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Bruno Fogliani, Saliou Bouraïma-Madjèbi, René Pineau, Pierre Cabalion. Screening of Fifty Cunoniaceae Species from New Caledonia for Inhibitors of Xanthine Oxidase and Scavengers of Superoxide Anions. *Pharmaceutical biology*, 2002, 40 (7), pp.526-533. 10.1076/phbi.40.7.526.14690 . hal-02912882

HAL Id: hal-02912882

<https://unc.hal.science/hal-02912882>

Submitted on 7 Aug 2020

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To cite this article: Bruno Fogliani, Saliou Bouraïma-Madjebi, René Pineau & Pierre Cabalion (2002) Screening of Fifty Cunoniaceae Species from New Caledonia for Inhibitors of Xanthine Oxidase and Scavengers of Superoxide Anions, *Pharmaceutical Biology*, 40:7, 526-533, DOI: [10.1076/phbi.40.7.526.14690](https://doi.org/10.1076/phbi.40.7.526.14690)

To link to this article: <https://doi.org/10.1076/phbi.40.7.526.14690>



Published online: 29 Sep 2008.



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Screening of Fifty *Cunoniaceae* Species from New Caledonia for Inhibitors of Xanthine Oxidase and Scavengers of Superoxide Anions

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Abstract

Two-hundred-and-four extracts (101 ethyl acetate extracts and 103 methanol extracts) from 50 species of *Cunoniaceae* from New Caledonia were screened for antioxidant properties due to free radical scavenging and/or xanthine oxidase (XOD) inhibitory activity. Of the crude extracts, 95.1% were active at a concentration of 50 µg/ml. Fifteen (27.8%) extracts showed more than 50% activity at 10 µg/ml and were studied for XOD inhibition. All were active against XOD at 50 µg/ml but only 6 (40%) showed up to 60% inhibition. Ethyl acetate extracts from the bark of *Weinmannia dichotoma* Brongniart & Gris, *Weinmannia monticola* Däniker and *Cunonia linearisepala* (Guillaumin) Bernardi and from the roots of *Codia incrassata* Pampanini, as well as methanol extract from the bark of *Pancheria brunhesii* Pampanini, exhibited the highest activities (between 70% and 86% XOD inhibition at 50 µg/ml). In view of these preliminary results, New Caledonian *Cunoniaceae* species appears to be promising material for the isolation of bioactive compounds.

Keywords: *Cunoniaceae*, ethyl acetate extract, free radical scavengers, methanol extract, New Caledonia, xanthine oxidase.

Introduction

In general, the biodiversity of New Caledonia is rich and unique. The flora, in particular, shows up to 80% endemism (Jaffre et al., 1995), due to the Island's long geographical isolation after physical separation from Australia about 65 million years ago.

The flowering plant family Cunoniaceae, in the order Rosales (Cronquist, 1982), comprises 26 genera including more than 250 species (Heywood, 1985). It occurs largely in the southern hemisphere and is an example of this rich endemism, since in New Caledonia, it contains 6 genera, *Acsmithia*, *Codia*, *Cunonia*, *Geissois*, *Pancheria* and *Weinmannia*, represented by more than 80 species, all of which are endemic (Guillaumin, 1948). Moreover, two of these genera, *Codia* and *Pancheria*, are endemic with 36 species all together.

Some members of the Cunoniaceae are reputed to have medicinal properties and are used to cure various diseases (Cambie & Ash, 1931; Rageau, 1957; Luis Diego Gomez, 1995). Because of this and the results of Bosisio et al. (2000), who showed that *Cunonia montana* Schlechter has anti-elastase and anti-xanthine oxidase activities, our laboratory decided to investigate several biological activities in this family, such as antibiotic effects, cytotoxicity and inhibition of different enzymes. Preliminary results show that some species present properties in all of these domains (Fogliani et al., 2000).

Xanthine oxidase (XOD) converts hypoxanthine to xanthine and finally to uric acid, the accumulation of which causes hyperuricacidemia associated with gout (Noro et al., 1983a; Hayashi et al., 1989). It is also responsible for oxidative damage to living tissues under conditions such as allergies, inflammation, diabetes, emphysema, heart ischemia-reperfusion, aging, atherosclerosis, etc. (Crastes de Poulet et al., 1994). Thus, free radical scavengers and/or specific inhibitors of XOD are expected to be therapeutically

Accepted: April 19, 2002

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useful for the treatment of these pathologies (Goodman & Gilman, 1990). Plant compounds such as xanthenes, flavonoids, coumarins and proanthocyanidins have been reported to be potent inhibitors of xanthine oxidase (Sumahara et al., 1977; Noro et al., 1983b) and to possess anti-oxidant properties.

