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Screening of the topical anti-inflammatory activity of some Central American plants

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Abstract

Hexane, chloroform and methanol extracts of seven herbal drugs used in the folk medicine of Central America against skin disorders (*Aristolochia trilobata* leaves and bark, *Bursera simaruba* bark, *Hamelia patens* leaves, *Piper amalago* leaves, and *Syngonium podophyllum* leaves and bark) were evaluated for their topical anti-inflammatory activity against the Croton oil-induced ear oedema in mice. Most of the extracts induced a dose-dependent oedema reduction. The chloroform extract of almost all the drugs exhibited interesting activities with ID_{50} values ranging between 108 and 498 μg/cm^2, comparable to that of indomethacin (93 μg/cm^2). Therefore, the tested plants are promising sources of principles with high anti-inflammatory activity.

Keywords: *Aristolochia trilobata*; *Bursera simaruba*; *Hamelia patens*; *Piper amalago*; *Syngonium podophyllum*; Anti-inflammatory activity

1. Introduction

Inflammation or phlogosis is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defence mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases. Therefore, the use of anti-inflammatory agents is helpful in the therapeutic treatment of these pathologies.

In this context, medicinal plants are widely used in folk medicine of many countries to treat different inflammatory conditions and, in particular, skin inflammations. However, for many of the plants in use the real efficacy and/or the relevant active principles are unknown. Consequently, experimental studies aimed to demonstrate the pharmacological properties of these plants and to identify the relevant active principles are needed.

To this aim, we screened the topical anti-inflammatory properties of seven herbal drugs obtained from five species growing in Central America: *Aristolochia trilobata* L. (Aristolochiaceae) leaves and bark, *Bursera simaruba* (L.) Sarg. (Burseraceae) bark, *Hamelia patens* Jacq. (Rubiaceae) leaves, *Piper amalago* L. (Piperaceae) leaves and *Syngonium podophyllum* Schott. (Araceae) leaves and bark. These plants are traditionally employed in the local folk medicine of Belize to prepare infusions, decoctions, baths or poultices used for their wound healing properties. Moreover, these preparations are also used to produce relief in several skin affections characterised by an inflammatory component, such as sores, rashes and burns (Domínguez and Alcorn, 1985; Cáceres et al., 1987; Zamora-Martinez and de Pascual Pola, 1992; Arvigo and Balik, 1993; Di Stasi et al., 1994).

In order to verify their topical anti-inflammatory potential, each herbal drug was extracted by solvents of increasing polarity grade and the obtained extracts were

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evaluated for their ability to inhibit the Croton oil-induced ear oedema in mice (Tubaro et al., 1985). As reference, the non-steroidal anti-inflammatory drug indomethacin was used.

2. Materials and methods

2.1. Plant materials and extraction

Plant materials were collected in Belize in February 1999 and authenticated by Professor M.J. Balick. Voucher specimens were dried and deposited at the New York Botanical Garden (NY, USA).

The air-dried and powdered plant materials (from 200 to 500 g) were submitted to sequential maceration with 2500 ml of *n*-hexane, chloroform and methanol at room temperature. After each step the extracts were filtered and the solvents were removed under vacuum at 30 °C until dry hexane, chloroform and methanol extracts were obtained.

2.2. Chemicals

Croton oil and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO, USA), whereas ketamine hydrochloride from Virbac (Milano, Italy). The other reagents, of analytical grade, were Carlo Erba products (Milano, Italy).

