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Antiinflammatory and antioxidant activity of plants used in traditional medicine in Ecuador

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Abstract

Ethanolic extracts from 15 plant species, representing eight different families, used in traditional medicine in Ecuador were evaluated for antiinflammatory and antioxidant activities. *Conyza floribunda*, *Eupatorium articulatum*, *Bonafousia longituba*, *Bonafousia sananho*, *Tagetes pusilla* and *Piper lenticellosum* extracts showed a significant antiinflammatory activity in vivo in the carrageenan-induced paw oedema model in mice. The extracts were also tested in vitro for their ability to inhibit lipid peroxidation and to scavenge superoxide and hydroxyl radicals. *E. articulatum* extract possesses both activities. *Baccharis trinervis*, *E. articulatum* and *Phytolacca rivinoides* extracts were active as antioxidants.

Keywords: Antiinflammatory activity; Radical scavenger; Lipid peroxidation; Traditional medicine; Ecuadorian plants

1. Introduction

The use of traditional medicine is widespread and plants still present a large source of structurally novel compounds that might serve as leads for the development of novel drugs. The present investigation represents a preliminary screening in an ongoing programme on plants used in traditional medicine in Ecuador in the treatment of different pathologies as antiinflammatory and digestive agents, although other uses have also been suggested (Basten, 1981; Gupta, 1995).

Plants collected were: *Baccharis trinervis* Pers., *Conyza floribunda* H. B and K., *Eupatorium articulatum* Hort., *Eupatorium glutinosum* Lam., *Neurolaena lobata* R. Br., *Tagetes pusilla* H. B and K. (Asteraceae); *Bonafousia sananho* Ruiz and Pav.,

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Bonafousia longituba Macbride. (Apocynaceae); *Croton mentodorum* Benth. (Euphorbiaceae), *Heisteria acuminata* Engl. (Olecaceae), *Marsdenia condurango* Nichols. (Asclepidaceae), *Phytolacca rivinoides* Kunth and Bouché, *Phytolacca bogotensis* H. B and K. (Phytolaccaceae), *Piper lenticellosum* C. D. C. (Piperaceae) and *Urena lobata* Linn. (Malvaceae).

A survey of the literature revealed that no studies on the potential antiinflammatory and antioxidant activities of these plants have been undertaken. The present paper deals with the search for antiinflammatory and antioxidant activity of the ethanolic extracts obtained from these plants in order to validate their popular use.

2. Materials and methods

2.1. Plant collection and extracts

The plant material was collected based on information about its use in folk medicine, in the flowering season in different geographical locations of Ecuador. Botanical samples were authenticated by Dr Chiriboga, and voucher specimens are deposited in the Botany Department Herbarium, School of Biochemistry and Pharmacy, at the Central University of Quito, Ecuador.

Each plant material was sun dried for 5–7 days and pulverised. A total of 50 g of plant was extracted with dichloromethane (12 h). The residue was extracted with ethanol 96% during 12 h, the suspension filtered, and the residue percolated with ethanol. The resultant extract was dried under reduced temperature and pressure using a rotary evaporator. The collection numbers and the percentage yields, in terms of w/w dry starting are: 0039 *B. trinervis* (2.6%), 0048 *C. floribunda* (2.4%), 0057 *E. articulatum* (2.1%), 0026 *E. glutinosum* (2.0%), 0045 *N. lobata* (2.0%), 0021 *T. pusilla* (2.5%), 0005 *B. sananho* (5.6%), 0035 *B. longituba* (2.4%), 0042 *C. mentodorum* (2.0%), 0034 *H. acuminata* (6.1%), 0067 *M. condurango* (10.0%), 0068 *P. rivinoides* (14.0%), 0025 *P. bogotensis* (10.0%), 0063 *P. lenticellosum* (4.6%), and 0054 *U. lobata* (5%).

2.2. In vivo antiinflammatory activity

Antiinflammatory activity was determined by the method of Winter et al. (1962), modified by Sugishita et al. (1981). Animals (female Swiss mice weighing 20 ± 5 g) were divided into groups of ten and had access to food and water ad libitum. Oedema was induced by subcutaneous injection of 0.05 ml of a solution of 3% w/v carrageenan type IV (Sigma) in 0.9% saline solution into the subplantar region of the left hind paw. The volume of each paw, up to the tibiotarsal articulation, was measured plethysmographically before the injection of carrageenan, and 3, 5, and 7 h later. The vehicle (Tween 80:ethanol:water, 2:2:20 v/v), dried extracts (at a dose of 1.25 g dry plant kg^{-1} body weight) and the reference drug (phenylbutazone 80 mg kg^{-1}, Sigma) were administered orally 1 h before carrageenan injection. The inhibition percentage of oedema was calculated for each animal group in comparison with its vehicle-treated group.