For these reasons, 50 species of *Cunoniaceae* from New Caledonia were assayed on XOD, using hypoxanthine and then xanthine as substrates. The objective was to select species with high activity and then to submit the active extracts for detailed phytochemical investigations in order to isolate the bioactive constituents.

Materials and methods

Plant material

Material was collected by Bruno Fogliani from localities in New Caledonia at different seasons but always from plants in flower or fruit. All accessible parts were sampled, including leaves, bark, flowers, fruits and roots, and herbarium voucher specimens were made. Identifications were made by comparison with named collections in the herbarium at the Institut de la Recherche pour le Développement (I.R.D.), Nouméa, and some were verified by Helen Fortune Hopkins (Lancaster University) and Jason Bradford (Missouri Botanical Garden) who are currently revising some genera of New Caledonian *Cunoniaceae*. Voucher specimens were deposited in our laboratory (L.B.P.V.A.) in the University of New Caledonia. Samples are referred to as BF 1–90.

Preparation of the crude extracts

Two samples of 10 g each of the air-dried powdered plant material were extracted separately by maceration for 24 h with ethyl acetate (100 ml) and methanol (100 ml). After filtration, the extracts were concentrated *in vacuo* at 45 °C and weighed for the determination of the w/w yield. The residue was dissolved in methanol to a concentration of 1 mg/ml for the tests.

Test solutions

Crude extracts in methanol were diluted in buffer to obtain the desired final concentrations of 50, 10 and 1 µg/ml in the reaction mixture as described by Gonzales et al. (1995).

Reagents

XOD (EC 1.1.3.22) from buttermilk (Grade III, 1.1 units/mg protein), hypoxanthine, xanthine, nitroblue tetrazolium, quercetin and all other reagents and chemicals were purchased from the Sigma Chemical Company. In the assay with hypoxanthine substrate, all products were dissolved and diluted with 0.05 M Tris-HCl buffer, pH 7.5, while in the case

of xanthine substrate, this was done in 0.1 M K⁺ phosphate buffer, pH 7.8.

Activity assay with hypoxanthine as substrate

At first, all the extracts were tested with hypoxanthine as substrate to find XOD inhibitory activity and/or antioxidant properties due to free radical scavenging. This phenomenon was measured spectrophotometrically following the conversion of nitroblue tetrazolium (NBT) to formazan, at 560 nm for 3 min, as reported by Bors et al. (1989).

The assay mixture consisted of 100 µl test solution, 500 µl of hypoxanthine (5 µM), and 100 µl of NBT (1 mM). After preincubation of the mixture at 25 °C for 15 min, the reaction was initiated by adding 100 µl of enzyme solution (1.67 U/ml) and stopped after 3 min of incubation by adding 100 µl of HCl (1 N). Variation of absorbance was measured against a blank prepared in the same way except that the enzyme solution was added to the assay mixture after adding HCl. Percent activity was calculated according to the following formula: $(1-B/A) \times 100$, where A is the absorbance without the test material and B the absorbance with the test material. All crude extracts with an activity up to 60% at 50 µg/ml were then tested at concentrations of 10 and 1 µg/ml. Extracts showing an activity up to 50% at 10 µg/ml were then assayed with xanthine as the substrate, in order to determine whether the activity was due to xanthine oxidase inhibition.

XOD activity assay

The enzyme activity was measured spectrophotometrically at 295 nm following the conversion of xanthine to uric acid for 3 min as reported by Robak et al. (1988), with the following modifications. The assay mixture contained 0.1 M K⁺-phosphate buffer, pH 7.8, 10 µM EDTA, 0.1 mM xanthine and 0.04 units/ml XOD, with a final volume of 1 ml. This mixture was preincubated for 15 min at 25 °C with the test material before adding the substrate. The reaction was stopped by adding 100 µl of 1 N HCl and the variation of absorbance was measured against a blank prepared in the same way except that the enzyme solution was added to the assay mixture after adding HCl. XOD inhibitory activity was expressed as the percentage of inhibition calculated as $(1-B/A) \times 100$, as described above. The selected extracts were tested at 50 µg/ml and extracts with activity up to 60% were then tested at 10 and 1 µg/ml. Quercetin (3.4 µg/ml) was used as reference inhibitor.

