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SCREENING OF PLANTS FROM NEW CALEDONIA AND VANUATU FOR INHIBITORY ACTIVITY OF XANTHINE OXIDASE AND ELASTASE

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ABSTRACT

A series of 38 plants (55 plant extracts) from New Caledonia and 22 plants (40 plant extracts) from Vanuatu (Efate and Erromango islands) were screened for xanthine oxidase (XOD) and elastase inhibitory activity. Of the crude extracts 82% were found to possess xanthine oxidase inhibitory activity, and 23% were active against elastase, at a concentration of 50 μg/ml. The methanol extracts of Cunonia montana *Schlechter (Cunoniaceae) and* Amyema scandens *Danser (Loranthaceae), bark and leaves, respectively, exhibited the highest activity in both the assays.* C. montana *bark extract at 50 μg/ml exhibited 85 and 84% inhibition of XOD and elastase, respectively. IC_{50} values were 23 ± 0.82 and 41 ± 3 μg/ml, respectively, for XOD and elastase.* A. scandens *leaf extract, at 50 μg/ml, exhibited 88 and 71% inhibition of XOD and elastase, respectively. IC_{50} values were 13 ± 0.48 and 44 ± 2.2 μg/ml respectively, for XOD and elastase.*

INTRODUCTION

Xanthine oxidase (XOD; xanthine:oxygen oxidoreductase) converts hypoxanthine to xanthine and then to uric acid, which can precipitate giving rise ultimately to gout. It is also the enzymic tissue generator of superoxide ions which react with hydrogen peroxide to generate hydroxyl radicals. Oxygen derived free radicals are involved in the tissue damage under several conditions such as inflammation, allergies, heart ischemia-reperfusion, atherosclerosis, diabetes, emphysema, aging, etc. (Crastes de Paulet et al., 1994).

Proteases, in particular human leukocyte elastase, are implicated in tissue destruction in chronic inflammatory diseases including pulmonary emphysema (Groutas et al., 1980) rheumatoid arthritis (Snider, 1981), and in aging (Hall, 1964). This enzyme is capable of degrading many compounds of the extracellular matrix such as elastin (Galdston et al., 1979; Janoff, 1985), collagen type I, II (Starkey et al., 1977) collagen type III and IV (Mainardi et al., 1980a,b) and proteoglycans (Starkey et al., 1977). Endogenous inhibitors (for example alpha-1-proteinase inhibitor) of proteolytic enzymes are normally present in the extracellular fluids but their activity can be drastically reduced by oxidation. Depletion of proteinase inhibitors by oxidants in cigarette smoke (Dean et al., 1989) and by oxygen free radicals has been reported (Hancock et al., 1987). Thus, an attractive approach to the treatment of pathologies where free radicals and proteases are concurrent components, may be the development of drugs with anti-xanthine oxidase and anti-elastase activity. Furthermore, a class of elastase inhibitors which combine anti-oxidant properties will fulfill the requirement of blocking the enzyme and protecting the endogenous inhibitors from oxidation.

Several compounds present in plants (flavonoids, xanthones, coumarins, proanthocyanidins) are reported to be inhibitors of xanthine oxidase (Gonzalez et al., 1995) and proteases (Parellada & Guinea, 1995) and to possess anti-oxidant properties.

Compounds which possess both XOD and elastase inhibitory activity were procyanidines isolated from *Vitis vinifera* seeds (Maffei Facino et al., 1994). The flora of New Caledonia and Vanuatu is extremely rich

Keywords: Xanthine oxidase inhibition; elastase inhibition; *Cunonia montana* bark, *Amyema scandens* leaf, New Caledonia, Vanuatu.

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and diverse. Plants in New Caledonia are 80% endemic and most of them are unstudied. Some of them have a reputation as medicinal plants and are used for various diseases (Bourdy et al., 1992; Singh, 1992). A series of 38 plants from New Caledonia and 22 plants from Vanuatu (Efate and Erromango Islands) were subjected to screening for anti-xanthine oxidase and anti-elastase activity with the purpose of selecting plant species highly active for further phytochemical investigation to isolate the active principle/s, by means of bioactivity-guided fractionation. The choice was made at random, on the basis of a preliminary chemical screening for plants rich with phenolic compounds.

