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TESTING PRAIRIE PLANTS WITH ETHNOBOTANICAL IMPORTANCE FOR ANTI-CANCER AND ANTI-AIDS COMPOUNDS

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ABSTRACT. — Literature research into ethnobotanical uses of North American prairie plants by Native Americans and early written accounts by travelers and doctors identified 203 native prairie species that have been used for medicine. We collected, identified, and made extracts from 22 of these species and subjected the extracts to biological screens to identify new anti-HIV and anti-cancer chemical leads. Our results show greater rates of activity for both aqueous extract anti-AIDS screens (60.0%) and organic extract anti-AIDS screens (13.6%) than rates previously determined through random screening of terrestrial plants (13.9% and 3.0%, respectively). In preliminary anticancer screening, 10 of 22 organic extracts showed at least moderate activity. This work demonstrates that native prairie plants (and probably those of other regions in North America) may provide new chemical leads, especially if the target list includes those species that have ethnobotanical use histories. We also believe that our work helps substantiate the idea that Native Americans were choosing many plants with pharmacologically active substances in their health and healing practices.

RESUMEN. — Una investigación bibliográfica acerca de los usos etnobotánicos de plantas de las praderas norteamericanas por parte de los indígenas, y las descripciones tempranas de viajeros y médicos, identificó 203 especies nativas de la pradera que han sido usadas como medicinas. Colectamos, identificamos y preparamos extractos de 22 de estas especies y sometimos los extractos a pruebas biológicas para indentificar nuevos candidatos químicos contra el SIDA y el cáncer. Nuestros resultados muestran tasas mayores de actividad anti-SIDA tanto en pruebas con extractos acuosos (60.0%) como extractos orgánicos (13.6%) que las tasas previamente determinadas a través de pruebas con plantas terrestres

seleccionadas al azar (13.9% y 3.0%, respectivamente). 10 de 22 extractos orgánicos mostraron por lo menos actividad moderada en pruebas preliminares anti-cáncer. Este trabajo demuestra que las plantas nativas de la pradera (y probablemente las de otras regiones de Norteamérica) pueden proporcionar nuevos candidatos químicos, especialmente si la lista seleccionada incluye aquellas especies que tienen una historia de uso etnobotánico. Creemos también que nuestro trabajo ayuda a substanciar la idea de que los indígenas norteamericanos estaban escogiendo en sus prácticas de salud y curación muchas plantas con sustancias farmacologicamente activas.

RÉSUMÉ.— Une recherche bibliographique sur les utilisations ethnobotaniques des plantes des prairies nord-américaines par les Amérindiens ainsi que les premiers écrits des voyageurs et médecins a permis d'identifier 203 espèces indigènes des prairies qui étaient utilisées comme médicaments. Nous avons collecté, identifié et préparé des extraits de 22 de ces espèces et avons soumis ces extraits à des examens biologiques pour identifier de nouveaux agents chimiques anti-V.I.H. et anti-cancéreux. Nos résultats montrent des taux d'activité plus élevés pour les examens des extraits aqueux antisida (60,0%) et pour les examens des extraits organiques antisida (13,6%) que les taux déterminés antérieurement par des examens de plantes terrestres faits au hasard (33,8% et 4,2% respectivement). Dans nos examens préliminaires anti-cancéreux, 10 des 22 extraits organiques ont montré une activité au moins modérée. Ce travail démontre que les plantes indigènes des prairies (et probablement celles d'autres régions d'Amérique du Nord) peuvent fournir de nouveaux agents chimiques, particulièrement si on inclut dans la liste cible les espèces qui ont une histoire ethnobotanique. Nous croyons aussi que notre travail vient soutenir l'idée que les Indiens d'Amérique choisissaient plusieurs plantes aves des substances pharmacologiques actives dans leurs pratiques hygiéniques et thérapeutiques.

INTRODUCTION

Literature research into the ethnobotanical uses of prairie plants by Native Americans, early travelers, traders, settlers, and doctors has identified 203 native prairie species that were used for medicinal purposes (Kindscher 1992) and 123 species that were used for food (Kindscher 1987) in the Prairie Bioregion (Figure 1). Conservation of tropical rain forests receives considerable attention because of the probable value of potential pharmaceutical agents (Balick and Mendelsohn 1992; Farnsworth and Soejarto 1991; Hodson, Englander, and O'Keefe 1995), and the National Cancer Institute's current large-scale plant collecting and screening program is focused on the tropics. By contrast, few prairie plants have ever been considered for use by the contemporary health industry (Kindscher 1992; Tyler 1993). We believe that this is an untapped resource that should be explored further.

