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Characterization of Topical Antiinflammatory Compounds in *Rosmarinus officinalis* L.

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The topical antiinflammatory activity of three extracts at increasing polarity (*n*-exane, chloroform, and methanol) from the leaves of *Rosmarinus officinalis* L. (Labiatae) has been tested using the croton oil ear test in mice. Both the *n*-hexane and the chloroform (CE-1) extracts from the leaves showed a dose-dependent activity, the last one possessing an antiinflammatory potency similar to that of indomethacin, the nonsteroidal antiinflammatory drug used as a reference drug (ID_{50} = 83 and 93 $\mu g/cm^2$, respectively). The bioassay-oriented fractionation of CE-1 led to the identification of tritepenes, ursolic acid, oleanolic acid, and micromeric acid as the main antiinflammatory principles. Furthermore, the CE-1 extract obtained from the residue of the steam distillation of the leaves (extract A) showed the same antiinflammatory potency of CE-1, suggesting this waste product as a source of antiinflammatory products.

KEYWORDS: Rosemary; *Rosmarinus officinalis* L; topical antiinflammatory activity; ursolic acid; oleanolic acid; micromeric acid

INTRODUCTION

Rosmarinus officinalis L. (Labiatae) is an edible evergreen shrub native to the Mediterranean area. The leaves of the plant are commonly used as a spice and as a source of antioxidant compounds employed in food conservation (*1*−*6*). Furthermore, it is used to obtain, by steam distillation, the essential oil used as a food additive. The production of essential oil leads to the accumulation of high amounts of residues containing nonvolatile compounds. The plant extracts are also used in traditional medicine for the antioxidant activity ascribable to diterpenoids and polyphenols (*5*, *7*, *8*).

A methanol (ME) extract of *R. officinalis* was shown to exert protective effects against carbon tetrachloride hepatotoxicity in rats (*9*). Oral administration of a rosemary extract was found to also protect against mouse skin tumorigenesis induced by 7,12-dimethlybenz(a)anthracene initiation and croton oil as the promoter (*10*). On the other hand, the major phenolic diterpene constituent carnosol was reported as a new potential anti-Parkinson's agent (*11*). Furthermore, Nolkemper et al. (*12*) reported an in vitro antiviral effect against herpes simplex virus types 1 and 2 for an aqueous extract of rosemary. Moreover, the essential oil of the leaves possesses antimicrobial and antiplasmid properties (*13*−*16*). The decoction of rosemary leaves can be used topically against eczema and other cutaneous diseases (*17*, *18*).

Recently, others members of Labiatae family, *Thymus* spp. and *Salvia officinalis* L., known for the biological properties of their essential oils, showed antiinflammatory activities due to their nonvolatile components (*19*−*24*). Thus, the aim of the present study was the characterization of the topical antiinflammatory compounds from another Labiatae species, such as *R. officinalis*.

The leaves of *R. officinalis* were submitted to sequential extractions using solvents of increasing polarity. Two lipophilic extracts were prepared from the leaves also after their steam distillation to obtain the essential oil. The extracts were submitted to a pharmacological and phytochemical investigation to study their topical antiinflammatory activity following a bioassay-oriented fractionation procedure. Croton oil-induced dermatitis in the mouse ear was used as a model of inflammation (*25*, *26*). This is an in vivo model useful to screen the topical antiinflammatory activity of synthetic and natural compounds (*27*, *28*). The inflammatory reaction induced by the phorbol esters of croton oil is an acute response, whose vascular and cellular components can be easily quantified, obtaining objective results by gravimetric determination of the edematous response and inflammatory cells measurement by an enzymatic way (*29*, *30*). The test is suitable to evaluate the antiinflammatory activity of natural compounds, often available in limited amounts during the bioassay-oriented fractionation procedure due to the small amount of substances necessary to carry out the assay.