MATERIALS AND METHODS

Plant Material

Plants were collected by Dudley Nicholls (DN) and Pierre Cabalion (PC), ORSTOM Center, Noumea, from different places in New Caledonia and Vanuatu (Erromango and Efate islands). Plant identity was verified at the herbarium of the ORSTOM Center of Noumea and voucher specimens were deposited at the Laboratory of Natural Substances of Biological Interest, Noumea, New Caledonia. Plants collected in New Caledonia bear the collection number DN 1–38, plants collected in Vanuatu are reported as PC 3134–3177 (Table 1).

Preparation of the Crude Extracts

The air-dried plant material (5 g) was powdered, delipidized with petroleum ether 40–60° and extracted twice with 30 ml of methanol. The extracts were filtered, concentrated *in vacuo* and weighed for the determination of the w/w yield. Extracts were kept at –20°C until use and dissolved in DMSO or DMSO: water, 1:1 to a concentration of 1 mg/ml for the biological tests.

Reagents

XOD (EC 1.1.3.22) from buttermilk (Grade III, 1.3 units/mg protein), xanthine, elastase (EC 3.4.21.36) from pig pancreas (PPE, Type IV, 70 units/mg prot.), N-suc-$(Ala)_2$-Pro-Leu-*p*-nitroanilide, 3,4-dichloroisocoumarin (3,4-DCI) and quercetin were obtained from Sigma Chemical Company. All other reagents and chemicals were from Merck and were of analytical grade.

Assay of XOD Activity

The enzyme activity was measured spectrophotometrically following the conversion of xanthine to uric acid at 295 nm for 3 min, as reported by Robak et al. (1988). The assay mixture contained 0.1 M K^+-phosphate buffer, pH 7.8, 10 μM EDTA, 0.1 mM xanthine, 0.04 units/ml XOD, final volume 1 ml. Crude extracts were tested at a concentration of 50 μg/ml. This concentration was selected on the basis of preliminary assays and according to Gonzales et al. (1995). Quercetin (10 μM) was used as reference inhibitor. The assay mixture was preincubated for 5 min at 25°C with the test material before adding the substrate. XOD inhibitory activity was expressed as the percentage of inhibition, calculated as (1-B/A) x 100 where A is the enzyme activity without the test material and B the enzyme activity with the test material.

Assay of PPE Activity

The method used was that described by Herbert et al. (1992). PPE activity was determined using N-Suc-$(Ala)_2$-Pro-Leu-*p*-nitroanilide as substrate. The assay mixture contained 0.3 mM substrate, 0.5 M NaCl, 100 mM Tris-HCl buffer, pH 8.8, 0.7 units/ml PPE, final volume 1 ml. Activity was determined by release of *p*-nitroaniline as indicated by the increase of optical density at A_{410nm} over 100 s at 25°C. Samples were preincubated with test materials for 5 min before adding the substrate. Crude extracts were tested at concentration of 50 μg/ml and inhibition was calculated as described for XOD activity. 3,4-DCI 5 μM was used as a reference inhibitor.

Statistical Analysis

Dose response curves were analyzed according to De Lean et al. (1978).

RESULTS AND DISCUSSION

Fifty-five crude extracts from 38 species of the flora of New Caledonia and 40 extracts from 22 species of the flora of Vanuatu were assayed for XOD and PPE inhibitory activity at a concentration of 50 μg/ml, and the results are shown in Table 1. Most of the crude extracts (82%) were found to possess XOD inhibitory activity and only a few (23%) were active against PPE. Plant materials collected in Vanuatu are far less active than the ones from New Caledonia. Among the extracts, the ones showing the highest activity against both the enzymes were from the bark of *Cunonia montana* Schlechter (Cunoniaceae) and the leaves of *Amyema scandens* Danser (Loranthaceae). PPE inhibition by *C. montana* and *A. scandens* was 84 ± 2.2 and 71 ± 7.5%, respectively (mean ± s.e., n = 4 assays,

two replications/assay); XOD inhibition was 85 and 88%, respectively (mean of two determinations). In our experimental conditions, XOD inhibition by quercetin 10 μM (3.4 μg/ml) was 76 ± 2.2% (mean ± s.e., n = 12 assays); PPE inhibition by 3,4-DCI 5 μM (1.1 μg/ml) was 73 ± 2.9 (mean ± s.e., n = 4 assays). Data from the literature reported IC_{50} for quercetin 10 μM (Robak, 1988) and IC_{50} for 3,4-DCI 8 μM (Powers & Kam, 1994).