Results and discussion

Two hundred and four extracts from 50 species of New Caledonian *Cunoniaceae* were assayed for anti-oxidant properties due to free radical scavenging and/or XOD inhibitory activity at a concentration of 50 µg/ml. The results are shown in Table 1. Of all the extracts assayed, 194 (95.1%) demonstrated an activity at 50 µg/ml. Among these, 54 (26.5%) extracts showed an activity above 60% at that concentration.

Table 1. Extraction yield (% starting material, w/w) and % of activity.

Genera/Species voucher specimen	Plant part	Extract	Yield	Activity (%) ^a		
				50 µg/ml	10 µg/ml	1 µg/ml
<i>Acsmithia</i>						
<i>Acsmithia elliptica</i> (Vieillard ex Pampanini) Hoogland (BF 58)	Leaves	AcOEt	2.3	50.1		
		MeOH	14.4	54.5		
	Bark	AcOEt	2.3	28.6		
		MeOH	17.1	64.4	26	1.4
<i>Acsmithia meridionalis</i> Hoogland (BF 70)	Leaves	AcOEt	6.6	10.2		
		MeOH	7.3	31.4		
	Bark	AcOEt	3.3	18.9		
		MeOH	5.7	41.7		
<i>Acsmithia pedunculata</i> (Schlechter) Hoogland (BF 69)	Leaves	AcOEt	1.9	19.1		
		MeOH	5.2	32.1		
	Bark	AcOEt	1.5	10.3		
		MeOH	4	40.3		
		MeOH	4	40.3		
<i>Acsmithia pubescens</i> Pampanini (BF 34)	Leaves	AcOEt	2.8	30.7		
		MeOH	13	34.1		
	Bark	AcOEt	3.8	23.9		
		MeOH	16.7	54.6		
		MeOH	16.7	54.6		
<i>Codia</i>						
<i>Codia albifrons</i> Vieillard ex Guillaumin (BF 83)	Leaves	AcOEt	2.5	12.6		
		MeOH	8.5	37.5		
	Bark	AcOEt	0.5	47.9		
		MeOH	4.7	37.8		
<i>Codia arborea</i> Brongniart ex Guillaumin (BF 14)	Leaves	AcOEt	0.9	31		
		MeOH	7.2	64.9	18.1	8.4
	Bark	AcOEt	0.7	81.1	78.2	45.5
		MeOH	4.2	73.7	18.7	12.5
		MeOH	4.2	73.7	18.7	12.5
<i>Codia discolor</i> (Brongniart & Gris) Guillaumin (BF 55)	Leaves	AcOEt	1.9	62.4	16.8	0
		MeOH	11.6	72.1	39.5	9.7
	Bark	AcOEt	1.7	47.6		
		MeOH	16.3	77.5	35.6	8.8
	Flowers	AcOEt	0.5	32.1		
		MeOH	3.4	40		
<i>Codia ferruginea</i> Brongniart & Gris (BF 88)	Leaves	AcOEt	2.3	1.2		
		MeOH	9.2	74.6	18	3.8
	Bark	AcOEt	0.7	68.8	6.7	0
		MeOH	8.2	46.3		
		MeOH	8.2	46.3		
<i>Codia incrassata</i> Pampanini (BF 36)	Leaves	AcOEt	1.3	9.4		
		MeOH	15.4	40.3		
	Bark	AcOEt	1.1	54.1		
		MeOH	11.6	70.5	42.6	14.2
	Roots	AcOEt	1.2	62.1	50.2	0
		MeOH	9.3	77.8	26.9	10.4
<i>Codia montana</i> Forster (BF 37)	Leaves	AcOEt	1.7	56.9		
		MeOH	9	77.9	47.2	9.5
	Bark	AcOEt	1.7	55.1		
		MeOH	4	76.1	20.2	0
	Roots	AcOEt	1.1	76.3	34.7	0
		MeOH	13.5	67.9	12.1	0
<i>Codia nitida</i> Schlechter (BF 62)	Leaves	AcOEt	1.8	49		
		MeOH	16	72.8	52.2	28.5
	Bark	AcOEt	1.1	59.7		
		MeOH	10.2	73.6	50.4	28.5
<i>Codia sp nov.</i> (BF 84)	Leaves	AcOEt	1.1	13.5		
		MeOH	12.9	41.3		
	Bark	AcOEt	1.5	12.5		
		MeOH	5.8	80.9	23.9	0
		MeOH	5.8	80.9	23.9	0