Several authors have obtained a higher proportion of active extracts from ethnobotanically targeted as opposed to random plant collections (Balick 1990; Cox *et al.* 1989; Lewis and Elvin-Lewis 1995; Spjut and Perdue 1976). McCutcheon *et al.* (1992, 1994) demonstrated that there is value in studying temperate North American plants for medicinal purposes. They determined that 85% of 96 extracts of native plants of British Columbia with reported ethnobotanical uses exhibited

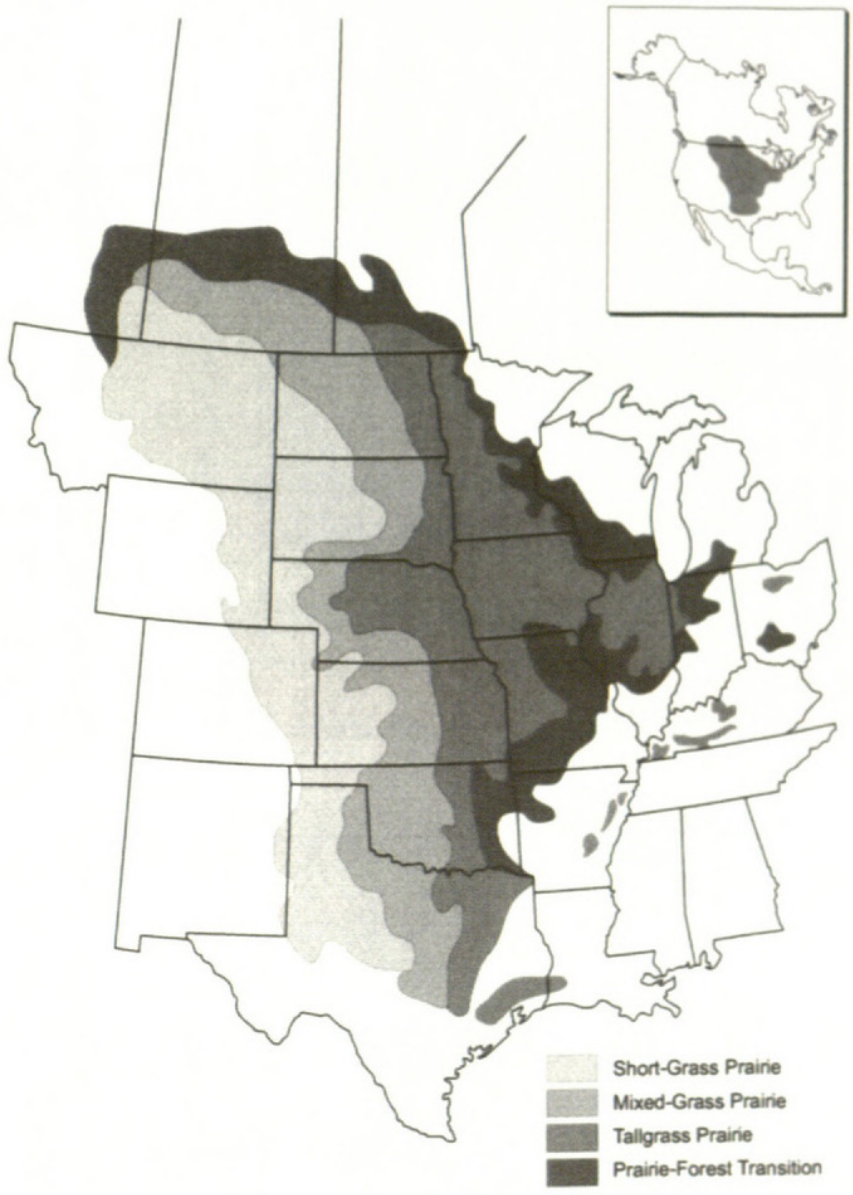

FIGURE 1. — Map of the Prairie bioregion

antibiotic activity (McCutcheon *et al.* 1992) and that 81% of these plant extracts exhibited antifungal activity (McCutcheon *et al.* 1994). They also recognized that the appeal of tropical ethnobotany had not extended to temperate North America, but asserted that the North American flora is worthy of ethnobotanically-based medicinal product exploration.

We conducted our study to: a) highlight the potential economic value of prairie and prairie plants; b) screen these plants for potential anti-HIV and anti-cancer bioactivity; and c) to determine if a greater number of plants with potential bioactivity can be found by choosing species that have an ethnobotanical history of use by Native Americans than by random screenings. While we knew it was unlikely that we would find a plant that was a cancer or AIDS cure, we hoped to show the promise of building upon the knowledge of Native Americans.

