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Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout

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Abstract

Xanthine oxidase (xanthine: oxygen oxidoreductase EC 1.2.3.2) inhibitory activity was assayed from 26 species belonging to 18 families traditionally used for the treatment of gout and related symptoms by Indigenous people of northeastern North America. The degree of inhibition was determined by measuring the increase in absorbance at 295 nm associated with uric acid formation. Eighty-eight percent of the plants were found to have inhibitory activity at 100 μg/ml, with 20% having greater than 50% inhibition. *Larix laricina* exhibited the highest activity with an inhibition of 86.33%. Of the species with the highest activity, Lineweaver–Burk plots showed that inhibition mode was of linear mixed-type. Inhibitory activity of the plants correlated positively with their phenolic content ($r = 0.52$ $P < 0.01$) and tannin content ($r = 0.59$ $P < 0.001$). © 1999 Published by Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Xanthine oxidase; Inhibitor; Gout; North america; Phenols; Medicinal plants

1. Introduction

Hyperuricemia, associated with gout, results from the overproduction or underexcretion of uric acid and is greatly influenced by a high dietary intake of foods rich in nucleic acids. The traditional diets of northeastern North American Amerindians were especially high in wild meat, which could be expected to contribute to gout. Meats (especially organ meats), leguminous seeds, some types of seafood and food yeasts are considered rich sources of nucleic acids (Sarwar and And Brulé, 1991). The catalysis of xanthine by the enzyme xanthine oxidase (XO) (EC 1.2.3.2) can lead to the accumulation of uric acid (xanthine + $O_2 + H_2O \rightarrow$ urate + H_2O_2), and ultimately cause gout. Allopurinol, a XO inhibitor prescribed for chronic gout, acts as a substrate for and competitive inhibitor of the enzyme, and at higher concentrations, is a noncompetitive inhibitor

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(Hardman and Limbird, 1975). Plants are used by Indigenous people for the treatment of gout, or diseases with associated symptomologies such as rheumatism or arthritis, and we postulate that these may contain XO inhibitors. XO inhibitors have been found in a wide variety of plants used in traditional herbal medicines for the treatment of gout and rheumatism from Chile (Theoduloz et al., 1991) Paraguay (Hayashi et al., 1989; Schmeda-Hirschmann et al., 1987; Theoduloz et al., 1988) and Panama (González et al., 1995). However, no investigations have been reported on the plants used for the same disorders by the Indigenous population of northeastern North America.

Flavonoids (Iio et al., 1985; Chang et al., 1993), and certain other phenols (Hatano et al., 1989), polyphenols (Costantino et al., 1992) and tannins (Hatano et al., 1990), as well as coumarins (Chang and Chiang, 1995), plant growth regulators (Sheu and Chiang, 1996) and folic compounds (Lewis et al., 1984) have all been reported to be potent XO inhibitors. The putative therapeutic effects of many traditional medicines have been ascribed to flavonoids in particular due to their enzyme inhibitory and antioxidant activity (Havsteen, 1983; Brandi, 1992). Therefore, we expect a positive correlation between XO inhibitory activity and phenolic content of plant extracts. In studying the XO inhibitory activity of flavonoids, Iio et al. (1985) determined that kaempferol and quercetin had 85 and 90% XO inhibitory activity at 50 μg/ml, respectively. These compounds are effective inhibitors of xanthine oxidase due to the high number of phenolic groups, which render them hydrophilic. The number and position of glycosyl groups on a flavonoid however, decreases inhibition, rendering them too bulky or too hydrophilic, resulting in reduced contact of the glycosidic flavonoid with the enzyme. Moreover, tannins, because of their protein-binding properties, may have interfered with our in vitro assays, and so a quantitative analysis of the test plants was performed to distinguish the contribution of non-tannin phenolics to XO inhibition from that attributable to tannins.

The objective of this study is therefore to determine the validity of plant remedies used for gout by examining their xanthine oxidase inhibitory activity and whether a relationship exists between enzyme inhibition and the total and water-soluble phenolic and tannin content.

2. Materials and methods

2.1. Plant collection

A review of the ethnobotanical literature was used to identify species of plants traditionally used by Indigenous people in northeastern North America and across the continent for the treatment of gout and related symptoms, excluding those applied externally. Although most plants were listed as antirheumatics, plants were selected if at least one reference mentioned their related therapeutic use in the northeast and at least one North American Indigenous group used the plant specifically for the treatment of gout. Table 1 includes the literature sources from which the plants were selected, along with the Indigenous nations which employed them. Plants were collected in the Montreal and James Bay regions, Qué, between May and September, 1996. Voucher specimens were verified by Dr T. DiTommaso, Department of Plant Science, McGill University, and deposited in the McGill University Herbarium.