Dose inhibition curves and IC_{50} values of the two extracts are shown in Fig. 1 and Table 2. The ability of the two extracts to inhibit elastase activity was comparable. In the dose-response experiments, it could be observed that total inhibition of the enzymes was never achieved. Maximal inhibition was in the range of 80–86%, in the presence of 100 μg/ml of extract. With the addition of larger amounts of extracts to XOD assay mixture, the degree of inhibition decreased, indicating a pro-oxidant effect. As far as PPE inhibition is concerned, there is a discrepancy between the results reported in Tables 1 and 2. The explanation for the lower inhibition found in the experiments for the IC_{50} determination was the change of the batch of elastase supplied by the producer. In other occasions we have observed inhibition depending on the batch of commercially available elastase (data not shown).

From these results it can be concluded that the bark of *Cunonia montana* Schlechter (Cunoniaceae) and the leaves of *Amyema scandens* Danser (Loranthaceae) are promising starting materials for the isolation of compounds with anti-elastase and anti-XOD activities, for application as anti-gout agents and in free radical and protease mediated tissue injuries.

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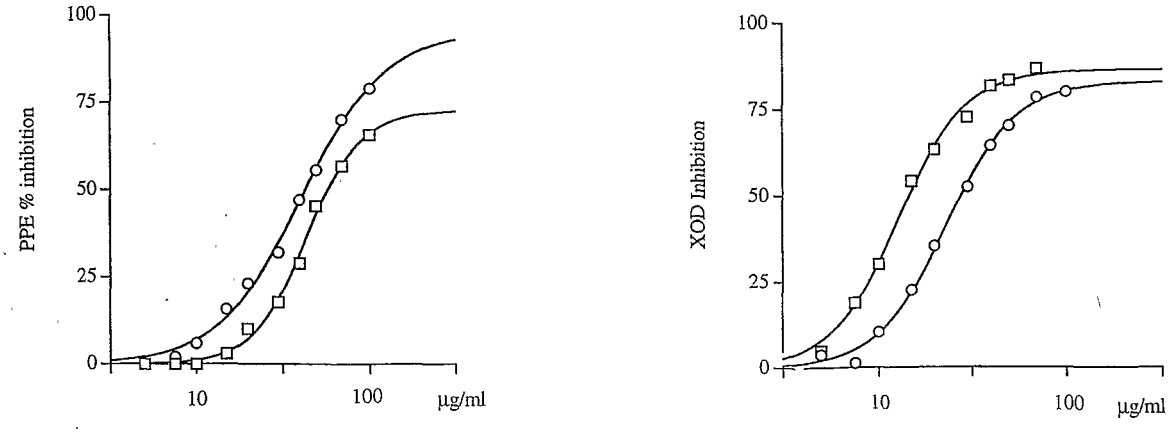

Fig. 1. Inhibition curves of PPE and XOD activities in the presence of increasing concentrations of *C. montana* (□) and *A. scandens* (○) extracts (5–100 mg/ml).

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Table 1. Extraction yield (% starting material, w/w) and enzyme inhibition.[d]

