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Antiviral activity of Hawaiian medicinal plants against human immunodeficiency Virus Type-1 (HIV-1)

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Summary

Hawaiian medicinal plants commonly used for the treatment of a variety of infections were screened for antiviral activity against human immunodeficiency virus type 1 (HIV-1). Sixty-one extracts derived from seventeen plants were tested for selective viral growth inhibition using the LAI (HTLV-III B) isolate. The greatest degree of antiviral activity was observed with aqueous extracts made from the bark of *Eugenia malaccensis* (L.) and the leaves of *Pluchea indica* (Less.) which had antiviral selectivity indices (50% cytotoxic concentration/50% effective antiviral concentration) of 109 and 94, respectively. These and other extracts conferred 100% cell protection against viral cytopathic effect when compared with control samples. Methanol and water extracts made from the *Pipturus albidus* (Gray) leaves and bark also achieved a high selective inhibition of virus replication with very low cytotoxicity. Plant extracts made from *Aleurites moluccana* (Willd.), *Psychotria hawaiiensis* (Gray), *Clermontia aborescens* (Mann), and *Scaevola sericea* (Forst.) also showed antiviral activity. These data provide a rationale for the characterization of antiviral natural products from these plants and related plant species.

Key words: ethnobotany, anti-viral, HIV-1, pharmacognosy, Polynesia, Hawaii

Introduction

Due to the rapid emergence of drug-resistant virus strains, the development of effective therapies for human immunodeficiency virus type 1 (HIV-1) infection is dependent upon the identification of novel therapeutic agents with low toxicity. Much attention has been directed at tropical rainforests for the identification of new natural product pharmaceuticals since an abundance of biodiversity might provide new natural products having a wide range of biological activity. Hawaiian plants represent a unique array of biodiversity since more than 90% of its native flora is endemic and is found within a relatively small region (Neal, 1948). Approximately 200 different plants have been used by Hawaiians to treat a variety of ailments, including infectious diseases (Kaaiakamanu and Akina, 1922; Handy et al., 1934; Tabrah and Eveleth, 1966; Nagata, 1971; Abbott and Shimazu, 1985). Some of these plants were shown to possess anti-bacterial properties (Bushnell et al., 1950), however additional characterization of the active constituents was not reported. Since ethnobotanical leads have been shown to be more successful than randomized screen-

ing for identifying biologically active plants (Farnsworth et al., 1985), Hawaiian medicinal plants were selected and screened for antiviral activity against HIV-1. Several plants had potent selective antiviral activity, one of which *(Aleurites moluccana)* is used by traditional medicinal practitioners to treat herpes virus infections. These plants and their relatives may provide new approaches for the identification of potential plant-derived antiviral agents.

Materials and methods

Plant material was collected on the islands of Hawai'i and O'ahu and voucher specimens were stored for deposition at the University of Hawaii at Manoa Department of Botany. All plant material was processed within two days of collection with storage at 4 °C during the interval between collection and processing. Plants were prepared for extraction by first drying plant material by lyophilization under vacuum pressure. Eight grams of dried plant material was extracted by using a Soxhlet extractor with solvents of increasing polarity beginning with hexane, followed in turn by methylene chloride, acetonitrile, methanol and lastly with water which efficiently removed lipid substances from cell and organelle membranes (i.e. chloroplasts). Extraction with each solvent was for 10–15 hours or until the solvent return ws colorless. The solvent was removed in a rotary evaporator operating at 4 °C under water pump pressure.

The antiviral screening was determined by evaluating cell death caused by plant extract cytotoxicity and viral cytopathic effect as previously described (Pauwels et al., 1988). Briefly, HIV-1 (strain III b/LAI) was grown in MT-4 cell lines using RPMI medium supplemented with 10% fetal bovine serum and 20 µg/ml gentamycin. Stock solutions (10 X final test concentration) of plant extracts were added in 25 µl volumes to two series of triplicate wells to determine their effect on HIV-infected and uninfected cells at the initiation of each experiment. Serial five-fold dilutions of plant extracts were made directly in flat bottom 96-well plastic microtiter trays (Becton Dickinson, Mountain View, CA) using a Biomek 1000 robot (Beckman, Fullerton, CA). Thus, each plant extract had final concentrations of 250, 50, 10, 2, and 0.4 µg in each experiment. 50 µl of HIV-1 stock at 100 $TCID_{50}$ (tissue culture infections dosage) or culture medium was added to either the infected or mock-infected part of the microtiter plate. The mock-infected cells were used to evaluate the effect of plant extracts on uninfected cells and to determine the concentration at which the plant extracts were cytotoxic. MT-4 cells in the log phase of growth were centrifuged at 5 minutes at 140 x g and the supernatant was discarded. The MT-4 cells were then resuspended at 6 x 10^5 cells/ml and under slight magnetic stirring, 50 µl volumes were dispensed into each well of the microtiter plate.