TABLE 1. — Ethnobotanical uses of prairie plants tested in anti-AIDS and anti-cancer screens. Scientific names of plants, families, collection numbers, common names, code for tribes that used the plant (code is below), references, and ailments treated by the tribes. Tribal Codes: AR, Arapaho; AS, Assiniboin; BA, Bannocks; BL, Blackfoot; CE, Cree (Plains); CH, Cheyenne; CO, Comanche; CK, Choctaws; CR, Crow; FH, Flathead; GR, Gros Ventre; KA, Kiowa-Apache; KI, Kiowa; KU, Kutenais; ME, Meskwaki (Fox); MN, Menomini; NP, Nez Perce; OJ, Ojibwa (Chippewa); OM, Omaha; OS, Osage; PA, Pawnee; PO, Ponca; PW, Potawatomi; SH, Shoshones; SI, Sioux; SI-DA, Dakota Sioux; SI-LA, Lakota Sioux; TE, Tewa; WI, Winnebago; ZU, Zuni.

Scientific Name	Common Name	Tribes (References)	Ailments and Uses
Achillea millefolium L. (ASTERACEAE) (Loring 01)	Yarrow	AS (Shemluck 1982); BL (Hellson 1974); CH (Grinnell 1962); CR GR (Shemluck 1982; SI-LA (Buechel 1983); OS (Munson 1981; PA (Dunbar 1880); WI (Gilmore 1977).	boils; burns; colds; coughing and throat irritations; diuretic; earaches; fainting; fever; general external afflictions; heart trouble and chest pains; open sores; respiratory diseases like tuberculosis; stomach com plaints; stops bleeding; swelling; toothache and sore gums; treat wounds.
Amorpha canescens Pursh (FABACEAE) (Loring 02)	Leadplant	AS (Bray & Bray 1976); SI-LA (Rogers 1980);ME (Smith 1928); OM (Gilmore 1977);PO (Gilmore 1977); PW (Smith 1933).	astringent for cuts and open wounds; eczema; intestinal worms; neuralgia; rheumatism.
Astragalus bisulcatus[1] (Hook.) A.Gray (FABACEAE) (Loring 03)	Milkvetch	BL (Hellson 1974); CH (Grinnell 1962); SI-DA (Gilmore 1977);SI-LA (Rogers 1980);OM PO (Gilmore 1977).	applied to cuts; chest and back pains; coughing; febrifuge for children; heart troubles; loss of appetite; poison ivy and other plant dermatitis; promotes milk production in mothers; spitting of blood; stomach pains.
Ceanothus herbaceus[2] Raf. var. *pubescens* (T. & G.) Shinners (RHAMNACEAE) (Loring 04)	New Jersey Tea	AR (Nickerson 1966); CK (Bushnell 1909); PW (Smith 1933). OJ (Densmore 1974); SI-DA (Bray & Bray 1976); SI-LA (Rogers 1980); ME MN OJ PW (Smith 1928); SH (Nickerson 1966).	chest colds; hemorrhage from the lungs; stomach and bowel troubles.
Conyza canadensis (L.) Cronq. (ASTERACEAE) (Loring 05)	Horseweed	SI-LA (Rogers 1980; Munson 1981); ME (Smith 1928); ZU (Stevenson 1915).	bowel pain and diarrhea, particularly in children; rhinitis.
Equisetum hyemale L. (EQUISETACEAE) (Loring 06)	Horsetail	BL (Hellson 1974); CE (Johnston 1970); ME (Smith 1928); MN (Smith 1923); TE (Youngken 1925).	clear up system after childbirth; cold and diarrhea remedy for babies; diuretic; gonorrhea; kidney troubles; menstrual irregularities; rashes under arm and in the groin.