Table 1. Continued

Genera/Species voucher specimen	Plant part	Extract	Yield	Activity (%) ^a		
				50 µg/ml	10 µg/ml	1 µg/ml
<i>Cunonia</i>						
<i>Cunonia alticola</i> Guillaumin (BF 59)	Leaves	AcOEt	2.1	51.5		
		MeOH	28.4	52.3		
	Bark	AcOEt	0.9	50.8		
		MeOH	9.5	53.1		
<i>Cunonia aoupiniensis</i> Hoogland (Lit 048*)	Leaves	AcOEt	2.9	21.6		
		MeOH	2.4	48.5		
<i>Cunonia atrorubens</i> Schlechter (BF 52)	Leaves	AcOEt	3.9	53.6		
		MeOH	12.5	44.6		
	Bark	AcOEt	2.2	18.1		
		MeOH	13	52.9		
<i>Cunonia austrocaledonica</i> Brongniart & Gris ex Guillaumin (BF 79)	Leaves	AcOEt	2.3	41.8		
		MeOH	9	26.4		
	Bark	AcOEt	0.9	72.6	50.3	44.7
		MeOH	0.6	48.7		
<i>Cunonia bullata</i> Brongniart & Gris (BF 67)	Leaves	AcOEt	4	14.2		
		MeOH	4.3	22.1		
	Bark	AcOEt	1.7	12.9		
		MeOH	3.2	26.8		
<i>Cunonia balansae</i> Brongniart & Gris (BF 54)	Leaves	AcOEt	1.7	37.6		
		MeOH	9.7	61.1	0	0
	Bark	AcOEt	1.3	33		
		MeOH	9.8	75.1	23.5	2.8
	Fruit	AcOEt	1.4	43.8		
		MeOH	5.3	85.2	28.4	3.1
<i>Cunonia deplanchei</i> Brongniart & Gris (BF 17)	Leaves	AcOEt	3.9	37.8		
		MeOH	5.6	11.4		
	Bark	AcOEt	0.7	9.3		
		MeOH	2.8	77.4	20.9	0
<i>Cunonia lenormandii</i> Viellard ex Brongniart & Gris (BF 66)	Leaves	AcOEt	1.4	8.7		
		MeOH	7.6	0.1		
	Bark	AcOEt	4.1	10.8		
		MeOH	2.2	28		
<i>Cunonia linearisepala</i> (Guillaumin) Bernardi (BF 81)	Leaves	AcOEt	2.6	20.1		
		MeOH	8.8	51.5		
	Bark	AcOEt	1.9	88.7	50.9	9.8
		MeOH	3.2	53.6		
<i>Cunonia macrophylla</i> Brongniart & Gris (BF 53)	Leaves	AcOEt	2.4	37.6		
		MeOH	18	76.9	27.8	10.3
	Bark	AcOEt	0.7	30.4		
		MeOH	13	76.1	20.6	0
	Flowers	AcOEt	2.3	61.8	9.2	2.2
		MeOH	17.5	88.7	27.1	25.6
<i>Cunonia montana</i> Schlechter (BF 57)	Leaves	AcOEt	0.7	48.3		
		MeOH	10.2	52.8		
	Bark	AcOEt	1.1	54.5		
		MeOH	16.4	57.1		
<i>Cunonia pulchella</i> Brongniart & Gris (BF 35)	Leaves	AcOEt	1.1	47.4		
		MeOH	8	16.8		
	Bark	AcOEt	0.6	52.3		
		MeOH	11	56.7		
<i>Cunonia pterophylla</i> Schlechter (BF 13)	Leaves	AcOEt	0.8	25.9		
		MeOH	3.9	49.1		
	Bark	AcOEt	0.6	21.1		
		MeOH	4.7	70.8	13	12.5

Table 1. Extraction yield (% starting material, w/w) and % of activity.