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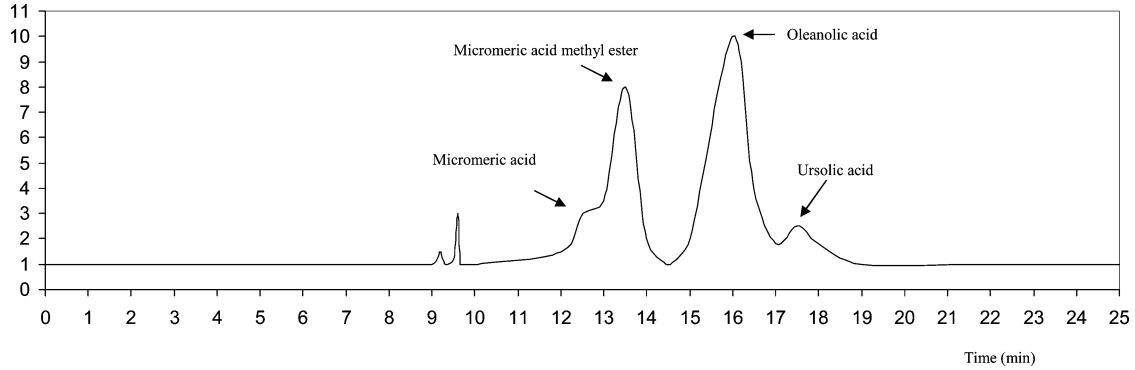

Figure 1. HPLC profile of fraction II from *R. officinalis*.

MATERIALS AND METHODS

Plant Material. A commercial sample of rosemary (*R. officinalis* L.), native from Macedonia, was supplied by the Institute for Medicinal Plant Research, Dr. Josif Pancic of Belgrade (Yugoslavia), where a voucher specimen (R.O. 1) was deposited at the herbarium.

Chemicals. Croton oil and indomethacin were Sigma products (St. Louis, MO). Ursolic acid and oleanolic acid were supplied by Indena S.p.A. (Milan, Italy) and Roth (Karlsruhe, Germany). Ketamine hydrochloride was purchased from Virbac S.r.l. (Milan, Italy). All other reagents of analytical grade were purchased from Carlo Erba (Milan, Italy).

Instruments. A Bruker DR-600 spectrometer operating at 599.2 MHz for ^{1}H and 150.9 for ^{13}C, using the NMR software package, was used for NMR measurements in CD_3OD solution. One- and two-dimensional NMR spectra were obtained by employing the conventional pulse sequences as previously described (*31*). Optical rotation was measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 1% w/v solution in ME. Electrospray ionization mass spectrometry (ESI-MS) was performed using a Finnigan LC-Q Deca instrument (Thermoquest, San Jose, CA) equipped with Xcalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump (capillary temperature, 220 °C; flow rate, 3 μL/min; capillary voltage, 5 V; spray voltage, 5 kV; and tube lens offset, 35 V).

Extraction and Fractionation Procedure. Dried leaves (45 g) were submitted to successive extractions with 600 mL of *n*-hexane, chloroform, or methanol in a Soxhlet apparatus for 6 h each. The extracts were filtered and concentrated in vacuum to give hexane (HE, 2.5 g, yield 5.6% w/w), chloroform (CE-1, 5.0 g, 11.1% w/w), and ME (3.5 g, 7.8% w/w) extracts, respectively.

The extracts were tested for their topical antiinflammatory activity. The most active one (CE-1) was checked by thin-layer chromatography (TLC) analysis (Si-gel, $CHCl_3$/MeOH 9:1) showing the presence of triterpenic acids as its major constituents. Thus, part of the CE-1 extract (1 g) was partitioned by consecutive extraction between Et_2O/H_2O containing 1% NaOH (10:50 mL for five times). The organic layer was dried in vacuum giving fraction I (172 mg, 17.2% w/w). The combined alkaline aqueous layers were acidified with CH_3COOH to pH 5.3 and then re-extracted with Et_2O (five times). The combined ether layers were dried in vacuum to give fraction II (738 mg, 73.8% w/w).

To isolate the pure compounds, part of fraction II (100 mg) was separated by high-performance liquid chromatography (HPLC) (**Figure 1**) on a μ-Bondapack C-18 (30 cm × 7.8 mm i.d.; flow rate rate, 1.8 mL/min), with MeOH−H_2O (9:1) as the solvent system, obtaining micromeric acid (2.5 mg, R_t =12.5 min), micromeric acid methyl ester (2.1 mg, R_t =13.5 min), oleanolic acid (*30*−*32*) (10.1 mg, R_t =16.0 min), and ursolic acid (*31*−*33*) (9.5 mg, R_t =17.5 min) (**Figure 2**). Micromeric acid and micromeric acid methyl ester were completely characterized by NMR and MS data in comparison with literature data (*33*−*37*).