Five days after infection, the viability of mock and HIV-infected cells were examined spectrophotometrically using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO), also known as the MTT assay. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan MCC, ICN Flow) at two wavelengths (540 and 690 nm). All data represent the average values for a minimum of three wells. The 50% cytotoxic dose (CC_{50}) was defined as the concentration of compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The percent protection achieved by the compounds in HIV-infected cells was calculated by the following formula:

$$\frac{(OD_c)_{HIV} - (OD_c)_{HIV}}{(OD_c)_{mock} - (OD_c)_{HIV}} \text{ expressed in \%}$$

where $(OD_c)_{HIV}$ is the optical density measured with a given concentration of the test compound in HIV-infected cells; $(OD_c)_{HIV}$ is the optical density measured for the control untreated HIV-infected cells; and $(OD_c)_{mock}$ is the optical density measured for control untreated mock-infected cells. The dose achieving 50% protection according to the formula above was defined as the 50% effective dose (EC_{50}). The selectivity index value was defined as CC_{50}/EC_{50}.

Results

Seventeen different plants that grow in Hawaii and were reported to have medicinal benefits were evaluated for antiviral activity against HIV-1 (Table 1). Sixty-one extracts were made from the parts of the plants claimed to possess medicinal properties and were simultaneously tested for cytotoxicity and anti-viral activity. Fifteen extracts made from seven different plants showed detectable antiviral activity, ten of which were quite active in that they had selectivity indices of ten or greater (Table 2). The most effective plant extracts were water extracts made from the bark of *E. malaccensis* and the leaves of *P. indica*, which had 50% effective concentrations (EC_{50}) of 2.3 and 1.7 µg/ml and selectivity indices (CC_{50}/EC_{50}) of 109 and 94, respectively. Both extracts showed complete cell protection (100% or greater) against HIV-induced cytopathic effect compared to control wells. Five different water extracts made from the leaves of *P. albidus* showed inhibition of HIV growth. These were found to have EC_{50} concentrations that ranged from 3.8 µg/ml to 29.6 µg/ml and selective indices that ranged from 8 to 68. Four of the five extracts afforded 100% cell protection against viral cytopathic effect while the remaining extract had 82% cell protection. The growth inhibition by this plant was not restricted to the use of water or to leaf extracts since both water and methanol extracts made from the bark and leaves also showed significant HIV growth inhibition. Other inhibitory plant extracts