Family/Species voucher specimen	Plant part	Yield	XOD[a]	PPE[a]	
ARALIACEAE					
Schefflera plerandroides Vig. (DN 20)	leaves	15	12	0	
BURSERACEAE					
Canarium harveyi Seemann (PC 3152)	leaves	nd	56	<10	
	bark	6.1	21	<10	
	resin	56	0	0	
BURSERACEAE					
Canarium indicum L. (PC 3141)	woody endocarp	2.0	32	<10	
CASUARINACEAE					
Casuarina collina Poisson ex Sebert+Pancher (DN 37)	leaves	16	42	<10	
	bark	18	55	<10	
CASUARINACEAE					
Casuarina equisetifolia L. (PC 3134)	leaves	8.8	45	0	
	bark	16	62	<10	
CELASTRACEAE					
Peripterygia marginata Loes. (DN 10)	leaves	nd	40	<10	
	bark	12	33	<10	
CLUSIACEAE					
Calophyllum neoebudicum Guillaumin (PC 3171)	leaves	18	42	<10	
	bark	12	43	12	
CLUSIACEAE					
Garcinia pseudoguttifera Seemann (PC3160)	leaves	14	18	<10	
	bark	14	23	<10	
CLUSIACEAE					
Montrouziera cauliflora Planchon & Triana (DN 19)	bark	18	33	<10	
COMBRETACEAE					
Lumnitzera littorea (Jack) Voigt (PC 3137)	leaves	nd	63	18	
	bark	9.8	44	<10	
CYPERACEAE					
Baumea deplanchei Boeck.(DN 9)	whole plant	7.0	38	0	
CYPERACEAE					
Lepidosperma perteres C.B. Clarke (DN 38)	whole plant	10	33	<10	
EBENACEAE					
Diospyros vieillardii (Hiern) Kostermans (DN 31)	leaves	15	60	77	
CUNONIACEAE					
Cunonia montana Schlechter (DN 15)	leaves	9.4	51	<10	
	bark	7.8	**85**	**84**	**2.2** [c]
EPACRIDACEAE					
Dracophyllum verticillatum Labillardière (DN 22)	leaves	7.4	41	0	
EUPHORBIACEAE					
Austrobuxus carunculatus (Baillon) Airy Shaw (DN 34)	bark	8.0	42	0	
EUPHORBIACEAE					
Breynia disticha J.R. & G. Forster (DN 32)	leaves	14	35	16	
	bark	22	44	32	
EUPHORBIACEAE					
Breynia disticha J. R. & G. Forster (PC 3170)	leaves	9.1	37	<10	
	bark	9.5	45	25	
FABACEAE: Mimosoideae					
Acacia spirorbis Labillardière (DN36)	leaves	24	49	<10	
	bark	14	51	10	
FABACEAE: Papilionoideae					
Sophora oblongata Tsoong (PC3159)	leaves	14	0	<10	
	bark	12	0	<10	
FLACOURTIACEAE					
Casearia silvana Schlechter (DN 21)	leaves	nd	22	0	
	bark	2.4	13	<10	
LORANTHACEAE					
Amyema artense (Montrouzier) Danser (PC 3164)	leaves	22	30	14	
	bark	14	48	13	
LORANTHACEAE					
Amyema scandens Danser (DN 18)	leaves	10	**88**	**71**	**7.5** [c]
MELIACEAE					
Dysoxylum bijugum (Labillardière) Seemann (DN 5)	leaves	13	<10	0	
MYRSINACEAE					
Maesa ambrymensis Guillaumin (PC 3147)	leaves	nd	18	<10	
	bark	8.3	33	<10	

Table 1 continues

Table 1 (continued)