3β-Hydroxy-ursa-12,20(30)-dien-28-oic Acid (Micromeric Acid). White amorphous powder. IR ν_{max} cm^{-1}: 3400 (OH), 3080, 3000 (C−H), 1695 (CO_2H), 1660 (C═C), 890 (C═CH_2), 822 (C═CH). ESI-MS

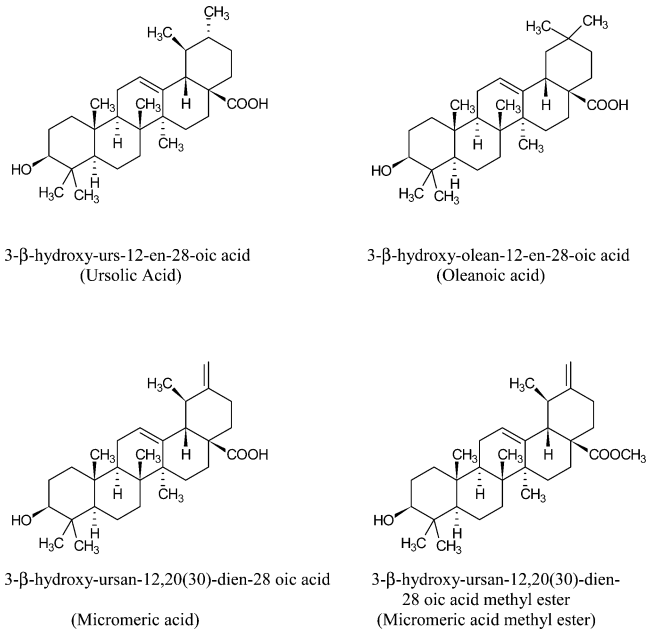

Figure 2. Chemical structures of the pure compounds isolated from the CE-1 extract of *R. officinalis*.

m/*z* 454 $[M]^+$, 436 $[M - H_2O]$*, 410, 409 $[(M - CO_2H]^+$, 232, 187 $[232\text{-}CO_2H)]^+$. For ^{1}H and ^{13}C NMR data, see **Table 1**.

Methyl 3β-Hydroxy-ursa-12,20(30)-dien-28-oate (Micromeric Acid Methyl Ester). White amorphous powder. IR ν_{max} cm^{-1}: 3450 (OH), 3080, 2950 (C−H), 1720 (CO_2Me), 1650 (C═C), 886 (C═CH_2), 825 (C═CH). ESI-MS *m*/*z* 468 $[M]^+$, 453 [M − Me]*, 409 $[(M - CO_2Me]^+$, 232, 187 $[232\text{-}CO_2H)]^+$. For ^{1}H and ^{13}C NMR data, see **Table 1**.

Steam Distillation of Dried Leaves and Extraction of Vegetable Residue. The dry plant leaves (37 g) were submitted to steam distillation in a Cleavenger type apparatus, according to the method for the determination of essential oil in medicinal plants reported in the European Pharmacopoeia (*38*) with 500 mL of water for 2 h (three time) to obtain the relevant essential oil.

The residue of steam distillation was separated from the water and dried. Two different parts of dried residue (5 and 13 g, respectively) were submitted to an extraction with 250 mL of $CHCl_3$ and 400 mL of Et_2O, respectively, in a Soxhlet apparatus for 6 h. The extracts were concentrated in vacuum to give, respectively, extract A and extract B.

Topical Antiinflammatory Activity. The topical antiinflammatory activity was evaluated as inhibition of the croton oil-induced ear edema in mice (*25*, *26*) at doses of 30−1000 μg/cm^2 for the extracts (**Tables 2**, **3**, and **6**) and at doses reported in **Tables 4** and **5** for fractions and pure compounds. Male CD-1 mice (28−32 g; Harlan-Italy, Udine, Italy) were kept for 1 week before the experiment, at constant conditions of temperature (21 ± 1 °C) and humidity (60−70%), with a fixed artificial light cycle (7.00−19.00 h). Inflammation was induced on the inner surface of the right ear (surface, about 1 cm^2) of mice, previously