2.3. Topical anti-inflammatory activity

Topical anti-inflammatory activity was evaluated as inhibition of the Croton oil-induced ear oedema in mice (Tubaro et al., 1985). Male CD-1 mice (28–32 g; Harlan Italy, S. Pietro al Natisone, Italy) were anaesthetised with ketamine hydrochloride (145 mg/kg, intraperitoneally) before the induction of the phlogosis. Cutaneous inflammation was induced by applying 80 μg of Croton oil dissolved in 15 μl of acetone (hexane and chloroform extracts) or suspended in the same volume of 42% aqueous ethanol (v/v) (methanol extracts) to the inner surface of the right ear of mice (surface: about 1 cm^2). The left ear remained untreated. The substances under testing were applied together with the Croton oil, except for control animals which received only the relevant irritant solutions. Six hours later, mice were sacrificed and a plug (6 mm Ø) was removed from both the treated and the untreated ears. The oedematous response was measured as weight difference between the two plugs and the anti-inflammatory activity was expressed as percentage of oedema reduction in treated mice with regard to control mice. At least two experimental groups of five animals were tested for each dose level. Animal experiments complied with the Italian D.L. n. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609 ECC).

2.4. Statistical analysis

The data were analysed by Student's *t*-test and a probability level lower than 0.05 was considered to be significant. The doses inhibiting the oedematous response by 50% (ID_{50}) were calculated by graphic interpolation of the dose–effect curves.

3. Results

The yields of the examined herbal drugs extracts are reported in Table 1 and the results on the anti-inflammatory activity are reported in Tables 2 and 3. At doses ranging from 100 to 1000 μg/cm^2, most of the extracts induced a significant dose-dependent reduction of the oedematous response, which sometimes was comparable to that of the reference drug indomethacin. The hexane extract of *A. trilobata* leaves, *B. simaruba* bark as well as *S. podophyllum* bark and leaves induced, at the highest tested dose, oedema reductions ranging between 50 and 83%, whereas that of the other drugs reached 17–48% reduction. The chloroform extracts were more active than the hexane ones and induced oedema inhibitions ranging between 50% (*A. trilobata* bark) and 93% (*A. trilobata* leaves). The mildest activity was observed for the methanol extracts, the highest dose of which provoked oedema inhibition ranging from 18% (*A. trilobata* leaves) to 54% (*H. patens* leaves) (Table 2).

The relationship between the administered doses and the observed effects allowed to estimate the anti-inflammatory potency of the extracts by calculating the relevant ID_{50} values (dose giving 50% oedema inhibition). The obtained results, reported in Table 3, show that, for each herbal drug, the chloroform extracts were the most active, followed by the hexane and by the methanol ones, with exception for *H. patens* and *P. amalago* which methanol extracts were more active than

Table 1
Yields of the plant extracts

Plant material	Starting amount (g)	Yield of extraction (w/w)		
		Hexane	Chloroform	Methanol
A. trilobata leaves	200	2.3(%)	1.7(%)	9.8(%)
A. trilobata bark	200	1.1(%)	1.1(%)	7.5(%)
B. simaruba bark	400	0.7(%)	0.7(%)	3.9(%)
H. patens leaves	300	3.1(%)	3.5(%)	7.7(%)
P. amalago leaves	470	1.2(%)	3.8(%)	4.4(%)
S. podophyllum leaves	500	1.7(%)	1.6(%)	2.0(%)
S. podophyllum bark	400	0.6(%)	0.5(%)	1.3(%)