Table 1. Hawaiian medicinal plants studied

Family and Botanical Name	Hawaiian Name	Traditional Uses
ASCLEPIADACEAE		
Calotropis gigantea R. Br.		purgative (poisonous, Baldwin, 1979)
BARRINGTONIACEAE		
Barringtonia asiatica Kurz.	Hutu	burns, wounds (Whistler, 1992)
BOMBACACEAE		
Adansonia digitata L.		(no reference)
GOODENIACEAE		
Scaevola sericea Forst.	Naupaka	deep cuts, cataracts, scaly skin, punctures (Whistler, 1992)
COMPOSITAE		
Pluchea indica Less.		acid stomach, diaphoretic for fever, dysentery, scabies, rheumatism (Perry, 1980)
CONVOLVULACEAE		
Ipomoea congesta R. Br.	Koali	purgative, healing broken bones (Abbott, 1992)
Cuscuta sandwichiana Choisy	Kaunao'a	several different applications (Whistler, 1992)
EUPHORBIACEAE		
Aleurites moluccana Willd.	Kukui	oral thrush, cold sores, skin ulcers, deep bruises, cathartic, laxative, sore throat (Kaaiakamanu and Akina, 1922; Whistler, 1992)
LOBELIACEAE		
Clermontia aborescens Mann	Oha Waihui	deep cuts, asthma, restoring milk in dry breasts (Kaaiakamanu and Akina, 1992; Nagata, 1971)
MORACEAE		
Ficus prolixa Forst.		boils, sore throat, stomatitis, bronchitis, cathartic (Whistler, 1992)
MYRTACEAE		
Eugenia malaccensis L.	Ohia'ai	general debility, sore throat, cuts, thrush, venereal disease, tuberculosis, digestive tract disorders (Kaaiakamanu and Akina, 1922; Whistler, 1992)
PIPERACEAE		
Piper methysticum Forst.	Awa	urinary tract infections, tranquilizer (Whistler 1992; personal communication)
ROSACEAE		
Rhaphiolepsis indica Lindl.		heart disease (personal communication)
RUBIACEAE		
Morinda citrofolia Chev.	Noni	healing broken bones, deep cuts, bruises, sores (Kaaiakamanu and Akina, 1922; Handy et al., 1934)
Psychotria hawaiiensis Gray	Kopiko	cuts, wounds (personal communication)
SOLANACEAE		
Solanum niger L.	Popolo	respiratory tract disorders, skin eruptions, cuts, wounds, sprains (Handy et al., 1934; Neal, 1948; Kaaiakamanu and Akina, 1922)
URTICACEAE		
Pipturus albidus Gray	Mamaki	general debility, expectant mother, blood purifier, mild laxative (Kaaiakamanu and Akina, 1922; Abbott, 1992; pers. communication)

included water extracts made from leaves of *C. aborecens* (EC_{50} of 10.2 µg/ml and a selectivity index of 24) and *S. sericea* (EC_{50} of 8.2 µg/ml and al selectivity index of 31); the bark of *P. hawaiiensis* (EC_{50} of 9.7 µg/ml and a selectivity index of 23); acetonitrile/water extract made from the leaves (EC_{50} 7.8 µg/ml and a selectivity index of 9) and an acetonitrile/dichloromethane extract made from the husk (EC_{50} 15.6 µg/ml and a selectivity index of 10) of *A. moluccana*.

Discussion

This is the first report of Hawaiian medicinal plants having anti-HIV activity. Of sixty-one plant extracts tested, ten of these extracts had a selectivity index of greater than 10 (7/17 plants studied, 35%). This relatively high occurrence of anti-viral activity may reflect the utility of using ethnobotanical leads to identify plants having biological activity. Six of these seven plants may have a correlation of plant use in the treatment of infections and the biological activity ob-