Table 1. ^{13}C NMR and ^{1}H NMR Data of Micromeric Acid and Its Methyl Ester (CD_3OD, 600 MHz)

		micromeric acid		micromeric acid methyl ester	
position[a]	DEPT	δ_C	δ_H (J_{HH} in Hz)[b]	δ_C	δ_H (J_{HH} in Hz)[b]
1	CH_2	39.9	1.66 m	39.7	1.65 m
			1.03 m		1.02 m
2	CH_2	28.5	1.61 m	28.3	1.60 m
3	CH	80.3	3.25 dd (11.1, 4.0)	80.1	3.23, dd (11.0, 4.0)
4	C	39.9		39.5	
5	CH	56.7	0.74 m	55.9	0.73 m
6	CH_2	18.3	0.95 m	18.0	0.94 m
7	CH_2	34.2	1.52 m	34.0	1.53 m
			1.36 m		1.32 m
8	C	40.7		40.2	
9	CH	48.8	1.55 m	48.3	1.56 m
10	C	38.2		37.9	
11	CH_2	24.6	1.96	24.3	1.95
12	CH	127.7	5.34 t (3.5)	127.2	5.29 t (3.5)
13	C	138.0		137.9	
14	C	43.3		43.1	
15	CH_2	29.1	1.90, 1.19 m	28.9	1.89, 1.20 m
16	CH_2	25.6	1.79 ddd (13.0, 12.0, 5.1),	25.4	1.78 ddd (13.0, 12.0, 5.1),
			2.23 ddd (12.0, 5.0, 3.1)		2.24 ddd (12.0, 5.0, 3.1)
17	C	48.0		48.1	
18	CH	56.1	2.31 br s	55.9	2.30 br s
19	CH	38.7	2.39 d (11.0, 6.2)	38.4	2.40 d (11.0, 6.2)
20	C	152.8		152.6	
21	CH_2	33.5	2.25, 2.31 m	33.0	2.26, 2.30 m
22	CH_2	40.1	1.92, 1.73 m	39.9	1.90, 1.74 m
23	CH_3	29.4	1.00 s	29.0	1.02 s
24	CH_3	16.9	0.79 s	17.0	0.80 s
25	CH_3	16.6	0.94 s	16.3	0.95 s
26	CH_3	18.3	0.78 s	18.0	0.76 s
27	CH_3	24.6	1.16 s	24.0	1.15 s
28	C	177.8		177.2	
29	CH_3	17.3	1.03 d (6.2)	17.1	1.00 d (6.2)
30	CH_2	106.5	4.65 br s	106.0	4.65 br s
			4.70 br s		4.70 br s
–OMe	CH_3			52.1	3.49 s

[a] Assignments confirmed by HSQC and HMBC experiments. [b] ^{1}H–^{1}H couplings were measured from COSY spectra in Hz.

Table 2. Antiinflammatory Activity of HE, CE-1, and ME Extracts from *R. officinalis* Leaves 6 h after Croton Oil-Induced Dermatitis in Mouse Ear

substance	dose (μg/cm^2)	no. of mice	edema (mg)[a]	edema reduction (%)	ID_{50}[b] (μg/cm^2)
HE extract		10	7.6 ± 0.2		
	30	10	6.0 ± 0.4*	21	
	100	20	4.5 ± 0.4*	41	265
	300	10	4.1 ± 0.3*	46	
	1000	10	2.3 ± 0.5*	79	
CE-1 extract		10	7.6 ± 0.2		
	30	10	6.0 ± 0.5*	21	
	100	20	3.8 ± 0.3*	50	83
	300	10	0.5 ± 0.1*	93	
	1000	10	0.2 ± 0.1*	97	
ME extract		10	7.6 ± 0.2		
	100	10	7.5 ± 0.5	1	>1000
	1000	10	5.5 ± 0.6*	27	
indomethacin		10	7.6 ± 0.2		
	30	10	6.2 ± 0.4*	18	93
	100	10	3.3 ± 0.3*	57	
	300	10	1.7 ± 0.2*	78	

[a] Edema values are expressed as means ± SE. [b] ID_{50}, doses inhibiting the edematous response by 50%; *$p < 0.05$ at the Student's t test.