2.2. Preparation of crude extracts

Specimens were air dried and ground using a Wiley mill with a 850 μm mesh. Samples were extracted with methanol (HPLC grade) (100 mg/700 ml) for approximately 1 week, shaken periodically, and dried under vacuum.

2.3. Assay of xanthine oxidase activity

The XO activities with xanthine as the substrate were measured spectrophotometrically using the procedure of Marcocci et al. (1994) with the following modifications. The xanthine solution (0.15 mM) was prepared by initially dissolving xanthine (Sigma) in a minimal volume of NaOH, and adjusting pH to 7.5. The XO solution was pre-

Table 1
List of families and species of northeastern North American plants administered internally for the treatment of gout related symptoms

Family/species and common name	Voucher ID	Locality (Qué)[a]	Plant part and extract yield (%)[b]	Indigenous nation and reference
APOCYNACEAE				
A. androsaemifolium L. Spreading dogwood	18	Eaton	Root (9.56)	ns Foster and Duke (1990), Hutchens (1991), le Strange (1977)
ARALIACEAE				
Aralia naudicaulis L. Wild sarsaparilla	26	Eaton	Root (5.00)	ns Foster and Duke (1990), Harris (1985), Hutchens (1991)
ASTERACEAE				
A. millefolium L. Yarrow	28	Eaton	Aerial (9.66)	Iroquois; ns Moerman (1986), Popik (1988)
Arctium lappa L. Burdock	4	Montreal	Root (12.17)	Cherokee Moerman (1981) Delaware, Mohegan Moerman (1981) ns Bolyard (1981), Foster and Duke (1990), Hutchens (1991), Scully (1970)
Artemisia vulgaris L. Mugwort	16	Montreal	Leaves (4.52)	Miwok Duke (1986), ns Hutchens (1991), le Strange (1977), Lust (1974), Scully (1970)
Chicorium intybus L. Chicory	7	Montreal	Root (12.15)	ns Hutchens (1991), le Strange (1977)
E. purpureum L. Joe-pye-weed	12	Waskaganish	Aerial (8.23) Root (6.80)	Cherokee Duke (1986), Moerman (1986) Chippewa, Iroquois Moerman (1986) ns Duke (1986), Foster and Duke (1990), Harris (1985)
Solidago canadensis L. Goldenrod	10	Montreal	Flower tops (11.74)	ns Hutchens (1991)
BERBERIDACEAE				
C. thalictroides (L.) Michx. Blue cohosh	2	Eaton	Root (16.84)	Cherokee Moerman (1981, 1986) Cree Arnason et al. (1981), Duke (1986)
CAPRIFOLIACEAE				
S. canadensis L. Elderberry	9	Eaton	Fruit (31.00)	Cherokee Duke (1986) Rappahannock Moerman (1986)ns Hutchens (1991), le Strange, 1977; Lust, 1974; Scully, 1970
CUPRESSACEAE				
Juniperus communis L. Juniper	14	Waskaganish	Cones (26.48)	Chippewa Creek, Delaware and Hopi Moerman (1986) Malecite Duke (1986) Maritime Arnason et al. (1981) Micmac Duke (1986), Hutchens (1991), Moerman (1986), Scully (1970)
Thuja occidentalis L. Eastern white cedar	6	Ile Bellevue	Leaves (8.65)	Algonquin Arnason et al. (1981), Duke (1986) Iroquois Moerman (1986) ns Foster and Duke (1990), Lust (1974)
EQUISETACEAE				
Equisetum arvense L. Horsetail	11	Eaton	Herb (4.78)	Cherokee Duke (1986) Iroquois, Potawatomi Moerman (1986) ns Foster and Duke (1990)

Table 1
(*Continued*)

