Vernacular name(s)	Traditional uses
ACANTHACEAE		
Justitia schimperiana (Hochst. ex Nees) T. Andres (syn. *Adhatoda schimperiana* (Hochst. ex Nees)	Sensel, Semmasa, Dumoga	Stomach complaints, hepatitis, venereal diseases (Abate, 1989); malaria, asthma, leishmaniasis (Fichtl and Adi, 1994); malaria, cough (Jansen, 1981)
COMBRETACEAE		
Combretum paniculatum Vent.	Baye, Gabai, Shaga	Eye diseases, leprosy (Fichtl and Adi, 1994; Jansen, 1981)
COMPOSITAE		
Artemisia abyssinica Schtz.-Bip ex Richard	Chiikogne, Jukun	Haemostatic (nose), tonsillitis (Abate, 1989); cold, constipation, rheumatism (Jansen, 1981)
Artemisia afra Jacq. ex Willd.	Arrity, Sakajo	Perfume smallpox, stomachache (Jansen, 1981)
Solanecio gigas (Vatke) C. Jeff. (syn. *Senecio gigas* Vatke)	Yeshekoko Gomen, Galti	Fumigant against typhoid fever, rheumatism, wound dressing (Abate, 1989); typhoid fever (Fichtl and Adi, 1994)
Vernonia galamensis (Cass.) Less.	Gerch	Skin infections, malaria (personal communication)
FLACOURTIACEAE		
Dovyalis abyssinica (Rich.) Warburg	Koshim, Semay Tero, Koshumo	Haemorrhoids (Abate, 1989); ulcers (Fichtl and Adi, 1994); swelling of throat (Jansen, 1981)
LABIATAE		
Ajuga integrifolia Ham.-Buch. (syn. *A. remota* Benth.)	Armagusa, Ungoquasot	Stomach disorders (Abate, 1989); dysentery, swollen legs, high blood pressure, diabetes (Jansen, 1981)
		Skin infections (personal communication)
Salvia leucantha Cav.	Not found	
LEGUMINOSAE		
Calpurnia aurea (Ait.) Bentham	Digita, Setara, Sotellu	Stomach complaints, headache (Abate, 1989); eye diseases, amoebic dysentery, scabies, insecticide (Jansen, 1981)
Melilotus elegans Salzm. ex Ser (syn. *M. abyssinica* Baker)	Egug, Gugi, Yeman-berri	Excavated sore, piles, ulcers (Abate, 1989); mouth infection, lacerated wounds, haemorrhoids, bronchial asthma (personal communication)
MALVACEAE		
Alcea rosea L. (syn. *Althaea rosea* (L.) Cav.)	Not found	Contagious diseases (personal communication)
MELIANTHACEAE		
Bersama abyssinica Fresen	Azamer, Bersama, Lolchissa	Rabies, ascariasis, ulcers (Abate, 1989); malaria (Abebe and Ayehu 1993); diarrhoea, cholera, ascariasis, amoebiasis, worm infestations (Getahun, 1976); dysentery, round worm (Jansen, 1981)
MENISPERMACEAE		
Stephania abyssinica (Quart. Dill. ex Rich.) Walp.	Yeait-Hareg, Aregait, Etse Eyesus, Idootuta	Stomach complaints, syphilis (Abate, 1989); syphilis, venereal illness, gonorrhoea (Getahun, 1976); diarrhoea, dysentery, vomiting, heart complaints, mastitis (Jansen, 1981)
OLEACEAE		
Ximenia americana L.	Enkoi, Huda, Mellau	Contagious diseases, stomach complaints, placenta expulsion (Abate, 1989); internal parasitism, worm infestations (Getahun, 1976); vermifuge (Jansen, 1981)
POLYGALACEAE		
Securidaca longepedunculata Fresen	Etse-Menahe, Temmenai	Stomach complaints, tuberculosis (Abate, 1989); wound dressing, rheumatism, syphilis, cough, diarrhoea (Jansen, 1981); syphilis, rheumatism, typhus (Neuwinger, 1996)
SAPINDACEAE		
Dodonaea angustifolia L.f. (syn. *D. viscosa* L. Jacq.)	Kitkita, Teramin, Kirtita, Tasos	Dressing for skin diseases of the head and face, haemorrhoids, (Abate, 1989); fever, wound dressing, sore throat (Jansen, 1981); fever, malaria, paludism, ague, angina, sore throat, cold, rhinitis, sinusitis, influenza, flu (Lemordant, 1971); piles (Tadesse, 1994)