Table 3. Antiinflammatory Activity of the Total and the CE-1 Extracts from *R. officinalis* Leaves 6 h after Croton Oil-Induced Dermatitis in Mouse Ear

substance	dose (μg/cm^2)	no. of mice	edema (mg)[a]	edema reduction (%)
controls		11	7.2 ± 0.2	
CE-1 extract	136.5	10	1.7 ± 0.3*	76
total extract	300	10	1.6 ± 0.1*	78

[a] Edema values are expressed as means ± SE; *$p < 0.05$ at the Student's t test.

anaesthetized with ketamine hydrochloride (145 mg/kg, intraperitoneally) by application of 80 μg of Croton oil, suspended in the appropriated vehicle. The left ear remained untreated. Control animals received only the irritant solution, whereas the other mice received both the irritant and the test substances. Used were the following vehicles: acetone (for HE and CE-1 extracts, extracts A and B, fractions I and II, pure compounds, and the relevant controls) or 42% aqueous ethanol (v/v) (for ME extract and its controls). At the maximum edematous response, 6 h later, mice were sacrificed and a plug (6 mm ∅) was removed from both the treated and the untreated ears. The edematous response was measured as the weight difference between the two ear plugs. The antiinflammatory activity was expressed as percent reduction of the edematous response in treated mice as compared to the control mice. As a reference, the nonsteroidal antiinflammatory drug indomethacin was used. All animal experiments complied with the Italian D.L. n. 116 of January 27, 1992, and associated guidelines in the European Communities Council Directive of November 24, 1986 (86/609 ECC).

Statistical Analysis. The pharmacological data were analyzed by Student's t test, considering a probability level lower than 0.05 as statistically significant. The doses inhibiting the edematous response by 50% (ID_{50}) were calculated by graphic interpolation of the dose–effect curves.

Table 4. Antiinflammatory Activity of Fractions I and II from the CE-1 Extract of *R. officinalis* Leaves 6 h after Croton Oil-Induced Dermatitis in Mouse Ear

substance	dose (μg/cm^2)	no. of mice	edema (mg)[a]	edema reduction (%)
controls		10	7.6 ± 0.3	
CE-1 extract	300	10	2.2 ± 0.3*	71
fraction I	52[b]	10	6.9 ± 0.4	10
fraction II	221[b]	12	1.2 ± 0.2*	85

[a] Edema values are expressed as means ± SE. [b] Dose equivalents to 300 mg of the CE-1 extract; *$p < 0.05$ at the Student's t test.

RESULTS

Extraction of Plant Material. The leaves of *R. officinalis* were submitted to the successive extraction in a Soxhlet apparatus with solvents of increasing polarity as indicated in the Materials and Methods to give the HE, CE-1, and ME extracts in the amounts of 2.5, 5.0, and 3.5 g (5.6, 11.0, and 7.8% with respect to dry plant material). Each extract was submitted to the croton oil ear test to verify its topical antiinflammatory activity.

Topical Antiinflammatory Activity of the Extracts. HE and CE-1 extracts were administered at the doses of 30, 100, 300, and 1000 μg/cm^2, while the ME extract was applied at doses of 100 and 1000 μg/cm^2. As shown in **Table 2**, CE-1 and HE extracts exerted a dose-dependent topical antiinflammatory activity. The ID_{50} (dose giving 50% edema inhibition) was 265 μg/cm^2 for HE and 83 μg/cm^2 for CE-1. The antiinflammatory potency of CE-1 was similar to that of the reference drug indomethacin ($ID_{50} = 93$ μg/cm^2). The ME extract was the less active one, inducing only 27% edema inhibition at the highest administered dose (1000 μg/cm^2). Consequently, its contribution to the activity of the crude drug seems to be limited.

To evaluate the contribution of the CE-1 extract to the topical antiinflammatory activity of whole herbal drug, its effect was compared to that of a virtual total extract, prepared by pooling HE (23.5%), CE-1 (45.5%), and ME (31.8%) extracts, according to the respective extraction yields. As reported in **Table 3**, 300 μg/cm^2 of total extract or the corresponding amount of CE-1 extract (136.5 μg/cm^2) exhibited similar activity (78 and 76% edema inhibition, respectively). Consequently, the CE-1 extract gives the highest contribution to the antiinflammatory activity of *R. officinalis* leaves.

Fractionation of the CE-1 Extract. TLC analysis of the CE-1 extract, the most active one, revealed the presence of triterpenic acids as its major constituents. Therefore, it was subjected to a preliminary separation to obtain a triterpenic acid-enriched fraction. By means of repeated partitions between diethyl ether and water containing 1% NaOH, two main fractions were obtained, fraction I (0.172 g) and fraction II (0.738 g), which represented 17.2 and 73.8% of the parent extract, respectively.