Genera/Species voucher specimen	Plant part	Extract	Yield	Activity (%) ^a		
				50 µg/ml	10 µg/ml	1 µg/ml
<i>Cunonia purpurea</i> Brongniart & Gris (BF 48)	Leaves	AcOEt	1.3	7.9		
		MeOH	10	13.9		
	Bark	AcOEt	1.4	78.3	54.6	35.9
MeOH		10	51			
<i>Cunonia rotundifolia</i> Däniker (BF 68)	Leaves	AcOEt	0.8	0		
		MeOH	7.1	78.9	50.2	9.7
<i>Cunonia varijuga</i> Hoogland (BF 28)	Leaves	AcOEt	0.7	52.2		
		MeOH	7.5	46.9		
	Bark	AcOEt	0.3	22.9		
MeOH		9.4	50.4			
<i>Cunonia vieillardii</i> Brongniart & Gris (BF 89)	Leaves	AcOEt	4.3	0		
		MeOH	10.6	87.6	27.9	5.5
	Bark	AcOEt	0.5	29.2		
MeOH		3	51.6			
<i>Geissois</i>						
<i>Geissois hippocastaneifolia</i> Guillaumin (BF 23)	Leaves	AcOEt	1.5	18.6		
		MeOH	18	31.3		
	Bark	AcOEt	0.4	20.7		
MeOH		14.5	51.6			
<i>Geissois hirsuta</i> Brongniart & Gris (BF 29)	Leaves	AcOEt	1.4	0		
		MeOH	3	39.3		
	Bark	AcOEt	3.1	75.6	28.9	1.6
MeOH		3.4	56.6			
<i>Geissois polyphylla</i> Lecard ex Guillaumin (BF 38)	Leaves	AcOEt	1.4	1.1		
		MeOH	8.5	24.1		
	Bark	AcOEt	0.4	55.3		
MeOH		10.2	54			
<i>Geissois pruinosa</i> Brongniart & Gris (BF 15)	Leaves	AcOEt	2.5	30.6		
		MeOH	4.8	36		
	Bark	AcOEt	0.4	34		
MeOH		5.9	49			
<i>Geissois magnifica</i> Baker (BF 24)	Leaves	AcOEt	1.1	30.1		
		MeOH	8.5	45.5		
	Bark	AcOEt	0.7	26.3		
MeOH		12	63.7	20	6.3	
<i>Weinmannia</i>						
<i>Weinmannia dichotoma</i> Brongniart & Gris (BF 40)	Leaves	AcOEt	4.4	36.8		
		MeOH	12.7	59.9		
	Bark	AcOEt	5.9	95.9	54.1	32.8
MeOH		14.4	53.8			
<i>Weinmannia monticola</i> Däniker (BF 39)	Leaves	AcOEt	5.2	43.2		
		MeOH	13.7	54		
	Bark	AcOEt	6.6	91.9	67.9	16.9
MeOH		13.6	58.7			
<i>Pancheria</i>						
<i>Pancheria alaternoides</i> Brongniart & Gris (BF 12)	Leaves	AcOEt	1.7	10.1		
		MeOH	12.9	60.6	9.6	0
	Bark	AcOEt	0.8	8.1		
MeOH		11.3	71.2	15.2	0	
<i>Pancheria brunhesii</i> Pampanini (BF 16)	Leaves	MeOH	4.4	50.7		
	Bark	MeOH	3.8	87.2	50.8	10.5
<i>Pancheria communis</i> Baker (BF 82)	Leaves	AcOEt	2.7	14.7		
		MeOH	8.1	41.9		
	Bark	AcOEt	0.6	24.2		
MeOH		6.5	33.9			