Table 1. Continued

Family, Botanical name	Vernacular name(s)	Traditional uses
SOLANACEAE		
Withania somnifera (L.) Dunal in DC.	Gizawa, Sebbere-gola, Hidi-Budawa, Agol	Dressing for headache (Abate, 1989); paludism, ague, fever, malaria (Heine and Brenzinger, 1988); headache, stomach ache, diuretic, (Jansen, 1981)
UMBELLIFERAE		
Coriandrum sativum L.	Dimbilal, Debo, Zagda	Stomach ache, excavated sore, rheumatism (Jansen, 1981)
VERBENACEAE		
Clerodendron discolour (Kolotzsch) Vatke	Yemisirch	Haemorrhoids (Abate, 1989)
Verbena officinalis L.	Attuch, Etse Mengist, Akkoragag, Serrufit	Abscess dressing, skin complaints, liver diseases (Abate, 1989); dysentery, vermifuge, throat inflammation, burns, respiratory complaints (Jansen, 1981)

concentration at which the plant extracts were cytotoxic. Exponentially growing MT-4 cells were centrifuged for 5 min at 1000 rpm and the supernatants were discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, using slight magnetic stirring, and 50 μL volumes transferred to the microtitre tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of the yellow coloured 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Ascent Reader, Labsystems, Helsinki, Finland), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of extract that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The concentration achieving 50% protection was defined as the 50% effective concentration (EC_{50}). The selectivity index value was defined as the CC_{50}/ EC_{50} ratio.

RESULTS AND DISCUSSION

Investigation of the anti-HIV activity of medicinal plants against different strains of HIV is relevant both in terms of promoting the traditional use of plants as anti-HIV agents and in the development of novel antiretrovirals based on the active ingredients of the plants. Unfortunately, often bioactivity of plant-derived drugs does not parallel therapeutic potential, since a number of natural products show non-selective toxicity to host cells (Farnsworth *et al.*, 1975; Vlietinck and Domisse, 1985). Evaluation of cytotoxicity, therefore, becomes critical when searching for potential chemotherapeutic agents so as to distinguish between specificity of action and non-selective toxicity. One way in which the selectivity of activity may be assessed is by comparing the desired biological activity with non-selective cytotoxicity.

Table 2 presents the results of *in vitro* anti-HIV-1 (III_B) and anti-HIV-2 (ROD) activities as well as cytotoxicity in MT-4 cells of 71 extracts prepared from 21 plant species used in Ethiopian traditional medicine for the treatment of a variety of ailments. Among the plant extracts examined in the present study, six of them exhibited significant inhibition of the replication of HIV-1 at subtoxic doses, while only two fractions showed activity against HIV-2 with a maximum selectivity index of 32. The selectively active extracts were obtained from different plant parts of *B. abyssinica*, *C. paniculatum*, *D. angustifolia* and *X. americana*, all of which are widely used in Ethiopian traditional medicine as remedies for different kinds of infectious diseases (Table 1). The most active fractions were the methanol extracts made from the root bark of *B. abyssinica* and the leaves of *C. paniculatum* which inhibited replication of HIV-1 at 50% effective concentrations (EC_{50}) of 3.1 and 5.2 μg/mL with a corresponding selectivity index of 3.8 and 6.4, respectively. The acetone fraction of the leaves of *C. paniculatum* also exhibited antiviral activity against both HIV-1 and HIV-2 with EC_{50} values of 15.0 and 3.0 μg/mL and selectivity indices of 6.4 and 32, respectively. The other three extracts obtained from the leaves of *D. angustifolia* and the stem bark of *X. americana* were found to have EC_{50} values against HIV-1 ranging from 8.3 to 27.7 μg/mL and selectivity indices that ranged from 3.9 to 4.9. The hydroalcohol extract of *X. americana* also displayed an inhibition of the replication of HIV-2 at concentrations that were non-toxic to the host cells (EC_{50}: 27.1 μg/mL).