Family/species and common name	Voucher ID	Locality (Qué)[a]	Plant part and extract yield (%)[b]	Indigenous nation and reference
ERICACEAE				
L. groenlandicum Oeder. Labrador tea	15	Waskaganish	Leaves (18.55)	Cree, Quinault Moerman (1986) ns Foster and Duke (1990), Hutchens (1991), Lust (1974)
FABACEAE				
M. alba Medikus. White sweet clover	21	Montreal	Aerial (9.94)	ns Lust (1974)
Trifolium repens L. White clover	24	Eaton	Herb, flower (11.68)	ns Foster and Duke (1990)
IRIDACEAE				
I. versicolor L. Blueflag	3	Waskaganish	Root (16.91)	Chippewa Moerman (1981) Delaware Duke (1986)Mohegan Moerman (1986) Montagnais Moerman (1981) ns Foster and Duke (1990), le Strange (1977)
LAMIACEAE				
Mentha canadensis L. Wild mint	25	Waskaganish	Herb (14.41)	ns Moerman (1981)
MENISPERMACEAE				
M. canadense L. Moonseed	5	Ile Bellevue	Root (10.57)	ns Foster and Duke (1990), Hutchens (1991), Lust (1974)
OLEACEAE				
Fraxinus americana L. White Ash	23	Montreal	Leaves (8.30)	ns Hutchens (1991), Lust (1974)
PINACEAE				
L. laricina (Duroi) K. Koch.	22	Waskaganish	Inner bark	Iroquois Moerman (1981)
Tamarack			(21.33)	ns Foster and Duke (1990), Hutchens (1991)
POACEAE				
Elytrigia repens (L.) Nevski.	17	Eaton	Rhizome	ns Hutchens (1991), Lust (1974)
Quackgrass			(14.22)	
ROSACEAE				
Fragaria virginiana Duchesne. Wild strawberry	27	Ile Claude	Herb, flower, fruit (22.51)	ns Foster and Duke (1990), Harris (1985), Popik (1988), Scully (1970)
SALICACEAE				
P. balsamifera L. Balsam poplar	1	Eaton	Inner bark (21.76)	Cherokee Duke (1986) Iroquois, Ojibwa Moerman (1986) ns Foster and Duke (1990), Hutchens (1991)
SCROPHULARIACEAE				
V. officinalis L. Common speedwell	8	Eaton	Whole plant (22.36)	ns Foster and Duke, 1990
URTICACEAE				
U. dioica L. Stinging nettle	19	Waskaganish	Aerial (7.80) Root (9.61)	Paite Duke (1986) Quinault, Shuswap, Tainarna Moerman (1986) ns Foster and Duke (1990), Hutchens (1991), le Strange (1977)

Additional data is included for nations from outside the reference area. The locality of the collected specimen, the part collected, the voucher specimen identification number, as well as the literature source from which the plant was cited is also included.
[a] Eaton 45° 21′N 71° 28′W; Ile Bellevue 45° 24′N 73° 57′W; Ile Claude 45° 24′N 73° 58′W; Montreal 45° 29′N 73° 36′W; Waskaganish 51° 37′N 78 °50′W.
[b] Numbers in parentheses indicate w/w yield in terms of initial dried material.
ns, not specified. The source either made general reference to American Indians, or stated that the plant was used traditionally.

pared by diluting XO from cow's milk (Sigma) to a final concentration of 0.2 U/ml in cold 50 mM potassium phosphate buffer (pH 7.5). The assay mixture consisted of 0.250 ml plant extract solution (0.4 mg/ml 50 mM potassium phosphate buffer, pH 7.5), 0.385 ml 50 mM potassium phosphate buffer (pH 7.5) and 0.330 ml xanthine solution, giving a final concentration of 100 μg plant extract per ml assay mixture. The reaction was initiated by adding 0.035 ml XO solution, and the change in absorbance recorded at 295 nm for 3 min at room temperature. Allopurinol (Sigma) was used as a standard inhibitor at a final concentration of 100 μg/ml in the assay mixture.

Xanthine oxidase activity was expressed as percent inhibition of xanthine oxidase, calculated as $(1 - B/A) \times 100$, where A is the change in absorbance of the assay without the plant extract (Δabs. with enzyme $-$ Δabs. without enzyme), and B is the change in absorbance of the assay with the plant extract (Δabs. with enzyme $-$ Δabs. without enzyme).

2.4. Lineweaver–Burk plots

Enzyme kinetics were carried out in the absence and presence of the plant extract with varying concentrations of xanthine as the substrate, using the XO assay methodology.

2.5. Quantitative determination of total phenols

Total phenolic content was determined spectrophotometrically at 750 nm using the procedure of Lowman and Box (1983) with the following modifications. Plant extracts (0.10 mg/ml) boiled in 50% v/v methanol were filtered and made up to 100 ml with 50% v/v methanol. After diluting 2.50 ml of the sample to 25 ml with deionized water, 1.50 ml sodium carbonate (BDH) (200 g/l) and 1N 0.50 ml Folin–Ciocalteu's phenol reagent (Sigma) was added, and the absorbance read after 1 h, using (+)-catechin (Sigma) as the standard.