Table 1. Continued

Genera/Species voucher specimen	Plant part	Extract	Yield	Activity (%) ^a		
				50 µg/ml	10 µg/ml	1 µg/ml
<i>Pancheria confusa</i> Guillaumin (BF 21)	Leaves	AcOEt	1.3	10.5		
		MeOH	13.6	39.7		
	Bark	AcOEt	1.3	12.4		
MeOH		12.1	68.7	31	9	
<i>Pancheria elegans</i> Brongniart & Gris (BF 78)	Leaves	AcOEt	3.9	4.8		
		MeOH	10.3	27.4		
	Bark	AcOEt	1.1	0		
MeOH		3.8	29.6			
<i>Pancheria elliptica</i> Pampanini (BF 20)	Leaves	AcOEt	3.9	37.8		
		MeOH	11.9	65	27.2	0.6
	Bark	AcOEt	1.3	29.5		
MeOH		10.2	92.1	33.9	5.5	
AcOEt		1.8	43.2			
<i>Pancheria engleriana</i> Schlechter (BF 56)	Leaves	MeOH	9.7	47.4		
		AcOEt	0.7	63.1	31.7	2.3
	Bark	MeOH	13.2	68.1	13.7	0
AcOEt		2.9	36.6			
<i>Pancheria ferruginea</i> Brongniart & Gris (BF 61)	Leaves	MeOH	15.3	75.7	50.7	15.7
		AcOEt	1.6	68.5	34.7	0
	Bark	MeOH	11.3	79	56.4	39.9
AcOEt		3.3	16.1			
<i>Pancheria hirsuta</i> Vieillard ex Pampanini (BF 22)	Leaves	MeOH	12.6	43.4		
		AcOEt	0.5	55.6		
	Bark	MeOH	2.2	21.9		
AcOEt		4	14.4			
<i>Pancheria obovata</i> Brongniart & Gris (BF 51)	Leaves	MeOH	10	43.1		
		AcOEt	0.9	80.2	50.1	26.6
	Bark	MeOH	10	33.6		
AcOEt		6.3	0			
<i>Pancheria phylliraeoides</i> Brongniart & Gris ex Guillaumin (BF 85)	Leaves	MeOH	11.2	52.3		
		AcOEt	0.5	62.4	10.7	0
	Bark	MeOH	12.7	49.4		
AcOEt		4.3	20.7			
<i>Pancheria reticulata</i> Guillaumin (BF 60)	Leaves	MeOH	19.7	49.9		
		AcOEt	0.6	89.2	22.8	15.2
	Bark	MeOH	13.4	71.4	50.49	0
AcOEt		5.8	0			
<i>Pancheria sebertii</i> Guillaumin (BF 86)	Leaves	MeOH	9.2	59.5		
		AcOEt	1.5	72.9	18.8	5.1
	Bark	MeOH	5.5	51.3		
AcOEt		5.2	1.1			
<i>Pancheria vieillardii</i> Brongniart & Gris (BF 87)	Leaves	MeOH	7.9	55.9		
		AcOEt	1.5	69	11.1	4.7
	Bark	MeOH	8	38		
AcOEt						

^a Mean 9 determinations. In control samples, Δ_{abs} was $0.303 \pm 0.05/\text{min}$ for activity with hypoxanthine as substrate (mean \pm s.e.; $n = 20$).

* plant powder from Mr Marc Litaudon, C.N.R.S center of Noumea.

All of these were then tested at 10 and 1 µg/ml. At 10 µg/ml, 53 (98.1%) of the extracts were active; 15 (27.8%) showed activity over 50%. At 1 µg/ml, 36 (66.6%) were still active.

It is clear from Table 1 that the genera *Acsmithia* and *Geissois* are the least interesting for further studies. In con-

trast, the endemic genera *Codia* and *Pancheria* appear to be good potential sources of bioactive compounds, as an activity of over 60% at 50 µg/ml was showed by, respectively, 87.5 and 75% of the species tested. These values represent 18 (47.4%) of the extracts from *Codia* species and 15 (32.6%)