It is interesting to note the variation in activities observed for the different fractions of *B. abyssinica*, *C. paniculatum* and *D. angustifolia*. The petroleum ether and dichloromethane fractions, like all the other non-polar extracts examined in the present study, were devoid of antiretroviral activity at concentrations that were non-toxic to MT-4 cells. Similarly, their total hydroalcohol extracts obtained by maceration with 80% methanol also failed to display activity at subtoxic concentrations. On the other hand, both the acetone and/or methanol fractions of each of these extracts achieved a relatively high selective inhibition of HIV-1 replication. Additionally, the acetone extract of *C. paniculatum* showed an inhibitory effect against HIV-2 with a relatively high selectivity index. These findings suggest that the toxicity caused by each of the hydroalcohol extracts of the above

Table 2. *In vitro* activities of some Ethiopian medicinal plants on the replication of HIV-1 (III_B) and HIV-2 (ROD) and their cytotoxicity in MT-4 cells

Family, Botanical name	Plant part[a]	Extract[b]	EC_{50}[c] HIV-1 (III_B)	EC_{50}[c] HIV-2 (ROD)	CC_{50}[d]	SI[e] HIV-1 (III_B)	SI[e] HIV-2 (ROD)
ACANTHACEAE							
Justitia schimperiana (Hochst ex Nees) T. Andres (syn. *Adhatoda schimperiana* Hochst. ex Nees)	LF	HA	>125	>125	>125	X1	X1
COMBRETACEAE							
Combretum paniculatum Vent.	LF	PE	>118.5	>118.5	118.4	<1	<1
		DM	>44.7	>44.7	44.6	<1	<1
		ACT	15.0	3.0	96.1	6.4	32.0
		ME	5.2	>24.6	24.5	4.7	<1
		HA	>23.5	>23.5	23.4	<1	<1
COMPOSITAE							
Artemisia abyssinica Schtz.-Bip. ex Richard	AP	HA	>103	>103	102.9	<1	<1
Artemisia afra Jacq. ex Willd.	AP	HA	>123.5	>123.5	123.4	<1	<1
Solanecio gigas (Vatke) C. Jeff.	RT	DM	>23.4	>23.4	23.3	<1	<1
(syn. *Senecio gigas* Vatke)		ACT	>120.5	>120.5	120.4	<1	<1
		ME	>250	>250	>250	X1	X1
	RB	DM	>28.1	>28.1	28.0	<1	<1
		ACT	>115.2	>115.2	115.1	<1	<1
		ME	>250	>250	>250	X1	X1
Vernonia galamensis (Cass.) Less.	LF	PE	>30.1	>30.1	30.0	<1	<1
		DM	>5.0	>5.0	4.9	<1	<1
		ACT	>22.5	>22.5	22.4	<1	<1
		ME	>110.8	>110.8	110.7	<1	<1
		HA	>22.9	>22.9	22.8	<1	<1
FLACOURTIACEAE							
Dovyalis abyssinica (Rich.) Warburg	LF	DM	>3.8	>3.8	3.7	<1	<1
		ACT	>4.5	>4.5	4.4	<1	<1
		ME	>176.5	>176.5	176.4	<1	<1
	SB	DM	>3.8	>3.8	3.7	<1	<1
		ACT	>4.5	>4.5	4.4	<1	<1
		ME	>25.5	>25.5	25.4	<1	<1
LABIATAE							
Ajuga integrifolia Ham.-Buch.	AP	PE	>125	>125	>125	X1	X1
(*A. remota* Benth.)		DM	>57.6	>57.6	57.5	<1	<1
		ACT	>125	>125	>125	X1	X1
		ME	>125	>125	>125	X1	X1
		HA	>125	>125	>125	X1	X1
Salvia leucantha Cav.	LF	HA	>115.8	>115.8	115.7	<1	<1
LEGUMINOSAE							
Calpurnia aurea (Ait.) Bentham	SD	HA	>125	>125	>125	X1	X1
Melilotus elegans Salzm.ex Ser.	AP	HA	>96.3	>96.3	96.2	<1	<1
MALVACEAE							
Alcea rosea L. (*Althaea rosea* (L.) Cav.)	LF	PE	>109.3	>109.3	109.2	<1	<1
		DM	>109.2	>109.2	109.1	<1	<1
		ACT	>120.5	>120.5	120.4	<1	<1
		ME	>250	>250	>250	X1	X1
MELIANTHACEAE							
Bersama abyssinica Fresen	LF	HA	>2.3	>2.3	2.2	<1	<1
	RB	ACT	>2.8	>2.8	2.7	<1	<1
		ME	3.1	>12	11.9	3.8	<1
		HA	>2.7	>2.7	2.6	<1	<1