Fragaria virginiana Duchn. (ROSACEAE) (Loring 07)	Wild Strawberry	BL (Hellson 1974); OJ PW (Smith 1932 1933).	diarrhea; stomachache in babies; stomach complaints; summer cholera that affected young children.
Glycyrrhiza lepidota Pursh (FABACEAE) (Hurlburt 32)	Wild Licorice	BA (Murphey 1959); BL (Hellson 1974); CH (Hart 1981); SI-LA (Munson 1981); PA (Gilmore 1914; Kindscher 1987). ZU (Camazine & Bye 1980)	chest pains and sore throat; cough; diarrhea and upset stomach; earache; fever in children; flu; spitting of blood; swellings; toothache.
Helianthus grosseserratus Martens (ASTERACEAE) (Loring 08)	Sawtooth Sunflower	ME (Smith 1928);ZU (Camazine & Bye 1980).	burns; rattlesnake bites.
Ipomoea leptophylla Torr. (CONVOLVULACEAE) (Hurlburt 30)	Bush Morning Glory	SI-LA (Rogers 1980); PA (Gilmore 1977).	fainting; nervousness and bad dreams; pain; stomach trouble.
Juniperus virginiana L. (CUPRESSACEAE) (Loring 09)	Eastern Red Cedar	BL (McClintock 1909; Johnston 1970; Hellson 1974); CH (Grinnelll 1962); CR (Hart 1976); SI-DA (Gilmore 1977); FH (Hart 1976); GR (Kroeber 1908); KI (Vestal & Schultes 1939); KU (Hart 1976);SI-LA (Gilmore 1977); ME (Smith 1928; NP (Hart 1976); OM (Fletcher & LaFlesche 1911); PA (Gilmore 1977); SI (Hart 1976).	arthritis; asthma; canker sores; cholera; cleansing and healing for women after childbirth; colds; control bleeding; convalescent; coughs; diarrhea; external sores; fevers; lung troubles; nervousness and bad dreams; pneumonia; rheumatism; sedative; speed childbirth; stiff back or backache; stop vomiting; tonic; tonsilitis; weakness.
Liatris punctata Hook. (ASTERACEAE) (Loring 10)	Gayfeather	BL (McClintock 1923); CO (Carlson & Jones 1939); KA (Jordan 1965); SI-LA (Rogers 1980); ME (Smith 1928); OM PA (Gilmore 1977); PW (Smith 1933). PO (Gilmore 1977); PW (Smith 1928).	abdominal troubles; bladder and kidney troubles; bloody urine; diarrhea in children; external inflamation; external wounds; gonorrhea; heart pains; loss of appetite; scabies; stomachache; swellings; swollen testes.
Monarda fistulosa[3] L. (LAMIACEAE) (Loring 11)	Beebalm	BL (McClintock 1923; Hellson 1974); CR (Hart 1976); SI-DA (Gilmore 1977; Andros 1883); FH (Blankenship 1905; Dunbar 1880); KI (Vestal & Schultes 1939); SI-LA (Munson 1981; Rogers 1980); ME (Smith 1928); PA (Dunbar 1880); SI (Blankenship 1905); WI (Gilmore 1977; Andros 1883).	abdominal pain; after childbirth; boils; catarrh; cholera; colds; cuts; eyewash; fevers; fainting; headaches; induce vomiting; insect bites and stings; pimples; respiratory problems; revive unconscious patient; soothe kidneys; sore eyes; stop external blood flow; swollen neck glands; whooping cough and other coughs.
Oenothera rhombipetala Nutt. ex T. & G. (ONAGRACEAE) (Hurlburt 29)	Evening Primrose	OM (Gilmore 1977).	heals bruises; inflamation; swelling and sores.

Pediomelum argophyllum (Pursh) J. Grimes (FABACEAE) (Loring 12)	Wild Alfalfa	CH (Grinnell 1962); SI-DA (Rogers 1980); ME (Smith 1928).	chronic constipation; external wounds; fever; horse medicine.
Pycnanthemum tenuifolium[4] Schrad. (LAMIACEAE) (Loring 13)	Mountain Mint	SI-LA (Rogers 1980); ME (Smith 1928).	ague; alterative; chills; coughing.
Rhus glabra L. (ANACARDIACEAE) (Loring 14)	Smooth Sumac	KI (Vestal & Schultes 1939); ME (Smith 1928);OM (Gilmore 1977; 1913a);OS (Wakefield & Dellinger 1936); PA (Gilmore 1977; 1913a).	appetizer; astringent; bloody diarrhea; painful menstruation; urination and retention of water; rubefacient; tuberculosis; wash sores.
Rubus flagellaris Willd. (ROSACEAE) (Loring 15)	Black Raspberry	OS (Hunter 1957).	abdominal pains; children with bowel trouble.
Silphium laciniatum L. (ASTERACEAE) (Loring 16)	Compass Plant	CR (Vogel 1970); ME (Smith 1928); OM PA PO SI-DA (Gilmore 1977).	emetic; general debility; head colds; rid horses of worms.
Silphium perfoliatum L. (ASTERACEAE) (Loring 17)	Cup Plant	ME MN OJ (Smith 1928, 1932); OM PO (Gilmore 1977); PW (Smith 1933); WI (Gilmore 1977).	alleviate vomiting during pregnancy; emetic; head colds; nerve pains; profuse menstruation; rheumatism
Solidago canadensis L. (ASTERACEAE) (Loring 18)	Goldenrod	ME (Smith 1928).	kidney trouble.
Verbena hastata L. (VERBENACEAE) (Loring 19)	Blue Vervain	OJ (Densmore 1974); SI-DA (Gilmore 1977);ME (Smith 1928); MN (Smith 1923); OM (Gilmore 1977).	cloudy urine; fits; nosebleed; stomachache.

[1]No specific reference to this species, so tribes and ailments used from related species: *Astragalus adsurgens*, *A. canadensis*, *A. gracilis*, and *A.racemosus*.