2.3. In vitro antioxidant activity

2.3.1. Microsomal lipid peroxidation

Microsomes were isolated from homogenised livers of freshly killed Wistar rats (either sex, 150–250 g) and their peroxidation in the presence of iron ions and ascorbic acid was measured by the thiobarbituric acid (TBA) method, essentially as described by Mansuy et al. (1986).

Briefly, reaction mixtures consisted of 1.0 ml suspensions containing 0.25 mg microsomal protein, 10 μl extract in phosphate buffer, and 10 mM KH_2PO_4-KOH buffer, pH 7.4. Peroxidation was started by adding $FeCl_3$ and ascorbic acid (final concentration 100 μM), followed by incubations at 37°C for 20 min. The extent of peroxidation was assessed using the TBA test: 1.0 ml of TBA (1% w/v in 0.05 M NaOH) and 1.0 ml 2.8% (w/v) trichloroacetic acid were added, the tubes heated at 100°C for 15 min, and after cooling and extraction of the chromogen into 3 ml n-butanol, the absorbance at 532 nm was read.

Table 1
Effects of reference drug and ethanolic extracts on carrageenan-induced mouse paw oedema

Oral treatment	Increase in paw volume (ml) (mean ± S.E.M.) (percent of reduction)		
	3 h	5 h	7 h
Control	0.36 ± 0.01	0.41 ± 0.02	0.26 ± 0.01
Phenylbutazone	0.10 ± 0.01** (71.4)	0.14 ± 0.01** (66.6)	0.11 ± 0.02** (59.2)
B. trinervis	0.31 ± 0.02 (12.6)	0.28 ± 0.02 (32.9)	0.20 ± 0.03 (21.8)
C. floribunda	0.20 ± 0.03** (44.6)	0.26 ± 0.03* (36.5)	0.18 ± 0.02 (29.6)
E. articulatum	0.15 ± 0.02** (59.2)	0.24 ± 0.02* (42.3)	0.12 ± 0.01** (53.6)
E. glutinosum	0.26 ± 0.04 (26.2)	0.25 ± 0.04* (38.8)	0.20 ± 0.02 (22.9)
N. lobata	0.36 ± 0.02 (—)	0.35 ± 0.05 (14.7)	0.21 ± 0.05 (19.5)
T. pusilla	0.21 ± 0.02* (40.7)	0.15 ± 0.01** (62.4)	0.09 ± 0.01** (64.3)
B. sananho	0.23 ± 0.04* (35.7)	0.22 ± 0.02** (47.4)	0.21 ± 0.03 (20.6)
B. longituba	0.21 ± 0.03* (42.8)	0.15 ± 0.01** (63.5)	0.13 ± 0.01** (48.5)
C. mentodorum	0.33 ± 0.05 (7.1)	0.35 ± 0.04 (15.0)	0.19 ± 0.03 (27.7)
H. acuminata	0.27 ± 0.02 (25.0)	0.27 ± 0.02* (35.2)	0.23 ± 0.03 (11.8)
M. condurango	0.30 ± 0.04 (17.3)	0.28 ± 0.03 (31.2)	0.24 ± 0.03 (7.7)
P. rivinoides	0.35 ± 0.03 (—)	0.30 ± 0.03 (27.1)	0.19 ± 0.02 (26.5)
P. bogotensis	0.35 ± 0.02 (—)	0.38 ± 0.05 (6.8)	0.26 ± 0.04 (—)
P. lenticellosum	0.19 ± 0.02* (46.4)	0.19 ± 0.01** (54.3)	0.24 ± 0.02 (7.7)
U. lobata	0.34 ± 0.05 (6.8)	0.33 ± 0.04 (20.6)	0.20 ± 0.03 (21.8)

Values in brackets represent percent reduction in paw volume compared with the group treated only with carrageenan.
Animals injected with the carrageenan vehicle displayed no change in paw volume throughout the experiment.
Significance relative to control values: * $P<0.05$, ** $P<0.01$ ($n=10$).