Table 2. Extraction yield (% starting material, w/w) and XOD inhibition.^a

Genera/Species voucher specimen	Plant Part	Extract	Yield	Inhibition (%) ^b			
				50 µg/ml	10 µg/ml	1 µg/ml	IC ₅₀
Codia							
<i>Codia arborea</i> Brongniart ex Guillaumin (BF 14)	Bark	AcOEt	0.7	44.1			
<i>Codia incrassata</i> Pampanini (BF 36)	Roots	AcOEt	1.2	69.9	17.1	0.8	41.8
<i>Codia nitida</i> Schlechter (BF 62)	Leaves	MeOH	16	50.6			
	Bark	MeOH	10.2	49.4			
Cunonia							
<i>Cunonia austrocaledonica</i> Brongniart & Gris ex Guillaumin (BF 79)	Bark	AcOEt	0.9	34.6			
<i>Cunonia linearisepala</i> (Guillaumin) Bernardi (BF 81)	Bark	AcOEt	1.9	81.7	10.2	1.8	33.1
<i>Cunonia purpurea</i> Brongniart & Gris (BF 48)	Bark	AcOEt	1.4	38.2			
<i>Cunonia rotundifolia</i> Däniker (BF 68)	Leaves	MeOH	7.1	62.1	0	0	nd
Weinmannia							
<i>Weinmannia dichotoma</i> Brongniart & Gris (BF 40)	Bark	AcOEt	5.9	82.2	18.9	2.8	22.4
<i>Weinmannia monticola</i> Däniker (BF 39)	Bark	AcOEt	6.6	86.1	19.2	3.4	30.2
Pancheria							
<i>Pancheria brunhesii</i> Pampanini (BF 16)	Bark	MeOH	3.8	72.1	10.6	1.5	28.7
<i>Pancheria ferruginea</i> Brongniart & Gris (BF 61)	Leaves	MeOH	15.3	30.3			
<i>Pancheria obovata</i> Brongniart & Gris (BF 51)	Bark	MeOH	11.3	31.7			
<i>Pancheria reticulata</i> Guillaumin (BF 60)	Bark	AcOEt	0.9	31.5			
	Bark	MeOH	13.4	54.1			

^aUnder the described experimental conditions, quercetin 10 µM (3.4 µg/ml) gave an inhibition of 58.8 ± 2.2% (mean ± s.e.; n = 12 assays).

^bMean of nine determinations. In control samples, Δabs was 0.251 ± 0.05/min for XOD activity (mean ± s.e.; n = 20 assays).

of those from *Pancheria* species. The genus with the highest activities, *Weinmannia*, was represented here by two species among the five existing in New Caledonia, although Hopkins (1998) considered there are only four species and placed *Weinmannia monticola* Däniker into synonymy with *Weinmannia dichotoma* Brongniart & Gris. Both species of *Weinmannia* tested here gave similar results, which may perhaps support this conclusion.

At this stage of our investigations, we arbitrarily decided that we should only conserve the 15 extracts with activity up to 50% at 10 µg/ml for further studies. All of them were tested for XOD inhibitory activity at 50 µg/ml; results are shown in Table 2. All demonstrated XOD inhibitory activity, but only 6 (40%) showed enzyme inhibition of over 60% at this concentration. The nine less active extracts probably possessed both free radical scavengers and inhibitors of xanthine oxidase.

The six with the highest XOD inhibitory activities were then tested at 10 and 1 µg/ml. Five of them were active at these concentrations; their IC₅₀ values were calculated and are given in Table 2. It is interesting to note that except for the extract obtained from the roots of *Codia incrassata* Pampanini, all are extracts of bark, and three are ethyl acetate extracts.

It thus appears that five species, *Codia incrassata* Pampanini, *Cunonia linearisepala* (Guillaumin) Bernardi, *Weinmannia dichotoma* Brongniart & Gris, *Weinmannia monticola* Däniker and *Pancheria brunhesii* Pampanini, are encouraging starting materials for the further isolation of compounds with anti-XOD activities. In our experimental conditions, XOD inhibition by 10 µM (3.4 µg/ml) quercetin was 58.8 ± 2.2% (mean ± s.e.; n = 12 assays), while the IC₅₀ for quercetin has been reported as 10 µM (Robak et al., 1988). In comparison, our extracts from the five species cited above,

showed XOD inhibitory activities between 70 and 86% at a concentration of 50 µg/ml.

In conclusion, based on these results, Cunoniaceae from New Caledonia seem to be a very promising source for the isolation of active molecules, through a bioassay guided fractionation, that could be used from a therapeutic perspective against gout and in the treatment of free radical tissues injuries.

Acknowledgements

We are grateful to the Province Sud de Nouvelle-Calédonie for financial support of this project and for the grant provided to Mr. Bruno Fogliani. We thank Mr. Jean-Marie Veillon and Mr. Tanguy Jaffre for their help on the primary identification of plants. Special thanks are due to Ms. Valérie Médevielle for her technical assistance and Ms. Danielle Saintpierre for her help with the translation of this manuscript.

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