[2]Tribes and ailments used from related species, *Ceanothus americanus*.

[3]For *Monarda fistulosa*, the Pawnee recognized four "species," while the Dakota, Omaha, and Ponca recognized two (Gilmore 1977). Since we only recognize one today, these uses are combined.

[4]Related species *Pycnanthemum virginianum* used for tribes and ailments.

METHODS

Plant collection. — Prairie plants (native species of grasses, forbs, and woody shrubs in the Prairie Bioregion; see Figure 1) were selected based on their ethnobotanical use (Table 1) and availability for collection while in flower. These 22 species represent 11 families and include six species of the Asteraceae and four of the Fabaceae (two of the largest families of prairie plants). Plant identification follows the *Flora of the Great Plains* (Great Plains Flora Association 1986) and nomenclature follows Kartesz (1996). Voucher specimens of all species collected are archived at the R.L. McGregor Herbarium at the University of Kansas. At least 2 kg of each species was harvested and air dried and subsequently shipped to the Chemistry Laboratory at the University of Northern Iowa.

Extraction. — The plant material (leaves, stems, or roots) was chopped into small pieces, placed in a small cloth sack and immersed in liquid nitrogen. Once completely frozen, samples were crushed and placed in a large beakers filled with CH_2Cl_2 and MeOH (1:1) and covered. After 24 hours the solvent was drained off and the plants were covered with pure MeOH. After an additional 24 hours, the MeOH was drained, combined with the CH_2Cl_2:MeOH extract, and the solvent was removed at reduced pressure using a rotary evaporator. The resultant solid material was designated the "organic extract." The remaining plant material was covered with water for an additional 24 hours, the water was drained and the resultant extract was placed on a rotary evaporator for a few minutes to remove any traces of organic solvent. The water was then quickly frozen in a CO_2 - acetone bath and freeze-dried. This extract was referred to as the "aqueous extract."

Anti-HIV testing. — The anti-HIV assay was carried out at the Laboratory of Drug Discovery Research and Development at the US National Cancer Institute (NCI) as described previously (Weislow *et al.* 1989). Since this was a preliminary screening, each plant extract was tested in duplicate rather than replicating the tests with many different samples. The assay tests the ability of plant extracts to inhibit the killing of T4 (CD4+) lymphoid cells (CEM-SS line) by HIV-1 (RF strain). Samples of 5.0 mg of extract were dissolved in 100 ml of dimethylsulfoxide and diluted in a cell assay to give a maximum test concentration of 250 mg/mL of cells. The extract was then serially diluted to a minimum concentration of 0.0079 mg/mL. The exponentially-growing cells were pelleted from the growth medium and infected at a multiplicity of infection of 0.05 at room temperature for 45 minutes with constant agitation. The cells were then diluted in growth medium to the desired cell concentrations to yield 5,000 cells/well after inoculation and inserted into wells of 96 micro-titer plates. Equal aliquots (50 mL) of the test solutions containing the plant extracts were added to the appropriate wells, and the plates were incubated for 6 days at 37° C. Plates were then analyzed for cellular viability using the XTT-tetrazolium method (Weislow *et al.* 1989).

The assay provides three important parameters. The EC_{50} is the concentration of extract at which the growth of the infected cells is 50% of the non-infected, extract-free control. The IC_{50} is the concentration at which the growth of non-infected white blood cells containing the extract is 50% of the control, and measures the extract's toxicity to healthy cells. The TI_{50} is the ratio of the EC_{50} to the IC_{50} and

can be considered a measurement of viricidal activity relative to cytotoxicity. A larger TI_{50} value represents a more viable drug candidate.

These tests are considered a preliminary screen; therefore, exact quantification of the EC_{50}, IC_{50}, and TI_{50} values is inappropriate at this stage. In reporting the results of the assay, we classify the extracts as "active," "moderate," or "inactive." We define an "active" extract as one that achieves an EC_{50} value at a concentration less than 250 mg/mL and an extract with "moderate" activity as one which shows growth of infected cells at less than a 50% value. An "inactive" extract either fails to enable infected cells to grow or is toxic to the uninfected control cells at concentrations less than 250 mg/mL.

To test whether the rate of activity obtained from our ethnobotanically-selected sample was different from that expected from a random sample of plants, we used expected frequencies obtained in the NCI's large-scale "modified random" screening program, which included both medicinal and non-medicinal plants (Lewis and Elvin-Lewis 1995). Because of our small sample sizes and the small expected number of active extracts, we calculated the exact binomial probabilities (of obtaining results equal to or better than ours) (Sokal and Rohlf 1995) using QuattroPro software (Novell, Inc. 1994).