2.6. Quantitative determination of water-soluble phenols

Water-soluble phenolic content was determined spectrophotometrically at 750 nm using the procedure of Box (1983) with the following modifications. Plant extracts (0.4 mg/ml) were centrifuged at 3500 rpm and filtered after sitting at room temperature for 24 h. The absorbance was read 1 h after the successive addition of 1.50 ml monohydrate sodium carbonate (200 g/l) and 2N Folin Ciocalteu's phenol reagent, using (+)-catechin as the standard.

2.7. Quantitative determination of tannins

Tannin concentration was determined spectrophotometrically at 510 nm using the protein precipitation method of Hagerman and Butler (1978). Concentration of plant extract solution was 1.0 mg/ml 50% v/v methanol.

3. Results

3.1. Xanthine oxidase inhibitory activity

Twenty-six plant species belonging to 18 families were assayed for xanthine oxidase inhibitory activity at 100 μg crude extract/ml. Of the extracts assayed, 23 (88.5%) demonstrated xanthine oxidase inhibitory activity, among these 5 (19%) had 50% or greater inhibition (Table 2). These include *Achillea millefolium* L., *Larix laricina* (Duroi) K. Koch., *Ledum groenlandicum* Oeder., *Populus balsamifera* L. and *Veronica officinale* L. Although the frequency of XO inhibition among the plants tested was high, the majority had a relatively weak effect upon the enzyme. The average inhibitory activity of the crude plant extracts was 24.89% at 100 μg/ml. Plant extracts considered to have minimal or no XO inhibitory activity ($< 3\%$) included *Apocynum androsaemifolium* L., *Caulophyllum thalictroides* (L.)Michx., *Eupatorium purpureum* L. herb and root, *Iris versicolor* L., *Melilotus alba* Medikus., *Sambucus canadensis* L. and the roots of *Urtica dioica* L.

The plant with the highest inhibitory activity was *L. laricina* (86.33%) whose inner bark was used in a compound decoction by the Iroquois as an antirheumatic (Moerman, 1986; Foster and Duke, 1990) and more broadly to treat gout (Hutchens, 1991). The herb of *A. millefolium*

Table 2
Xanthine oxidase inhibitory activity, total phenolic, water-soluble phenolic and tannin content of plants used for the treatment of gout