Table 2. Continued

Family, Botanical name	Plant part[a]	Extract[b]	EC_{50}[c] HIV-1 (III_B)	EC_{50}[c] HIV-2 (ROD)	CC_{50}[d]	SI[e] HIV-1 (III_B)	SI[e] HIV-2 (ROD)
MENISPERMACEAE							
Stephania abyssinica (Quart. Dill.ex Rich.) Walp.	LF	HA	>53.8	>53.8	53.7	<1	<1
		AK	>12.7	>12.7	12.6	<1	<1
	TW	AK	>12.5	>12.5	12.4	<1	<1
	RT	HA	>85.6	>85.6	85.5	<1	<1
		AK	>71.9	>71.9	71.8	<1	<1
OLEACEAE							
Ximenia americana L.	SB	HA	8.3	27.1	37.7	4.5	1.4
POLYGALACEAE							
Securidaca longepedunculata Fresen	LF	HA	>81.5	>81.5	81.4	<1	<1
	RB	HA	>2.8	>2.8	2.7	<1	<1
SAPINDACEAE							
Dodonaea angustifolia L.f.	LF	PE	>0.6	>0.6	0.5	<1	<1
(*D. viscosa* (L.) Jacq.)		DM	>4.7	>4.7	4.6	<1	<1
		ACT	27.7	>108.7	108.6	3.9	<1
		ME	21.3	>104.3	104.2	4.9	<1
		HA	>4.9	>4.9	4.8	<1	<1
	SD	HA	>125	>125	>125	X1	X1
SOLANACEAE							
Withania somnifera (L.) Dunal in DC.	RT	HA	>22.7	>22.7	22.6	<1	<1
UMBELLIFERAE							
Coriandrum sativum L.	FT	PE	>109	>109	108.9	<1	<1
		DM	>233.3	>233.3	233.2	<1	<1
		ACT	>250	>250	>250	X1	X1
		ME	>250	>250	>250	X1	X1
VERBENACEAE							
Clerodendron discolour (Kolotzsch) Vatke	LF	HA	>91.3	>91.3	91.2	<1	<1
Verbena officinalis L.	AP	PE	>78	>78	77.9	<1	<1
		DM	>92.1	>92.1	92.0	<1	<1
		ACT	>125	>125	>125	X1	X1
		ME	>125	>125	>125	X1	X1
		HA	>125	>125	>125	X1	X1
	RT	PE	>83.6	>83.6	83.5	<1	<1
		DM	>103	>103	102.9	<1	<1
		ACT	>125	>125	>125	X1	X1
		ME	>125	>125	>125	X1	X1
		HA	>125	>125	>125	X1	X1

[a] AP, aerial part; FT, fruit; LF, leaf; RB, root bark; RT, Root; SB, stem bark, SD, seed; TW, twig.
[b] ACT, acetone; AK, total alkaloids; DM, dichloromethane; HA, 80% methanol; ME, methanol; PE, petroleum ether.
[c] 50% Effective inhibitory concentration in μg/mL.
[d] 50% Cytotoxic concentration in μg/mL.
[e] Selectivity index (CC_{50}/EC_{50}).

three plant species is likely to be due to substances other than the bioactive principles which do not seem to be soluble in acetone or methanol. It seems essential, therefore, when such screening is undertaken, to perform activity testing on the total extract as well as on the different solvent fractions so that the bioactive natural products are not overlooked.

In the case of the hydroalcohol extract of *X. americana*, antiviral activity was achieved at concentrations (EC_{50}: 8.3 μg/mL against HIV-1 and 27.1 μg/mL against HIV-2) that were lower than those necessary to produce cytotoxicity (CC_{50}: 37.7 μg/mL). However, it is possible that the active principle(s) and the toxic component(s) of the extract are different from each other in which case fractionation of the total extract should help determine the fraction with the highest selectivity index. Unfortunately, fractionation of this particular extract could not be carried out due to paucity of plant material. Previous phytochemical investigations of extracts of *X. americana* resulted in the isolation of polyphenols such as proanthocyanidins (Mwangi *et al.*, 1994) and a triterpenoid saponin (D'Agostino *et al.*, 1994). Condensed tannins such as procyanidin B_2 have been reported as potent inhibitors of reverse transcriptase obtained from avian myeloblastosis virus (Kakiuchi *et al.*, 1991). Similarly, triterpenoid saponins and sapogenins have been shown to exhibit potent *in vitro* anti-HIV effects by different mechanisms of action. Saponins such

as glycyrrhizin interfere with virus adsorption (Ito *et al.*, 1988) while some urosilic acid-type sapogenins exert their effects by inhibiting HIV-1 protease (Xu *et al.*, 1996). The antiviral activity of *X. americana* obtained in the present study warrants further investigation because of the selectivity it showed against each of the viral strains tested.