Anti-cancer screening. — Anti-cancer screening was carried out at Laboratory of Drug Discovery Research and Development. The two-day bioassays using 60 human tumor cell lines were performed as described previously (Boyd 1989). Each extract was tested at a maximum concentration of 250 mg/mL of cells and serially diluted to a minimum concentration of 0.018 mg/mL. The cells were allowed to incubate for 48 hours, at which time cell growth was measured as described in Boyd (1989). Three parameters were then measured: GI_{50} (the concentration of extract at which 50% of the tumor cells are inhibited in their growth relative to non-extract treated cells), GI_{100} (the concentration at which 100% of the tumor cells' growth has been inhibited), and LC_{50} (the concentration of extract at which 50% of the tumor cells are killed relative to the control). In addition to these three parameters, specificity was also measured. Specificity is observed when an extract demonstrates an exceptional amount of activity for one particular cell line relative to the others. Usually this activity is at least one order of magnitude greater than that for the average of all other cell lines. The human tumor cell lines tested were: leukemia, non-small cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast. A thorough discussion of data interpretation from the National Cancer Institute screen can be found in Boyd and Paull (1995).

Like the anti-HIV assay, the anti-cancer assay was run in duplicate with the same sample. We will again use "active," "moderate," and "inactive" to report our results. Samples that achieve an LC_{50} with at least 50% of the cell lines responding will be classified as "active," while extracts with "moderate" activity must achieve an LC_{50} with at least 20% of the cell lines tested responding.

TABLE 2. — Results of anti-HIV assay for prairie plants. A = "active" extract (achieves $EC_{50,}$ test concentration at which growth of infected cells is 50% of non-infected control); M = "moderate" activity (extract shows growth of infected cells at less than 50% of control); I = "inactive" (extract shows no growth of infected cells or toxicity to uninfected control cells at concentration less than 250 mg/ml); T= toxic to uninfected control cells at very low concentration. Overall rate of activity is 60.0% for aqueous extracts and 13.6% for organic extracts.

Scientific Name	Aqueous	Organic
Achillea millefolium	A	M
Amorpha canescens	A	I
Astragalus bisulcatus	I	I
Ceanothus herbaceus	A	I
Conyza canadensis	A	M
Equisetum hyemale	I	I
Fragaria virginiana	I	I
Glycyrrhiza lepidota	I	A
Helianthus grosseserratus	A	I
Ipomoea leptophylla	A	A
Juniperus virginiana	T	I
Liatris punctata	A	I
Monarda fistulosa	A	I
Oenothera rhombipetala	A	A
Pediomelum argophyllum	not tested	I
Pycnanthemum tenuifolium	A	I
Rhus glabra	not tested	M
Rubus flagellaris	A	I
Silphium laciniatum	A	I
Silphium perfoliatum	I	M
Solidago canadensis	I	I
Verbena hastata	I	I

RESULTS

Anti-HIV aqueous assay. — Aqueous extracts of 20 of the 22 plants collected were tested for anti-HIV activity. Twelve extracts met the criteria for "active" (Table 2). *Juniperus virginiana* showed an exceptionally low IC_{50} (the concentration at which 50% of the non-infected white blood cells are killed), but showed no protection to infected cells. This indicates a very high toxicity to healthy cells. At the other end of the activity spectrum was *Oenothera rhombipetala,* which had the lowest EC_{50} concentration of 0.56 mg/ml. *Helianthus grosseseratus,* with a TI_{50} value of >250, never showed toxicity to uninfected cells. The 60.0% activity rate in these extracts is significantly higher ($p < .001$) than the 13.9% rate reported for terrestrial plants by the NCI in its large-scale screening program (Cardellina *et al.* 1993).

Anti-HIV organic assay. — Twenty-two organic extracts were tested for anti-HIV activity. Only three plants achieved an EC_{50} (*Ipomoea leptophylla, Glycyrrhiza lepidota,* and *Oenothera rhombipetala*). This results in 13.6% of the extracts being classified as "active," a proportion which is significantly greater ($p = .03$) than the 3.0% rate for

TABLE 3. — Results of anti-cancer screen for prairie plants. A = "active" extract (achieves LC_{50}, test concentration at which 50% of tumor cells are killed relative to control, with at least 50% of cell lines responding); M = "moderate"(achieves LC_{50} with at least 20% of cell lines responding); I = "inactive."