Species	XO inhibition (%) at 100 μg/ml[a]	Total phenols (mg of catechin equivalents/ g plant extract)	Water-soluble phenols (mg of catechin equivalents/ g plant extract)	Tannin concentration (mg tannin/ g plant extract)
L. laricina	86.33 ± 0.45^{a}	292.73 ± 8.12	122.27 ± 5.09	86.46 ± 0.63
A. millefolium	66.64 ± 0.56^{b}	96.16 ± 3.31	79.38 ± 2.69	11.58 ± 0.38
P. balsamifera	51.29 ± 2.01^{c}	285.52 ± 19.54	115.72 ± 5.09	46.94 ± 0.35
L. groenlandicum	49.55 ± 2.78^{cd}	250.05 ± 11.72	105.24 ± 2.06	105.50 ± 0.25
V. officinalis	49.44 ± 0.67^{cd}	180.32 ± 8.12	90.33 ± 1.52	14.50 ± 1.19
A. vulgaris	47.82 ± 1.86^{cd}	126.82 ± 2.71	63.00 ± 1.46	14.42 ± 1.42
M. canadensis	45.24 ± 1.32^{d}	105.78 ± 1.50	92.34 ± 2.03	10.70 ± 1.50
F. virginiana	45.03 ± 1.90^{d}	193.85 ± 0.60	101.42 ± 4.97	107.23 ± 3.82
T. repens	37.56 ± 5.42^{e}	73.92 ± 4.51	54.58 ± 1.68	16.77 ± 1.11
A. lappa	36.35 ± 2.72^{e}	61.30 ± 6.91	43.32 ± 2.15	10.24 ± 1.04
E. arvense	33.13 ± 4.00^{ef}	46.27 ± 0.30	34.55 ± 1.67	8.28 ± 0.31
S. canadensis	32.46 ± 2.65^{ef}	122.91 ± 0.60	102.11 ± 2.49	18.46 ± 1.61
J. communis	28.36 ± 3.01^{fg}	64.60 ± 1.80	54.10 ± 2.61	15.98 ± 0.40
T. occidentalis	22.45 ± 1.08^{gh}	138.54 ± 9.62	67.94 ± 2.89	61.93 ± 1.21
F. americana	21.19 ± 1.25^{h}	95.56 ± 1.50	74.36 ± 0.60	11.07 ± 1.86
A. naudicaulis	14.10 ± 1.89^{i}	62.80 ± 1.80	45.01 ± 0.64	11.05 ± 0.54
Cichorium intybus	9.16 ± 1.59^{ij}	44.16 ± 0.60	27.61 ± 0.79	8.85 ± 0.15
U. dioica (Herb)	7.38 ± 0.88^{jk}	75.72 ± 10.52	58.76 ± 1.48	14.64 ± 0.67
Elytrigia repens	6.46 ± 1.52^{jkl}	27.33 ± 3.01	18.15 ± 1.12	8.59 ± 0.15
M. canadense	4.73 ± 2.72^{jklm}	257.56 ± 8.42	105.33 ± 0.37	8.61 ± 0.15
Eupatorium purpureum (Herb)	2.85 ± 0.45^{klmn}	58.89 ± 2.71	35.44 ± 0.86	8.59 ± 0.46
I. versicolor	2.43 ± 0.10^{klmn}	29.14 ± 0.60	19.75 ± 2.19	11.45 ± 1.55
S. canadensis	2.38 ± 2.27^{klmn}	100.07 ± 1.20	90.14 ± 2.35	9.45 ± 0.36
A. androsaemifolium	0.66 ± 0.72^{lmn}	86.24 ± 1.80	58.93 ± 1.39	8.57 ± 0.17
E. purpureum (Root)	-0.35 ± 0.63^{mn}	92.56 ± 0.90	63.14 ± 0.92	9.66 ± 0.65
C. thalictroides	-1.52 ± 0.75^{n}	92.25 ± 3.60	59.61 ± 1.63	12.06 ± 0.86
U. dioica (Root)	-1.96 ± 1.84^{n}	32.74 ± 3.01	19.88 ± 0.59	7.69 ± 0.21
M. alba	-2.05 ± 2.40^{n}	155.07 ± 4.51	68.39 ± 3.56	15.40 ± 0.25
	99.68 ± 1.09			

All tests were performed in triplicate.
[a] Means with the same letter are not significantly different.

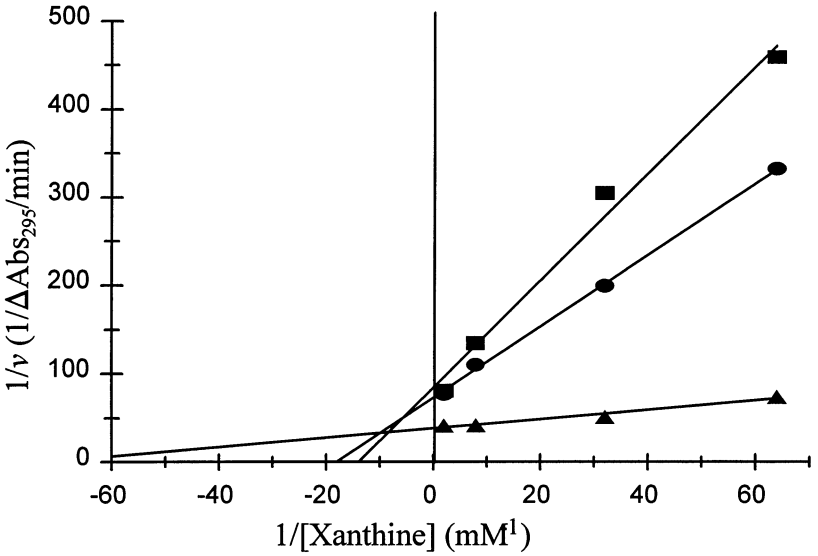

Fig. 1. Lineweaver–Burk plots Allopurinol in the absence (▲-▲) and in the presence of *A. millefolium* (100 μg/ml, ●-●) and *L. laricina* (100 μg/ml, ■-■) with xanthine as the substrate.

(66.64%) and inner bark of *P. balsamifera* (51.29%) were similarly used as teas by the Iroquois to treat rheumatism (Moerman, 1986). The latter was also used historically to treat gout (Bolyard, 1981), and by the Cherokee to treat rheumatism using a tincture of the buds (Duke, 1986). A tea made from the leaves of *L. groenlandicum* (49.55%) was used by the Cree (Moerman, 1986; Foster and Duke, 1990) and more broadly to treat gout (Lust, 1974; Hutchens, 1991).