Separation of fraction II by reverse-phase HPLC (**Figure 1**) revealed the presence of ursolic acid, oleanolic acid, micromeric acid, and micromeric acid methyl ester whose identities and purities were established by MS and NMR (**Table 1**) spectra in comparison to the literature data (*31*−*37*).

Antiinflammatory Activity of Fractions I and II. Fractions I and II were evaluated for their antiinflammatory activity at 52 and 221 μg/cm^2, corresponding to 300 μg/cm^2 of the parent CE-1 extract, on the basis of the fractionation yield. Fraction II reduced the edematous response by 85%, showing an activity much higher than that of fraction I, which provoked only 10% edema reduction (**Table 4**). Thus, fraction II, containing triterpenes and representing 73.8% of the parent extract, can completely justify the biological activity of the parent CE-1 extract.

Table 5. Antiinflammatory Activity of Pure Componds Isolated from the CE-1 Extract of *R. officinalis* leaves 6 h after Croton Oil-Induced Dermatitis in Mouse Ear

substance	dose (μg/cm^2)	no. of mice	edema (mg)[a]	edema reduction (%)
ursolic acid		9	8.0 ± 0.4	
	25	10	6.1 ± 0.3*	23
	50	10	4.5 ± 0.1*	43
	100	10	2.0 ± 0.3*	76
	200	10	0.8 ± 0.1*	90
oleanolic acid		9	8.0 ± 0.4	
	50	10	6.4 ± 0.4*	20
	100	10	4.4 ± 0.5*	45
	200	10	2.8 ± 0.3*	65
	400	9	1.7 ± 0.1*	78
micromeric acid		10	7.8 ± 0.2	
	50	10	5.5 ± 0.3	29
	100	10	4.5 ± 0.3*	42
micromeric acid methyl ester		10	7.8 ± 0.2	
	50	9	7.3 ± 0.4	5
	100	7	6.5 ± 0.3	17

[a] Edema values are expressed as means ± SE; *$p < 0.05$ at the Student's t test.

Antiinflammatory Activity of Pure Compounds. Ursolic acid, oleanolic acid, micromeric acid, and micromeric acid methyl ester, isolated from fraction II, were also studied for their antiinflammatory activity. Because of the very little amount available, micromeric acid and its methyl ester were tested only at the doses of 50 and 100 μg/cm^2 (corresponding to 0.10 and 0.20 μmol/cm^2).

As already observed (*19*), a significant dose-dependent inhibition of the edematous response was induced by both ursolic and oleanolic acids (**Table 5**); ursolic acid was more potent than oleanoic acid (ID_{50} = 56 and 132 μg/cm^2, respectively, corresponding to 0.12 and 0.29 μmol/cm^2) as well as than indomethacin ($ID_{50} = 93$ μg/cm^2, corresponding to 0.26 μmol/cm^2). Micromeric acid showed a significant antiinflammatory activity: The highest administrated dose (100 μg/cm^2, corresponding to 0.22 μmol/cm^2) reduced the edematous response by 42% (**Table 5**), similar to oleanolic acid at the same dose. On the contrary, its methyl ester was inactive at both the tested doses.

Extraction of the Residue of Steam Distillation. The dry plant leaves (37 g) were submitted to steam distillation to give the essential oil (0.7 mL, 1.87% yield with respect to dry plant material). The residue was separated in two parts and extracted with two different organic solvents, CE-1 and diethyl ether, to give extract A (0.98 g, 19.6% of the residue material) and extract B (1.67 g, 12.9% of the residue material).

Topical Antiinflammatory Activity of Essential Oil and of Extracts A and B. The topical antiinflammatory activity of the essential oil obtained from *R. officinalis* leaves and of extracts A and B was evaluated by the croton oil ear test. The essential oil, as reported in **Table 6**, did not show any antiinflammatory effect after topical application. On the contrary, extracts A and B (300 μg/cm^2) were able to reduce the edema response by 87 and 80%, respectively. Their activity was comparable to that exerted by the CE-1 extract (71% edema reduction), obtained by the extraction of rosemary leaves without previous steam distillation (**Table 6**).