Table 2
Anti-inflammatory activity of the plants extracts

Plant material (reference drug)	Dose (μg)	Hexane extract		Chloroform extract		Methanol extract	
		Oedema (mg) m±S.E.	% Red.	Oedema (mg) m±S.E.	% Red.	Oedema (mg) m±S.E.	% Red.
A. trilobata leaves	0	8.4±0.3	–	8.4±0.3	–	7.6±0.7	–
	100	4.6±0.3*	45	4.0±0.7*	52	7.2±0.3	6
	300	4.1±0.8*	51	3.3±0.5*	61	6.9±0.5	10
	1000	4.0±0.6*	52	0.6±0.2*	93	6.2±0.7*	18
A. trilobata bark	0	7.8±0.4	–	7.8±0.4	–	7.6±0.7	–
	100	7.4±0.3	5	5.1±0.8*	35	6.7±0.8	12
	300	5.5±0.3*	29	4.8±0.4*	38	6.8±0.2	11
	1000	5.1±0.4*	35	3.9±0.5*	50	6.1±0.3	20
B. simaruba bark	0	7.0±0.4	–	7.0±0.4	–	7.2±0.3	–
	100	4.8±0.4*	31	4.1±0.5*	41	5.6±0.3*	22
	300	2.9±0.4*	59	2.3±0.5*	67	5.1±0.5*	29
	1000	1.2±0.2*	83	1.9±0.4*	73	4.3±0.5*	40
H. patens leaves	0	6.5±0.3	–	6.5±0.3	–	7.2±0.3	–
	100	6.8±0.7	−5	4.1±0.4*	37	5.8±0.3*	19
	300	4.9±0.4*	25	3.7±0.2*	43	4.7±0.4*	35
	1000	3.4±0.7*	48	1.2±0.1*	81	3.3±0.5*	54
P. amalago leaves	0	6.5±0.2	–	6.5±0.3	–	7.2±0.3	–
	100	6.3±0.4	3	6.9±0.4	−6	6.9±0.5	4
	300	6.7±0.6	-3	5.0±0.5*	23	5.8±0.5*	19
	1000	5.4±0.6	17	1.3±0.2*	80	4.1±0.4*	43
S. podophyllum leaves	0	7.6±0.2	–	7.6±0.2	–	7.7±0.6	–
	100	4.8±0.5*	37	4.7±0.5*	38	6.7±0.5	13
	300	3.4±0.6*	55	3.1±0.4*	59	5.8±0.6*	25
	1000	2.3±0.6*	70	2.5±0.4*	67	3.4±0.6	48
S. podophyllum bark	0	7.0±0.3	–	7.0±0.3	–	7.7±0.6	–
	100	5.6±0.4*	20	5.4±0.5*	23	6.7±0.5	13
	300	4.6±0.7*	34	2.6±0.4*	63	6.7±0.6	13
	1000	2.1±0.4*	70	2.3±0.6*	67	4.6±0.6*	40
(Indomethacin)	0	7.0±0.4	–				
	45	5.1±0.4*	27				
	90	3.9±0.3*	44				
	180	1.7±0.2*	76				

*$P < 0.05$ at the Student's t-test.

Table 3
ID_{50} values of the extracts (μg/cm^2)

	Hexane	Chloroform	Methanol
A. trilobata leaves	354	108	> 1000
A. trilobata bark	> 1000	> 1000	> 1000
B. simaruba bark	221	143	> 1000
H. patens leaves	> 1000	255	779
P. amalago leaves	> 1000	498	> 1000
S. podophyllum leaves	236	211	> 1000
S. podophyllum bark	460	295	> 1000
Indomethacin		93	

the hexane ones. The chloroform extract of the most active drug (*A. trilobata* leaves) showed an anti-inflammatory effect comparable to that of indomethacin: its ID_{50} value was 108 μg/cm^2 whereas that of the reference drug is 93 μg/cm^2. Also the chloroform extracts of the other drugs revealed interesting anti-inflammatory properties, being characterised by relatively low ID_{50} values: 143 μg/cm^2 (*B. simaruba* bark), 211 μg/cm^2 (*S. podophyllum* leaves), 255 μg/cm^2 (*H. patens* leaves), 295 μg/cm^2 (*S. podophyllum* bark) and 498 μg/cm^2 (*P. amalago* leaves). Only *A. trilobata* bark chloroform extract showed a mild activity, being its ID_{50} value higher than 1000 μg/cm^2.

4. Discussion

The data presented in this study demonstrate that almost all the reported herbal drugs possess significant topical anti-inflammatory properties, supporting their traditional use for the treatment of different skin affections. Indeed, most of their extracts were able to inhibit the Croton oil-induced ear oedema in mice, after topical application.

In general, the most suitable solvent for the extraction of the anti-inflammatory principles from these plants was chloroform, being the chloroform extracts the most active. These lipophilic extracts can be regarded as a potential source of highly active anti-inflammatory

agents and, among them, the most promising one was that of *A. trilobata* leaves, which antiphlogistic potency is close to that of the pure non-steroidal anti-inflammatory drug indomethacin. This is quite unusual for crude extracts since their active compounds are often diluted by other bulk substances. Therefore, in order to reach the potency of indomethacin, the active constituents of an extract should be more active than the reference compound or should exert their activity in a synergistic way.