3.2. Inhibition mechanism

Kinetic analysis using Lineweaver–Burk plots revealed that all of the plants that displayed high inhibitory activity did so through a linear mixed-type mode of inhibition (between uncompetitive and noncompetitive type inhibition) (Fig. 1.). Therefore, the majority of the plant extracts which inhibited XO did so by binding either with the free enzyme or the enzyme-substrate complex.

3.3. Correlation between XO inhibition and the plant phenolic and tannin content

Analysis of residual values indicated an adequate fit for an existing linear relationship between both the % XO inhibitory activity and the plant total phenolic and tannin content expressed as log catechin equivalents and log tannin concentration respectively. The positive correlation between XO inhibitory activity and total phenolic content of plant extracts ($r = 0.52$ $P < 0.01$ with a 95% confidence limit of a population parameter between 0.16 and 0.76) was weakened by the evident number of plants rich in phenols displaying low XO inhibitory activity (Fig. 2), suggesting high variation in the possible mechanisms for enzyme inhibition. Of particular interest are species with relatively low phenolic content and high XO inhibition, in particular *A. millefolium*. Similarly, a positive correlation exists between XO inhibition and water-soluble phenolic content in roughly the same relationship as that of total phenols.

The positive correlation between XO inhibitory activity and the tannin content of plant extracts was not significantly different from that of total phenols, yet had a higher level of significance from zero correlation ($r = 0.59$ $P < 0.001$ with a 95% confidence limit of a population parameter between 0.38 and 0.74). The majority of plants assayed (85.71%) however had a relatively low tannin content, yet

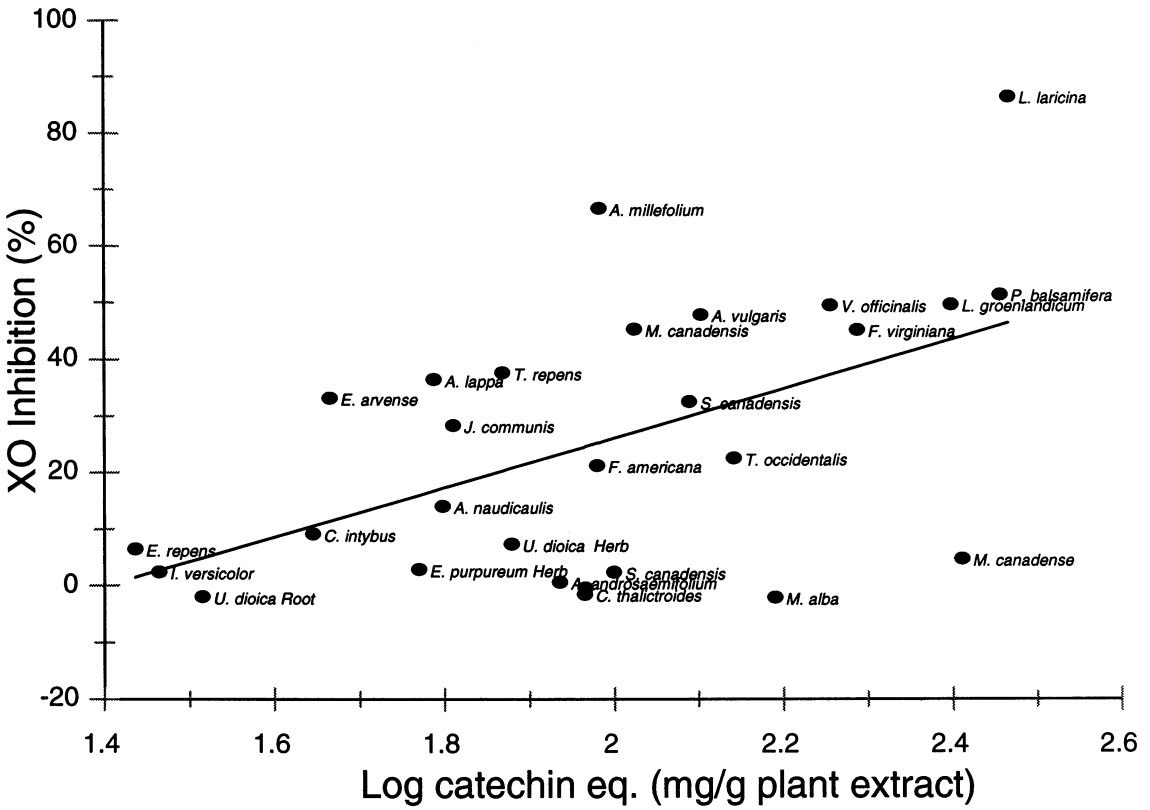

Fig. 2. Correlation between the xanthine oxidase inhibitory activity and the total phenolic content expressed as log catechin equivalents of plants assayed ($r = 0.54$ $P < 0.01$, $0.16 \leq \rho \leq 0.76$).