Table 6. Antiinflammatory Activity of Essential Oil and of Extracts A and B Obtained from Residue of Steam Distillation of *R. officinalis* Leaves 6 h after Croton Oil-Induced Dermatitis in Mouse Ear

substance	dose (μg/cm^2)	no. of mice	edema (mg)[a]	edema reduction (%)
essential oil	0	10	7.9 ± 0.2	
	300	10	7.1 ± 0.3	11
extract A	0	10	7.9 ± 0.2	
	300	10	1.0 ± 0.1*	87
extract B	0	10	7.9 ± 0.2	
	300	10	1.6 ± 0.2*	80

[a] Edema values are expressed as means ± SE; *$p < 0.05$ at the Student's *t* test.

DISCUSSION

R. officinalis is a well-known aromatic edible plant producing an essential oil with an antimicrobial effect (*13*−*16*). In traditional medicine, its leaf extracts and decoctions are used as antioxidant and antiinflammatory remedies against eczema and other cutaneous diseases after topical application (*17*, *18*).

The presented data on the in vivo topical antiinflammatory proprieties of *R. officinalis* leaf extracts, as well as of the extracts obtained from their residue after steam distillation, support the traditional use of the plant leaves against cutaneous inflammatory diseases. A potent topical antiinflammatory activity was observed for the rosemary CE-1 extract (ID_{50} = 83 μg/cm^2) obtained by maceration of the leaves. The potency of CE-1 was similar to that of indomethacin (ID_{50} 93 μg/cm^2) and three times more potent than that of the HE extract (ID_{50} = 265 μg/cm^2). Moreover, the antiphlogistic effect of CE-1 seems to account for the antiinflammatory activity of the whole herbal drug since 300 μg/cm^2 of a virtual total extract or the corresponding amount of CE-1 (136.5 μg/cm^2) exhibited similar activity (78 and 76% edema inhibition, respectively).

The bioassay-oriented fractionation of the CE-1 revealed that the activity is related to its triterpene constituents, since a triterpene enriched fraction (fraction II) showed an antiinflammatory potency similar to that of the parent extract (**Table 4**). Ursolic, oleanolic, and micromeric acids were identified as its antiinflammatory principles. Similar to previous observations for some *Thymus* and *Salvia* species (*19*, *21*, *23*, *20*), ursolic acid was the most active constituent: Its potency (ID_{50} = 0.12 μmol/cm^2) was two-fold higher than that of indomethacin (ID_{50} = 0.26 μmol/cm^2) and about three times higher than that of oleanolic acid (ID_{50} = 0.29 μmol/cm^2). Micromeric acid seems to possess an activity similar to that of oleanolic acid; in fact, at the same dose (100 μg/cm^2, corresponding to 0.22 μmol/cm^2), the two compounds inhibited the edema response by 42 and 45%, respectively. The strong activity shown by ursolic, oleanolic, and micromeric acids (**Table 6**) seems to account for the effect of both the CE-1 extract and the triterpene-enriched fraction II.

A topical antiinflammatory activity of triterpenic acids has already been reported (*39*−*41*), and interestingly, ursolic and oleanolic acids have been found to be the main antiinflammatory principles of other species belonging to the Labiatae family, such as *Salvia officinalis* (*20*), *Thymus willdenowii* (*19*), *Thymus broussonettii* (*21*), and *Thymus satureioides* (*23*). Their antiinflammatory effects have been attributed to the inhibition of different events of inflammatory reactions, such as histamine release, cyclooxygenase-2,5-lypooxygenase, elastase activity, complement activity, and nitric oxide production (*42*−*48*). Furthermore, to the best of our knowledge, this is the first report on the topical antiphlogistic activity of micromeric acid. Although more lipophilic and therefore better absorbed, micromeric acid methyl ester is less active than the free acid, indicating an important role of the free carboxylic group in the antiinflammatory activity of these compounds.

The topical antiinflammatory activity evidenced for the rosemary leaves was also detected in the residue obtained after steam distillation of the drug. In fact, the CE-1 (extract A) and diethyl ether (extract B) extracts, obtained from the leaf distillation residue, showed a topical antiinflammatory activity similar to that of CE-1, whereas the rosemary essential oil did not induce a significant reduction of edematous response.

In conclusion, even though *R. officinalis* is generally considered for its content in essential oil and traditional medicine preparations, an interesting pharmacological activity was observed for the nonvolatile constituents, which provides support to the use of the plant against topical inflammatory disorders. Furthermore, the residue, waste of the steam distillation after the essential oil production, could also be a cheap primary material to obtain fractions or compounds with topical antiinflammatory properties.

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