Scientific Name	Aqueous	Organic
Achillea millefolium	I	M
Amorpha canescens	I	I
Astragalus bisulcatus	I	I
Ceanothus herbaceus	slight activity	I
Conyza canadensis	I	I
Equisetum hyemale	I	I
Fragaria virginiana	I	I
Glycyrrhiza lepidota	I	M
Helianthus grosseserratus	I	A
Ipomoea leptophylla	I	A
Juniperus virginiana	I	A
Liatris punctata	I	M
Monarda fistulosa	I	M
Oenothera rhombipetala	I	I
Pediomelum argophyllum	not tested	I
Pycnanthemum tenuifolium	I	I
Rhus glabra	not tested	I
Rubus flagellaris	I	I
Silphium laciniatum	I	M
Silphium perfoliatum	I	M
Solidago canadensis	I	A
Verbena hastata	I	I

terrestrial plants reported by the NCI in their screening program (Cardellina *et al.* 1993). Four plants (*Achillea millefolium, Conyza canadensis, Rhus glabra,* and *Silphium perfoliatum*) showed moderate protection from the HIV virus in infected cells.

Anti-cancer aqueous screen. — Only one aqueous extract of the twenty tested, *Ceanothus herbaceus,* achieved an LC_{50} value. Its activity was slight, with only two of the 60 cell lines showing sensitivity to this extract.

Anti-cancer organic screen. — Twenty-two organic extracts were tested in the anti-cancer screen (see Table 3). Four extracts were active (*Helianthus grosseserratus, Ipomoea leptophylla, Juniperus virginiana,* and *Solidago canadensis*) and six extracts showed moderate activity (*Achillea millefolium, Glycyrrhiza lepidota, Liatris punctata, Monarda fistulosa, Silphium laciniatum,* and *Silphium perfoliatum*). This difference between activity of the organic and aqueous extracts may be due to the fact that the non-polar molecules of organic extracts more easily enter the cell through the non-polar cell membrane. *Juniperus virginiana* showed the highest activity. This extract achieved a GI_{50} and GI_{100} when tested with all 60 cell lines, while it achieved a LC_{50} with 83% of the cell lines. Its GI_{50} was 0.062 mg/mL. None of the plants tested met the criteria for specificity.

DISCUSSION

Although these are preliminary results from a small data set, we found that a relatively high proportion of prairie plants with historical ethnomedical uses were active in anti-cancer and anti-AIDS screening. Further testing is needed to quantify the data, including replication and testing with different cell lines and different viral strains of HIV.

The relatively high number of aqueous extracts we found to be active in the AIDS screen is likely to be due to the antiviral activity of sulfated polysaccharides (Beutler *et al.* 1993) or the potent reverse transcriptase inhibitors of polyphenolic tannins (Tan *et al.* 1991). Because these substances are already known and therefore are not of interest in the screening process (Cardellina *et al.* 1993), our active extracts should be further screened using alcohol mediated precipitation to eliminate the polysaccharides and polyamide adsorption to eliminate false positive results from tannins.

In the anti-cancer screen, *Juniperus virginiana* organic extract's GI_{50} of 0.062 mg/mL is impressively low in comparison with the value obtained for organic extract of *Camptotheca acuminata*. *Camptotheca* produces the known anti-tumor compound camptothecin and its GI_{50} was 3.0 mg/mL (Mike Boyd, personal communication, National Cancer Institute, 1997).

The failure of any of our extracts to meet the criteria for specificity is not surprising, since fewer than 1% of the plants tested by the NCI show evidence of selective cytotoxicity (Cragg *et al.* 1994). We would suggest that the ten extracts that achieved at least moderate activity should be further examined (*Achillea millefolium, Glycyrrhiza lepidota, Helianthus grosseserratus, Ipomoea leptophylla, Juniperus virginiana, Liatris punctata, Monarda fistulosa, Silphium laciniatum, Silphium perfoliatum*, and *Solidago canadensis*).

Several of the genera we tested were screened in the NCI's pre-1982 program and were excluded from further testing based on the large number of extracts screened (Spjut 1985). Our results show some anti-cancer activity for the organic extracts from these genera. Spjut stated that unless a different screening method were used, there were diminishing returns from additional collections of these genera. Our positive results suggest that re-evaluation of some of the plants tested before 1982 is merited. Ethnobotanical targeting may help identify promising candidates.

When comparing the rates of activity in a sample of ethnomedically targeted plants with a random sample, it is important that the term "activity" be clearly defined, and that the most appropriate data set be used for comparison. The most appropriate comparative data set for our work would have been a random sample of prairie plants, but we were unable to test the larger number of extracts that this would require. We chose to compare our percent of active extracts with the data from the NCI's primary AIDS screening program reported by Cardellina *et al.* (1993). These researchers, using data obtained through October 1992, reported that the proportion of terrestrial plants "selected for initial follow-up" was 13.9% for aqueous and 3.0% for organic extracts. Their criterion for activity was any extract achieving an EC_{50} at a concentration less than 250 mg/mL (Cardellina *et al.* 1993).