Family/Species voucher specimen	Plant part	Yield	XOD[a]	PPE[a]
MYRSINACEAE				
Maesa novacaledonica Mez (DN 7)	leaves	13	<10	<10
MYRTACEAE				
Syzygium nomoa Guillaumin (PC 3169)	leaves	17	26	0
	bark	12	43	0
MYRTACEAE				
Tristaniopsis callobuxus Brongniart & Gris (DN 17)	bark	7.1	56	51
OLACACEAE				
Ximenia americana L. (DN 2)	leaves	17	21	<10
	bark	22	62	60
ONCOTHECACEAE				
Oncotheca balansae Baillon (DN 11)	bark	7.9	60	37
PANDANACEAE				
Pandanus tectorius S. Parkinson ex Zucc. (PC 3135)	fruits	nd	17	30
PLUMBAGINACEAE				
Plumbago zeylanica L. (PC3173)	whole plant	6.2	19	0
PROTEACEAE				
Grevillea exul Lindley (DN 13)	leaves	6.9	22	<10
PROTEACEAE				
Grevillea gillivrayi Hook. &Arn. (DN 12)	leaves	8.2	nd	0
	bark	5.0	41	0
RANUNCULACEAE				
Clematis glycinoides A. DC. (PC 3145)	leaves	nd	<10	0
	branches	nd	0	0
RHAMNACEAE				
Alphitonia neocaledonica Guillaumin (DN 8)	leaves	19	41	<10
RHAMNACEAE				
Gouania richii A. Gray (PC 3140)	leaves	10	<10	0
RHIZOPHORACEAE				
Crossostylis grandiflora Brongniart & Gris (DN 14)	bark	7.7	12	0
RUBIACEAE				
Ixora cauliflora Beauvisage (DN 3)	leaves	19	37	0
RUBIACEAE				
Ixora collina Beauvisage (DN 33)	leaves	28	25	<10
RUBIACEAE				
Ixora francii Schlechter (DN 29)	leaves	22	35	23
RUBIACEAE				
Uncaria orientalis Guillaumin (PC 3150)	leaves	8.8	22	0
	bark	nd	32	0
RUTACEAE				
Acronychia laevis J.R.& G. Forster (DN 6)	leaves	nd	11	0
	bark	18	41	<10
RUTACEAE				
Myrtopsis novae-caledoniae (Viellard) Engler (DN 28)	leaves	12	37	17
SANTALACEAE				
Elaphanthera baumannii (Stauffer) Hallé(DN 23)	leaves	26	42	<10
	bark	6.3	38	0
SANTALACEAE				
Exocarpos neocaledonicus Schlechter & Pilger (DN 27)	leaves	31	38	<10
	bark	19	54	0
SAPINDACEAE				
Elattostachys falcata (A. Gray) Radlkofer (PC3149)	leaves	8.0	25	<10
	bark	6.0	22	13
	fruits	9.3	44	0
SAPINDACEAE				
Guioa glauca var. glauca (Labillardière) Radlkofer (DN 16)	leaves	6.8	50	47
	bark	15	54	28
SAPINDACEAE				
Guioa villosa Radlk. (DN 24)	leaves	12	51	76
	bark	12	51	0
SAPINDACEAE				
Pometia pinnata J. R. & G. Forster (PC3177)	leaves	12	28	0
	bark	12	28	<10
SAPOTACEAE				
Beccariella baueri (Montrouzier) Aubréville (DN 25)	leaves	20	30	<10

Table 1 continues

Table 1 (continued)

Family/Species voucher specimen	Plant part	Yield	XOD[a]	PPE[a]
SAPOTACEAE				
Beccariella seberti (Pancher) Pierre (DN 26)	leaves	14	0	<10
	bark	11	38	0
SAPOTACEAE				
Mimusops elengi L.*var. parvifolia*	leaves	20	66	<10
(R. Brown) H. J. Lam (PC 3158)	bark	26	39	0
STERCULIACEAE				
Commersonia bartramia (L.) Merril (PC3161)	leaves	8.8	44	<10
	bark	6.0	38	0
THYMELAEACEAE				
Lethedon balansae (Baillon) Kostermans (DN 4)	bark	18	<10	0
TILIACEAE				
Grewia crenata Schinz & Guillamin (PC3176)	leaves	nd	0	0
	bark	3.7	<10	0
ULMACEAE				
Trema orientalis (L.) Blume (DN 35)	leaves	18	61	<10
	bark	16	<10	66

[a] Enzyme inhibition (% of control) was evaluated at concentration of 50 μg/ml of crude extract. In control samples, Δ Abs was 0.280 ± 0.009/min for PPE activity (mean ± s.e., n = 15); Δ Abs was 0.312 ± 0.005/min for XOD activity (mean ± s.e.; n = 10).
[b] nd = not determined
[c] mean ± s.e of 4 assays, two replications/assay
[d] Under the described experimental conditions quercetin 10 μM gave an inhibition of the XOD activity of 75 ± 2.2% (mean ± s.e., n = 12 assays); PPE inhibition by 3,4-DCI 5μM was 73 ± 2.9 (mean ± s.e., n = 4 assays)

Table 2. IC_{50} values (μg/ml) for XOD and PPE inhibition.

Plant	XOD	PPE
C. montana bark	23 ± 0.82	41 ± 3.5
A. scandens leaves	13 ± 0.48	44 ± 2.2

The data are the mean ± s.e. of 3 assays, two replications/assay.