Table 2. Hawaiian medicinal plants studied

Botanical Name (Family)	Plant Part[a]	Solvents[b]	EC_{50}[c]	CC_{50}[d]	Selectivity[e]	% Protection[f]
Adansonia digitata L. (Bombaceae)	FL	H_2O	22.5 ± 9.3	114.8 ± 42.0	5	74
		MeOH	46.9 ± 32.4	69.1 ± 37.8	2	61
Aleurites moluccana Willd. (Euphorbiaceae)	ST	H_2	162.2 ± 76.9	162.2 ± 76.9	1	4
		MeOH	> 250.0	> 250.0	1	4
	LF	H_2O	> 250.0	> 250.0	1	5
		MeOH	138.4 ± 32.3	138.4 ± 32.3	1	8
		ACN/H2O	7.8 ± 6.6	100.4 ± 128.4	9	100
	HK	H_2O	> 250.0	> 250.0	1	6
		MeOH	193.9 ± 7.0	193.9 ± 7.0	1	25
		ACN/DCM	15.6 ± 3.8	116.8 ± 10.6	10	100
	SD	H_2O	> 250.0	> 250.0	1	23
Barringtonia asiatica Kurz. (Myrtaceae)	FL	MeOH	17.7 ± 3.6	126.6 ± 20.2	5	96
Calotropis gigantea R. Br. (Asclepiadaceae)	FL	H_2O	> 250.0	> 250.0	1	7
		ACN	0.6 ± 0.5	0.8 ± 0.2	1	23
Clermontia aborescens Mann (Lobeliaceae)	LF	H_2O	10.2 ± 8.6	> 250.0	24	134
		MeOH	> 250.0	> 250.0	1	11
Cuscuta sandwichiana Choisy (Convolvulaceae)	ST	H_2O	29.7 ± 6.3	124.6 ± 10.4	3	68
		MeOH	26.8 ± 11.3	> 223.1 ± 46.6	9	91
Eugenia malaccensis L. (Myrtaceae)	LF	H_2O	115.0 ± 13.3	115.0 ± 13.3	1	32
		MeOH	22.7 ± 2.2	54.9 ± 43.4	2	56
		ACN	72.4 ± 69.7	195.7 ± 49.7	3	53
	BK	H_2O	24.2 ± 8.5	130.0 ± 12.4	5	101
			2.3	> 250.0	109	103
		MeOH	20.7 ± 4.0	81.6 ± 82.1	4	63
		ACN	14.6 ± 13.3	54.1 ± 45.4	3	50
Ficus bengalensis L. (Moraceae)	FT	H_2O	207.8 ± 54.7	207.8 ± 54.7	1	19
Ipomoea congesta (Convolvulaceae)	SD	H_2O	> 250.0	> 250.0	1	8
		MeO	Hb> 250.0	> 250.0	1	5
Morinda citrofolia Chev. (Rubiaceae)	FT	H_2O	± 250.0	> 250.0	1	0
		DCM	> 250.0	> 250.0	1	8
Piper methysticum Forst. (Piperaceae)	LF	H_2O	> 250.0	> 250.0	1	8
		MeOH	68.8 ± 53.5	138.8 ± 7.3	2	42
		ACN	> 250.0	> 250.0	1	7
	ST	H_2O	208.8 ± 58.3	208.8 ± 58.3	1	9
	RT	H_2O	> 250.0	> 250.0	1	50
Pipturus albidus Gray (Urticaceae)	BK	H_2O	32.4 ± 12.1	> 250.0	8	68
		MeOH	29.7 ± 6.3	124.6 ± 10.4	4	81
	LF	H_2O	14.3 ± 0.9	235.4 ± 20.6	15	171
			3.8 ± 1.8	208.9 ± 58.2	66	261
			5.9 ± 4.5	> 250.0	68	128
			13.8 ± 6.1	248.7 ± 2.7	18	105
Pipturus albidus Gray (Urticaceae)	LF	H_2O	29.6 ± 12.8	> 250.0	8	82
		MeOH	20.9 ± 5.3	128.8 ± 14.7	7	130
		ACN	185.5 ± 81.9	185.5 ± 81.9	1	18
	ST	H_2O	132.9 ± 147.6	> 250.0	1	51
		MeOH	130.3 ± 21.4	130.3 ± 21.4	1	32
Pluchea indica Less. (Asteraceae)	LF	H_2O	1.7 ± 0.2	160.3 ± 24.6	94	100
		MeOH	199.0 ± 77.3	199.0 ± 77.3	1	93
Psychotria hawaiiensis Gray (Rubiaceae)	BK	H_2O	9.7 ± 6.2	223.3 ± 4.6	23	154
		MeOH	161.3 ± 68.2	229.5 ± 29.0	1	34
		ACN	101.6 ± 95	154.0 ± 20.8	2	21
	LF	H_2O	> 250.0	> 250.0	1	25
		MeOH	247.4 ± 3.7	247.4 ± 3.7	1	7

Table 2. Hawaiian medicinal plants studied (continued).

Botanical Name (Family)	Plant Part[a]	Solvents[b]	EC_{50}[c]	CC_{50}[d]	Selectivity[e]	% Protection[f]
Rhaphiolepis indica Lindl. (Rosaceae)	LF	H_2O	163.0 ± 122.3	> 250.0	1	14
		MeOH	165.3 ± 72.1	233.0 ± 24	1	29
Scaevola sericea Forst. (Goodinaceae)	LF	H_2O	8.2 ± 0.6	> 250.0	31	92
		MeOH	> 250.0	> 250.0	1	5
		ACN	> 250.0	> 250.0	1	6
	FT	H_2O	34.5 ± 14.0	> 250.0	8	59
		MeOH	> 250.0	> 250.0	1	20
Solanum niger L. (Solanaceae)	LF	H_2O	> 250.0	> 250.0	1	7
		MeOH	124.3 ± 11.2	124.3 ± 11.2	1	10
	ST	H_2O	128.7 ± 4.4	128.7 ± 4.4	1	43
		MeOH	103.3 ± 11.0	103.3 ± 11.0	1	4
		ACN	> 250.0	> 250.0	1	6