2.3.2. Scavenging of superoxide

Superoxide anions were generated by preparing a mixture of hypoxanthine and xanthine oxidase (de las Heras and Hoult, 1994). Reaction mixtures of 1.0 ml contained the following: 50 mM KH_2PO_4-KOH pH 7.4, 1 mM EDTA, 100 μM hypoxanthine and 100 μM cytochrome-*c*, type III. Reaction was started by adding 0.066 U of xanthine oxidase (freshly diluted in 100 μl of the above phosphate buffer), and the rate of cytochrome-*c* reduction was measured at 550 nm in a recording spectrophotometer at 25°C. The results are expressed as percentage of inhibition of cytochrome-*c* reduction.

Control experiments were carried out to determine whether these extracts themselves directly reduce cytochrome-*c* or inhibit xanthine oxidase. Thus, they were added to solutions containing 100 μM cytochrome-*c* and the OD_{550} was measured. Their actions on xanthine oxidase was tested by measuring uric acid formation, using xanthine as substrate and absorbance measured at 295 nm. Results are expressed as percentages of inhibition of uric acid production.

2.3.3. Scavenging of hydroxyl radical

Hydroxyl radicals were generated by incubating the following reagents at the indicated final concentrations in 1.0 ml 10 mM KH_2PO_4-KOH buffer pH 7.4 at 37°C for 60 min: 1.42 mM H_2O_2, $FeCl_3$-(20 μM) and 2.8 mM deoxyribose (Laughton et al., 1989). The extent of deoxyribose degradation by the formed hydroxyl radical was measured directly in the aqueous phase by the TBA method as described above.

2.4. Statistical analysis

Results are expressed as means ± S.E.M. The statistical significance of the data was analysed using Student *t*-test.

3. Results

A total of 15 plant species belonging to eight different families have been evaluated. Anti-inflammatory activity of the plant extracts are

Table 2
Inhibition of microsomal lipid peroxidation by ethanolic extracts

Extract	% Inhibition at 100 μg ml^{-1}
B. trinervis	74.8 ± 1.3*
C. floribunda	11.5 ± 2.4
E. articulatum	61.7 ± 1.1*
E. glutinosum	82.2 ± 1.7*
N. lobata	21.9 ± 4.8
T. pusilla	33.4 ± 1.6
B. sananho	—
B. longituba	14.8 ± 1.2
C. mentodorum	1.6 ± 0.9
H. acuminata	5.4 ± 1.1
M. condurango	23.5 ± 2.8
P. rivinoides	66.8 ± 2.6
P. bogotensis	41.0 ± 3.3
P. lenticellosum	23.6 ± 1.5*
U. lobata	15.1 ± 0.5
Reference compound: butylated hydroxy toluene (BHT) (100 μM)	100

Results show means ± S.E.M. for six tests.
* Statistically significant inhibition, $P<0.01$ by Student's unpaired t-test.

Table 3
Scavenging by ethanolic extract of superoxide generated by the hypoxanthine-xanthine oxidase system

Extract	% Inhibition at 100 μg ml^{-1}
B. trinervis	78.5 ± 1.3*
C. floribunda	21.1 ± 2.7
E. articulatum	70.6 ± 1.1*
E. glutinosum	15.9 ± 3.2
N. lobata	3.1 ± 0.9
T. pusilla	55.8 ± 1.6*
B. sananho	12.0 ± 0.4
B. longituba	6.3 ± 1.7
C. mentodorum	34.2 ± 3.1
H. acuminata	13.1 ± 1.2
M. condurango	12.0 ± 3.2
P. rivinoides	68.3 ± 1.7*
P. bogotensis	46.7 ± 1.2*
P. lenticellosum	23.1 ± 3.3
U. lobata	8.5 ± 2.6
Reference compound: allopurinol (0.5 μg ml^{-1})	93.2 ± 0.5*

Results show means ± S.E.M. for six tests.
* Statistically significant inhibition, $P<0.01$ by Student's unpaired t-test.

shown in Table 1. *C. floribunda*, *E. articulatum*, *T. pusilla*, *B. longituba*, *B. sananho* and *P. lenticellosum* extracts exhibited a significant antiinflammatory activity in vivo by reducing the oedematous response induced by carrageenan, comparable with phenylbutazone (80 mg kg^{-1}) used as a reference drug.