However, plants belonging to the genus *Aristolochia* were reported to contain aristolochic acids (Chen and Zhu, 1987; Hashimoto et al., 1999). These compounds were shown to possess immunostimulatory and anti-inflammatory properties (Möse, 1966; Bartfeld, 1977; Kluthe et al., 1982; Vishwanath et al., 1988) as well as inhibitory activity against phopholipase A_2, a key enzyme involved in the formation of pro-inflammatory mediators (Vishwanath et al., 1988; Rosenthal et al., 1989). On the other side, they are also responsible for severe toxic effects, particularly on kidney and liver, that induce to neglect the therapeutic use of plants preparations containing such compounds (De Smet, 1992; Hashimoto et al., 1999). Nevertheless, aristolochic acids were not detected by TLC and NMR analysis of the fractions obtained after chromatographic purification of hexane and chloroform extracts on silica-gel column. On the other hand, a number of pure aristolochic acids derivatives were isolated from methanol extract after purification on Sephadex LH-20, followed by HPLC and detected by TLC and NMR (data not shown). Therefore, aristolochic acids are not involved in the high anti-inflammatory activity of the lipophilic extracts.

The bark of the same plant yielded less active extracts. Therefore, the use of *A. trilobata* bark in the traditional medicine as a remedy for skin affections depends probably from other pharmacological properties, different from the anti-inflammatory one.

Concerning the other drugs, *B. simaruba* bark was the second most active. An anti-inflammatory activity of this plant had been previously reported by Abad et al. (1996) but, contrary to our results, no antiphlogistic activity was recorded for the plant bark. In particular, while a hexane extract of the leaves was shown to inhibit the adjuvant-carrageenan-induced inflammation in rats after oral administration, the more hydrophilic ethanol extracts of both leaves and bark were inactive in that model of chronic inflammation (Abad et al., 1996). Therefore, besides the different experimental models and administration routes, the differences in the results could be related to a different chemical composition of the extracts. In fact, while the ethanol extract reported by Abad et al. (1996) could be characterised by the presence of polar compounds with relatively low antiphlogistic activity, the chloroform one should contain higher concentrations of anti-inflammatory components of apolar nature. Compounds of such polarity, in particular triterpenes, steroids and lignans, had been previously isolated from *B. simaruba* resin (Peraza-Sánchez and Peña-Rodríguez, 1992; Peraza-Sánchez et al., 1995). Anyway, their presence in the bark chloroform extract as well as their role in the anti-inflammatory activity can be only hypothesised.

S. podophyllum and *H. patens* revealed a similar degree of activity whereas *P. amalago* was less active: the chloroform extracts of the first two species were about 2–3-fold less active than indomethacin, while that of *P. amalago* was about 5-fold lower. Anyway, no reports on an anti-inflammatory action or related biological activities were described for these species. On the contrary, some phytochemical studies on *H. patens* and *P. amalago* had been reported. In particular, the investigation of the aerial parts and of the leaves of *H. patens* lead to the isolation of triterpenic and steroidic compounds, flavonoids and alkaloids (Ripperger, 1978; Aquino et al., 1990; Chaudhuri and Thakur, 1991). These compounds could be involved in the observed antiphlogistic activity and bioassay-oriented fractionation studies are in progress to define their role as possible anti-inflammatory principles of the chloroform extract of *H. patens* leaves.

Concerning *P. amalago*, piperine-like amides and sesquiterpenes were identified in the plant roots (Achenbach et al., 1984, 1986; Domínguez et al., 1986). Being relatively apolar compounds, their presence also in the chloroform extract of the leaves and a possible role in its anti-inflammatory activity can be hypothesised.

In conclusion, the obtained results confirm the presence of anti-inflammatory principles in most of the examined herbal drugs, giving a rational support to their use by traditional medicine of Belize.

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