[a] LF, leaf; ST, stem; BK, bark, RT, bark; FL, flower; FT, fruit; HK; husk
[b] MeOH, methanol; ACN; acetonitrile; ACT, acetone; DCM, dichloromethane
[c] 50% Effective inhibitory concentration EC_{50} (µg/ml)
[d] 50% Cytotoxic concentration CC_{50} (µg/ml)
[e] CC_{50}/EC_{50}
[f] Percent protection of viral cytopathic effect compared to control wells at EC_{50}

served here *in vitro*. In traditional Hawaiian medicine, the latex sap of *A. moluccana* is used to treat herpes virus infections, scrofulous sores, and skin ulcers (Kaaiakamanu and Akina, 1922); *C. aborencens* was used to treat cuts and wounds, while *E. malaccensis* was used in the treatment of venereal disease, cuts and wounds *(ibid); S. sericea* and *P. hawaiiensis* were also used to treat cuts and wounds (Whistler, 1992; personal communication); and *P. albidus* was used as a blood purifier and for general debility (Kaaiakamanu and Akina, 1922; Abbott, 1992).

Recently, a phorbol ester called prostratin was isolated from the *A. moluccana*-related Samoan medicinal plant *Homolanthus nutans* and showed anti-HIV activity *in vitro* (Gustavson et al., 1992). This plant belongs to the same family (Euphorbiaceae) as *A. moluccana* but it remains to be determined if compounds of a similar nature are responsible for the observed anti-HIV activity. In a separate study, *A. moluccana* plant extracts were not active when tested against herpes simplex virus (HSV, Types 1 and 2), vesicular stomatitis virus (VSV), Semliki forest virus (SFV), polio virus, or Coxsackie B 3 virus *in vitro*, suggesting that the antiviral activity observed with this plant is HIV-specific (Locher et al. submitted for publication). In contrast, extracts made from *E. malaccensis, S. sericea*, and *P. hawaiiensis* were active against HSV and VSV, suggesting that these plants have a broader range of antiviral activities. Although each of the viruses inhibited (HIV, HSV, and VSV) were enveloped viruses, it may not necessarily mean that these extracts are selective for enveloped viruses since SFV (also an enveloped virus) was not inhibited by these same extracts.

It is noteworthy that much of the antiviral activity observed in this study was with water extracts made from the leaves of plants, indicating that the active ingredients are hydrophilic compounds. It is known that sulfated polysaccharides such as heparin can inhibit HIV adsorption to cells *in vitro* (Baba et al., 1988). In other studies it was shown that some carbohydrates and polysaccharides can affect the glycoprotein synthesis of enveloped viruses (reviewed in Che, 1991; Vlietinck and Vanden Berghe, 1991). Moreover, a phase 1 clinical trial showed that acemannan, a polymannoacetate isolated from *Aloe barbadensis*, diminished symptoms associated with AIDS in twenty-four of forty-one patients (McDaniel et al., 1988). It is possible that the anti-HIV activity observed with some of the water extracts in this study is due to products of a carbohydrate nature. Alternatively, polyphenolic compounds such as tannins are known to non-specifically bind to proteins and may have interfered with HIV-1 gp 120 binding to its CD 4 receptor on the cell surface. Experiments are underway to determine if polysaccharides or polyphenolic compounds were responsible for the observed antiviral activity with the Hawaiian medicinal plants studied here.

In previous alkaloid screening studies of Hawaiian plants, it was shown that *C. aborecens* tested positive using six different alkaloid reagents, while *A. moluccana, P. albidus, E. malaccensis, P. hawaiiensis* and *S. sericea* were negative (Swanholm et al., 1959; Swanholm et al., 1960; Scheuer et al., 1962). Therefore, the antiviral activity observed with *C. aborecens* may be due to alkaloid natural products. At least 32 different alkaloids have shown antiviral activity *in vitro* (reviewed in Che, 1991; Vlietinck and Vanden Berghe, 1991).

This study supports the concept that traditional medicine may be important in the potential discovery of natural product pharmaceuticals. This approach is supported by

the idea that societies which existed without contact with Western Medicine used locally grown plants as sources of medicine (Cox and Ballick, 1994). New strategies which involve collaboration with tribal healers are now being used to identify plant sources of medicinal compounds (Stix, 1993). In this respect, ethnobotany in Hawaii and Polynesia may be useful in the development of new, effective pharmaceutical alternatives to existing drugs.

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