displayed a large variance between individual plants in the degree of XO inhibition (Fig. 3). Tannins explain 35% of the apparent XO inhibition (phenols, in comparison, explain 27%), suggesting that XO inhibition was as well determined by other factors. Although there was large variation between individual plants, we can state, in general, that the phenolic and tannin content of plants extracts made an important contribution to the inhibition of XO.

4. Discussion

The flavonoids apigenin and luteolin have been reported to be strong inhibitors of xanthine oxidase (Noro et al., 1983). Derivatives of these flavonoids are present in *A. millefolium* (Chandler et al., 1981) and *V. officinalis* (Glasby, 1991), two plants which had relatively high XO inhibitory activity. If we exclude the possibility that tannins in *L. laricina* were responsible for non-specifically binding to the enzyme, then the most likely XO inhibitor within the species would be phenylpropanoid *p*-coumaric acid (Niemann, 1969) which has been reported to have XO inhibitory activity (Chiang et al., 1994). Kaempferol-3-glucoside (Niemann and Bekooy, 1971) and other phenolic glucosides (Glasby, 1991) with unreported XO inhibitory activity may also have had an inhibitory effect. Moreover, the flavonoids procyanidin B-2 (Hatano et al., 1990), procyanidin B-3 (Cho et al., 1993) (+)-catechin (Cho et al., 1993) and (−)-epicatechin (Hatano et al., 1990), isolated from the bark of other *Larix* species (Shen et al., 1986; Glasby, 1991) have also been found to be active against XO.