The different parts of *B. abyssinica* have previously been subjected to phytochemical and biological investigations. The fruits have yielded a series of bufadienolides with anti-tumour activity (Kupchan *et al.*, 1969; Kupchan *et al.*, 1971). The alcohol extract of the stem bark was reported to be devoid of anti-tumour activity although two bufadienolides together with sterols and a xanthone have been isolated from it (Bowen *et al.*, 1985). The leaf extracts possessed cardiogenic (Lock, 1962) and spasmolytic (Makonnen and Hagos, 1993) activities, but failed to show any antibacterial activity (Taniguchi and Kubo, 1993). The extract of the root bark afforded abyssinin, a potent insect antifeedant and a further three bufadienolides (Kubo and Matsumoto, 1984; 1985). All the four bufadienolides demonstrated antibacterial activity (Taniguchi and Kubo, 1993). In the present investigation the methanol extract of the root bark of *B. abyssinica* exhibited potent inhibition of HIV-1 replication (EC_{50}: 3.1 μg/mL) with some degree of selectivity. The above mentioned components of *B. abyssinica* may account for its anti-HIV activity. However, the role of other (known and unknown) compounds cannot be ruled out.

In Ethiopian traditional medicine, the leaves of *C. paniculatum* are widely used as a remedy for leprosy (Table 1), particularly in the south-western part of the country. However, no phytochemical studies conducted on its constituents or biological activity testing carried out on its extracts could be found in the literature. Preliminary phytochemical screening performed on the hydroalcohol extract has indicated that the leaf extracts of *C. paniculatum* are rich in tannins. This was not unexpected since several plants belonging to the family Combretaceae are known to contain hydrolysable tannins (Hegnauer, 1989). Hydrolysable tannins isolated from *Terminalia chebula* (Combretaceae) have been shown to possess anti-HIV activity (Nonaka *et al.*, 1990). Furthermore, the antiretroviral activity of some of these compounds such as chebulagic acid and punicalin has, in part, been ascribed to inhibition of viral adsorption to the cells (Weaver *et al.*, 1992). Whether the anti-HIV activity of *C. paniculatum* extracts observed in the present study was due to tannins or other constituents of the plant is not yet known. Tannins may contribute to the inhibitory effect displayed by the extracts. But, the possible antiretroviral action of the other secondary metabolites occurring in the plant must also be investigated.

A few phytochemical investigations have been carried out on *D. angustifolia* which were summarized by Ghisalberti (1998). From the resin that covers the leaves of a number of xerophilic *Dodonaea sp*. including *D. angustifolia* the bicyclic diterpenes hautriwaic acid as well as an ent-labdane furan and an ent-clerodane furan together with flavones, particularly 3-methoxyflavones, have been reported. The flowers, seeds and stems of *D. angustifolia* have been shown to contain esterified derivatives of R1-barrigenol whilst the stem bark yielded R1-barrigenol together with jegosapogenol. The 3-methoxyflavones might contribute to the antiviral activity of the leaf extract of *D. angustifolia* observed in the present study, since a number of these compounds revealed pronounced antiviral activity against picornaviruses (polio- and rhinoviruses) (Vlietinck *et al.*, 1995).

From the present study and other anti-HIV screening tests already carried out on some Ethiopian plants, it appears that most of the plant extracts are more effective against HIV-1 in comparison with HIV-2. Moreover, much of the antiviral activity was demonstrated by the polar extracts, which indicates that the active principles are also polar compounds. This observation is consistent with earlier reports in the literature (Lednicer and Sander, 1991; Weislow *et al.*, 1989).

In conclusion, the results obtained from the present study do seem to justify the traditional uses of some of the plants for the treatment of infectious diseases of viral origin. They further indicate that these plants might be of value as sources or leads for novel antiretroviral compounds which can be useful in the continuing fight against HIV. Hence, phytochemical investigation aimed at the isolation and characterization of the active principles of those extracts that showed selective inhibition of HIV replication is currently underway.

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