The extracts (at 100 μg ml^{-1}) were tested for their ability to inhibit non-enzymatic lipid peroxidation in rat liver microsomes stimulated by $FeCl_3$-ascorbate. *B. trinervis* and *Eupatorium* extracts inhibited lipid peroxidation, showing *E. glutinosum* to have the greatest activity (Table 2).

We then investigated the ability of these extracts to scavenge superoxide anion (Table 3). Using a mixture of hypoxanthine and xanthine oxidase at pH 7.4, the generated superoxide anions can be detected by their ability to reduce ferricytochrome-*c* to ferrocytochrome-*c*. Of the extracts studied, *B. trinervis*, *E. articulatum*, *T. pusilla* and *P. rivinoides* extracts, tested at 100 μg ml^{-1} markedly scavenged superoxide O_2^- generated in this system. At this concentration, these extracts did not reduce cytochrome-*c* by themselves.

Experiments were also carried out to determine if these extracts, which apparently scavenged superoxide, might in fact have done so by inhibiting xanthine oxidase (Table 4). This was determined by measuring their ability to affect the generation of uric acid from xanthine. However, *B. trinervis* and *P. rivinoides* had a weak inhibitory action on xanthine oxidase, insufficient to account for their

Table 4
Effects of ethanolic extracts on xanthine oxidase

Extract	% Inhibition at 100 μg ml^{-1}
B. trinervis	24.7 ± 3.1
E. articulatum	81.2 ± 2.1*
P. rivinoides	3.8 ± 0.8
Reference compound: allopurinol (0.5 μg ml^{-1})	89.3 ± 0.3*

Results show means ± S.E.M. for six tests.
* Statistically significant inhibition, $P<0.01$ by Student's unpaired t-test.

Table 5
Scavenging of hydroxyl radical by ethanolic extracts

Extract	% Inhibition at 100 μg ml^{-1}
B. trinervis	31.2 ± 1.8*
C. floribunda	6.3 ± 1.2
E. articulatum	15.4 ± 0.9*
E. glutinosum	20.8 ± 2.4
N. lobata	1.4 ± 0.2
T. pusilla	1.7 ± 0.3
B. sananho	7.4 ± 2.1
B. longituba	3.6 ± 0.9
C. mentodorum	3.1 ± 0.7
H. acuminata	6.3 ± 1.6
M. condurango	15.6 ± 1.3*
P. rivinoides	8.9 ± 0.7
P. bogotensis	1.9 ± 0.6
P. lenticellosum	1.4 ± 0.7
U. lobata	11.4 ± 1.6
Reference compound: mannitol (50 mM)	58.3 ± 1.3*

Results show means ± S.E.M. for six tests.
* Statistically significant inhibition, $P<0.01$ by Student's unpaired t-test.

superoxide-scavenging activities. Only *E. articulatum* extract inhibited xanthine oxidase activity.

Table 5 shows results obtained when the extracts were tested as possible scavengers of hydroxyl radical. *B. trinervis* extract was the most active, while *E. articulatum* and *M. condurango* extracts exhibited a weak inhibitory activity on the hydroxyl generation.

4. Discussion

The preliminary results obtained revealed that some of these plants are endowed with antiinflammatory activity in vivo (Table 1), which supports their use in traditional medicine in Ecuador. Thus, the effectiveness of these plant extracts may be related to their ability to suppress inflammatory responses.

Numerous pathological events such as the inflammation process and ageing phenomena are associated with the generation of reactive oxygen species and the induction of lipid peroxidation (Cross et al., 1987). The antioxidant action of plant constituents has been found to be related to polyphenolic compounds (Hatano et al., 1989). Literature data report the presence of these type of compounds in some of these plant genera (Farnsworth et al. 1980; Gupta, 1995). Results obtained by our group through TLC techniques indicate the presence of several constituents (flavonoids, tannins, sesquiterpene lactones and saponins) in these plants (Ortega et al., 1996).

The antioxidant activity shown by *B. trinervis*, *E. articulatum* and *P. rivinoides* ethanolic extracts could be related to the presence of phenolic compounds such as tannins and flavonoids. Although direct evidence of the mechanism of these extracts was not obtained here, it has been reported that sesquiterpene lactones and flavonoids exhibit anti-inflammatory activity (Alcaraz and Jiménez, 1988), so these constituents could have contributed to this activity. Further studies intended to confirm these activities, as well as the isolation of the active principles responsible, are being conducted.

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