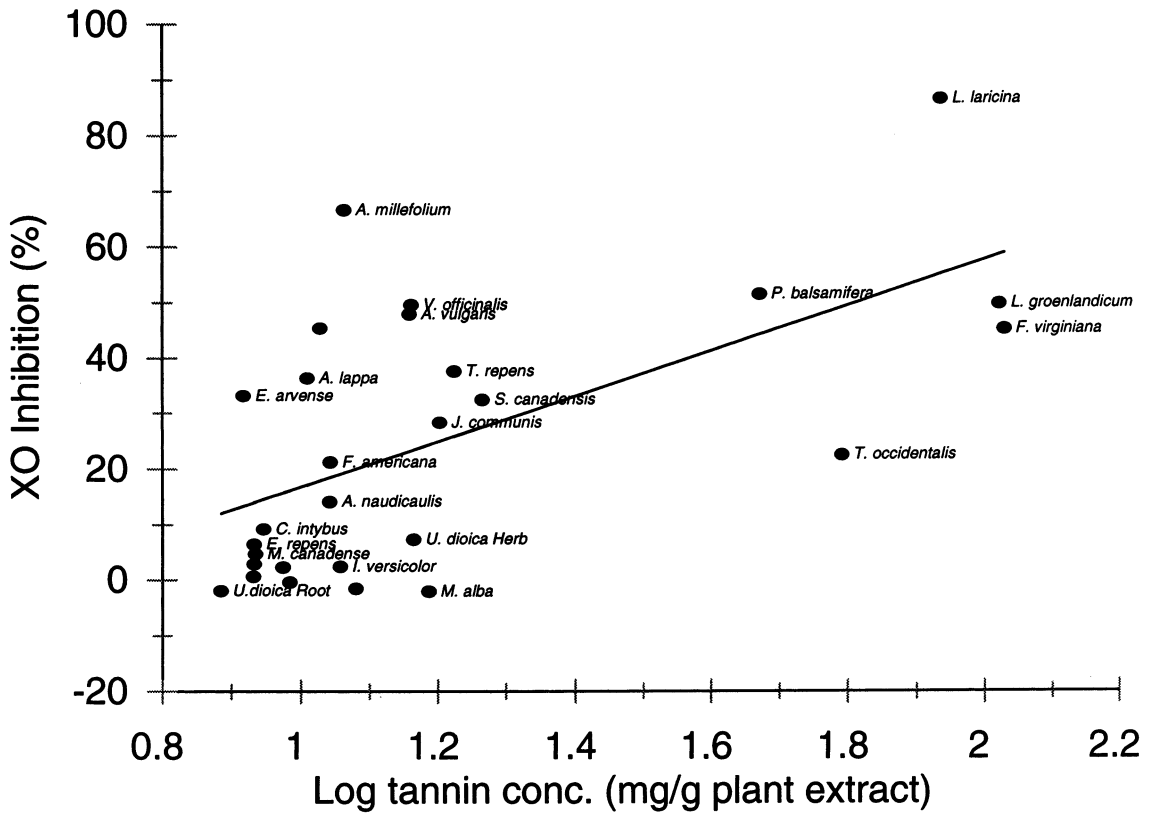

Fig. 3. Correlation between the xanthine oxidase inhibitory activity and the log tannin concentration of plants assayed ($r = 0.59$ $P < 0.001$, $0.38 \leq \rho \leq 0.74$).

Lineweaver–Burk plots revealed that plants with high XO inhibition did so through a linear mixed-type inhibition with respect to xanthine as a substrate. This is consistent with the inhibition mechanism of quercetin, kaempferol, apigenin and luteolin (Noro et al., 1983; Iio et al., 1985) and supports the assumption that flavonoids were responsible for XO inhibition in our assays.

Tannins and related polyphenols which are astringent via precipitation of proteins and mucopolysaccharides in mucous secretions, are the active principles of several herbal remedies (Haslam, 1989; Luck et al., 1994). Based on the astringent, antioxidant, antitumour, radioprotective and antimutagenic activities of tannins, plants high in tannins have been widely employed as treatments for conditions including inflammation, liver injury, kidney problems, arteriosclerosis, hypertension, stomach disorders, and nervous and hormonal problems (Zhu et al., 1997). High XO inhibition in *L. laricina*, *L. groenlandicum* and *F. virginiana* could have been attributed to the high tannin content of the plant, since tannins are known for their ability to non-specifically react with protein enzymes. Omitting these three particular plants from a correlation analysis resulted in a loss of correlation between both the tannin and phenolic content of the plant extract in relation to XO inhibitory activity. Although Hatano et al. (1990) demonstrated no correlation between XO inhibitory activity and binding activity to haemoglobin, suggesting that the inhibitory effects of tannins and related compounds were not due to the non-specific binding of tannins to XO,

the possibility of non-specific binding of tannins to XO in our assay cannot be ruled out. The results suggest that both total phenols and tannins contributed significantly to the inhibition of XO, however the 95% confidence interval for the r-value of tannins versus XO inhibition is tighter (0.38–0.74) with a smaller P-value ($P < 0.001$) than that of total phenols (0.16–0.76, $P < 0.01$) suggesting that tannins had a slightly greater influence on enzyme inhibition.

Because most tannin-protein complexes formed in the gastrointestinal tract are difficult to dissociate and pass out in the faeces (Haslam, 1989), it is likely that in vivo, XO inhibition would be attributed to absorbable non-tannin phenolics (Hollman et al., 1995) or other possible bioactive agents in the plant.

Preparation methods of medicinal remedies involving dosage would determine their activity and effectiveness. Most of the plants used to treat gout were administered as decoctions and infusions, so the biologically active compounds were most likely water-soluble. This is consistent with the positive correlation between the water-soluble phenolic content and the XO inhibitory activity of the plants demonstrated in our study. Some plants included in this paper are known to be toxic (e.g. *A. androsaemifolium*, *I. versicolor* and *Menispermum canadense*) and their usefulness would depend on their dosage and preparation. Nonetheless, the prevalence of XO inhibitors in these remedies suggest that, consistent with their traditional uses, many of them may have offered some degree of effectiveness towards treating gout.

5. Conclusions

Quantitative determination of the total and water-soluble phenolic and tannin content of plants traditionally used for treating gout revealed a positive correlation with XO inhibitory activity. Some phenolic compounds, particularly flavonoids, have been reported to be strong inhibitors of XO (Noro et al., 1983; Iio et al., 1985; Hatano et al., 1989; Chang et al., 1993) and may have been an important contributing factor in our assay. However, tannins may also have had an inhibitory effect by non-selectively binding to the enzyme. Kinetic analysis using Lineweaver–Burk plots revealed that the mode of inhibition was of linear mixed-type with respect to xanthine.

Our findings generally support existing ethnobotanical data on the use of these plants for the therapeutic treatment of gout by the Indigenous People of northeastern North America, since the majority of the tested plants contained XO inhibitors.

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