A more recent comparative data set is available in Cragg *et al.* (1994). This group, using data obtained through August 1993 (medicinal and non-medicinal plants combined), reported "percent active" rates of 33.8% for aqueous and 4.2% for organic extracts. The criterion for activity used by this group was any extract showing an EC_{50} at a concentration less than 1000 mg/mL (Gordon M. Cragg, personal communication, 1997). The difference in criteria used by these authors accounts for the difference in "percent active" from the two groups using the same assay. In our study we have used an EC_{50} concentration of <250 mg/mL to define our "percent active." Our data is therefore more comparable to Cardellina *et al.* (1993). Authors of other published literature have not always stated explicitly what criteria they used to determine whether or not a plant extract is "active," making comparisons between studies difficult.

Although several authors have attempted to show that ethnobotanically-targeted plant collections result in higher rates of active extracts being identified, the data from the NCI's large-scale screening program show no difference in rates of activity between medicinal plants and non-medicinal plants (Cragg *et al.* 1994). These data may seem discouraging to those who advocate using an ethnobotanical approach to collect plants in the search for new drugs, but we believe it means that ethnobotanists need to do a better job of targeting our collections and accurately matching ethnnomedical uses and practices to our screening methods. Balick (1994) suggests that the ethnobotanical approach will be most successful in small programs that are focused on collecting plants used by indigenous healers for the diseases they actually treat. Several authors have pointed out the difficulty in using ethnomedical data to identify anti-cancer agents, since cancer is not a well-defined disease in most traditional medical systems (Farnsworth 1990; Balick 1994). It is also important to attempt to match extraction procedures to the methods of administration used by healers so the active compounds actually used by healers are captured by the screening process (Cox 1990). Finally, it must be acknowledged that much of the historical ethnomedical information is poorly documented (Farnsworth 1990). It is not surprising that Lewis and Elvin-Lewis (1995) obtained a significantly higher rate of preliminary anti-AIDS activity in plants they selected based on primary (i.e., interviews) rather than secondary (i.e., literature and historical) ethnobotanical data, and specifically for traditional antiviral use as opposed to other ethnomedical uses. Primary data and specific uses are probably more accurate and reliable bases for identifying useful new compounds.

Although some authors have found higher rates of activity among plants with ethnomedical uses, expecting to identify novel therapeutic compounds from traditional medicinal plants is not necessarily realistic. Native traditional practitioners were, and continue to be, sophisticated in their ability to identify plants with biological activity, and to use them therapeutically. However, they did not use them in the context of Western medicine and Western disease concepts. The goals of native healers — finding plants that work for the medical problems of their communities — may not be identical to those of modern screening programs (finding novel compounds which can be used in Western medicine).

Finally, the issue of intellectual property needs to be considered. The plants we collected for this study fall into what Kloppenburg and Balick (1996) call the "middle ground" of intellectual property rights, that is plants "used regionally, by

more than one community or social group, and [having] different uses in different communities." We used secondary data gathered over a broad range of time and the entire Prairie Bioregion to target the plants for study. Nevertheless, we believe that Native people in the region should benefit if a new therapeutic agent were identified from a prairie plant that they traditionally used. We are looking for suggestions for how to do this. The use of royalties, an approach often called for by advocates of indigenous intellectual property rights, would be problematic in this case because for most tribes, commercialization of their knowledge is a violation of spiritual beliefs. Other ways to give something in return might be the establishment of a scholarship fund for Native American students at universities or other institutions, or funding medicinal plant gardens or ecological restoration on the Indian reservations in the region.

CONCLUSIONS

The Indian tribes of the Prairie Bioregion in North America used at least 203 species of medicinal plants (Kindscher 1992). These plants were not used against AIDS because native people did not encounter this disease historically. In addition, cancer was typically not identified by them as a specific disease. However, these plants were used for 78 different types of diseases and illnesses (Table 1). We believe that these uses suggest potentially active medicinal constituents and a broad knowledge base of plant use for health and in healing systems.

By collecting plants with a history of medicinal uses, we have increased our proportion of plants active in the NCI anti-HIV in-vitro screening assay. "Modified random" collection of plants world-wide (37,500 species) has lead to a 13.9% rate of activity for aqueous extracts and a 3.0% rate for organic extracts (Cardellina *et al.* 1993). Our data from 22 species has produced a significantly higher rate of activity of 60% for the aqueous extracts (12 of 20) and 13.6% for the organic extracts (3 out of 22). Although the higher percentage of activity does not mean that useful compounds will be found, it does show the promise that these plants potentially offer. Traditional knowledge of Native Americans should not only be studied (perhaps more appropriately stated as "learned"), but should be honored for the valuable insights it can offer, one of which is leads for finding plants that have active medicinal constituents. In addition, we believe that plants of native prairies and other ecosystems in our own continent merit further exploration and study.

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