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Topical anti-inflammatory activity of *Thymus willdenowii*

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Abstract

The topical anti-inflammatory activity of *Thymus willdenowii* Boiss (Labiatae) leaves, a herbal drug used in Moroccan folk medicine, has been studied using the croton oil ear test in mice. A bioassay-oriented fractionation procedure showed that the activity concentrates in the chloroform extract, which has a potency similar to that of indometacin, the non-steroidal anti-inflammatory drug used as reference (ID50 (dose giving 50 % oedema inhibition) = 83 μg cm^{-2} and 93 μg cm^{-2}, respectively). The main compounds responsible for the anti-inflammatory activity of *T. willdenowii* are ursolic acid and oleanolic acid. The flavonoids luteolin-3′-*O*-glucuronide and eriodictyol-7-*O*-glucoside were found for the first time in the genus *Thymus*.

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Introduction

Many species of *Thymus* (Labiatae), and particularly *Thymus communis* L., are widespread aromatic plants of the Mediterranean flora, commonly used as flavourings and medicinal remedies against a variety of diseases (Hernandez et al 1987; Corticchiato et al 1995). The biological activity of some members of the *Thymus* genus are well known and their extracts have been studied for spasmolytic (Van Den Broucke & Lemli 1981; Van Den Broucke et al 1982) and antioxidant activity (Miura & Nakatami 1989) ascribable to their flavonoid content, while thyme essential oils possess antimicrobial activity (Lattaoui et al 1993; Lattaoui & Tantaoui-Elaraki 1994) due to their monoterpenic content. However, despite extensive searching, neither phytochemical nor pharmacological investigation was found in the literature on *T. willdenowii* Boiss, a typical species endemic to Morocco and Tunisia, with the trivial name z'itra. Since the leaves of the plant are currently employed in traditional medicine as an anti-inflammatory remedy against bronchitis, colitis and rheumatism (Bellakhadar et al 1991; Thiri 1996), as a part of our program on Moroccan medicinal plants, a leaf extract was submitted to a phytochemical and pharmacological study to verify its therapeutic potential. A bioassay-oriented fractionation of *T. willdenowii* leaves was carried out using the croton oil ear test in mice as a model of acute inflammation (Tubaro et al 1985). This in-vivo inflammatory model possesses the advantage of using very small amounts of extracts or pure compounds and, consequently, is particularly suitable for bioassay-oriented fractionation studies.

This procedure has shown that the anti-inflammatory activity was concentrated in the *T. willdenowii* chloroform extract, which accounted for the total activity

of the plant. Bioactivity-directed screening and fractionation of the chloroform extract has led to the identification of ursolic and oleanolic acids as its active anti-inflammatory principles. Furthermore, some flavonoids, never previously reported in the genus *Thymus*, were isolated from the methanol extract.

Materials and Methods

Instruments

A Bruker DRX-600 spectrometer operating at 599.2 MHz for ^{1}H and 150.9 for ^{13}C, using the UXNMR software package, was used for NMR measurements in CD_3OD solutions. 1D and 2D NMR spectra were obtained by employing the conventional pulse sequences as previously described (Saturnino et al 1997). Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 1% w/v solutions in methanol. Fast atom bombardment mass spectra (FABMS) were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (XE atoms of energy of 2–6 KV). HPLC separations were performed with a Waters model 6000A pump equipped with a U6K injector and a Model 401 refractive index detector.

Plant material

T. willdenowii Boiss was collected in April 1998 in the region I Franc, Morocco. The plant was identified by Dr Ibn Tatou and a voucher specimen was deposited at the herbarium of the Scientific Institutes, Université Mohammed V, Rabat, Morocco.

Chemicals

Croton oil and indometacin were Sigma products (St Louis, MO). The standards of ursolic and oleanolic acids were supplied by Indena S.p.A. (Milano, Italy) and Roth (Karlsruhe, Germany). Ketamine hydrochloride and TLC plates were purchased respectively from Virbac S.r.l. (Milano, Italy) and Merck (Darmstadt, Germany).

Fractionation procedure

The air-dried leaves of *T. willdenowii* (417 g) were chopped into small pieces, defatted with *n*-hexane, and then consecutively extracted by maceration with chloroform, chloroform–methanol (9:1) and methanol. The extracts were filtered and concentrated in vacuum to give dry hexane, chloroform, chloroform–methanol (9:1) and methanol extracts, respectively.

Part of the methanol extract (7 g) was chromatographed on a Sephadex LH-20 column (100 × 5 cm; Amersham Pharmacia Biotech, Uppsala, Sweden) eluted with methanol. Fractions of 8 mL were collected, checked by TLC (Si gel plates in *n*-butanol–acetic acid–water 60:15:25 and chloroform–methanol–water 80:18:2) and combined to give three main fractions: A (0.84 g), B (4.95 g) and C (1.21 g) (Figure 1). Part of fraction B (100 mg) was subjected to RP-HPLC separation on a μ-Bondapak C-18 column (30 cm × 7.8 mm i.d., flow rate 1.5 mL min^{-1}; Waters Corporation, Milford, USA) with methanol–water (4:6) as a solvent system, whereas part of fraction C (100 mg) was separated using methanol–water (1:1) as a solvent system. Four pure compounds were isolated from fraction B (rosmarinic acid, luteolin-3′-*O*-glucuronide, luteolin-7-*O*-glucoside and eriodyctiol-7-*O*-glucuronide), whereas three pure compounds were isolated from fraction C (luteolin, eriodyctiol and thymonin). They were identified by their experimentally derived NMR, MS and UV spectra in comparison with literature data (Hernandez et al 1987; Tomas-Barberan & Wollenweber 1990; Mahmood et al 1993; Corticchiato et al 1995; Encarnacion et al 1999; Heitz et al 2000).

Part of the chloroform extract (0.84 g) was partitioned by consecutive extractions between ethanol–water containing 1% NaOH (100:250 mL, 5×). The organic layer was dried in vacuum giving fraction I. The combined alkaline aqueous layers were acidified with acetic acid to pH 5 and then re-extracted with ethanol (5×); the combined ethanol layers were dried in vacuum to give fraction II (Figure 1). To isolate the pure compounds, part of fraction II (30 mg) was separated by HPLC on a μ-Bondapak C-18 column (30 cm × 7.8 mm i.d., flow rate 1.5 mL min^{-1}), with methanol–water (9:1) as a solvent system, obtaining oleanolic acid and ursolic acid, identified by their experimental derived NMR spectra in comparison with literature data (Doddrell et al 1974; Seo et al 1975).

Topical anti-inflammatory activity

The topical anti-inflammatory activity was evaluated as inhibition of the croton-oil-induced ear oedema in mice (Tubaro et al 1985). All 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).

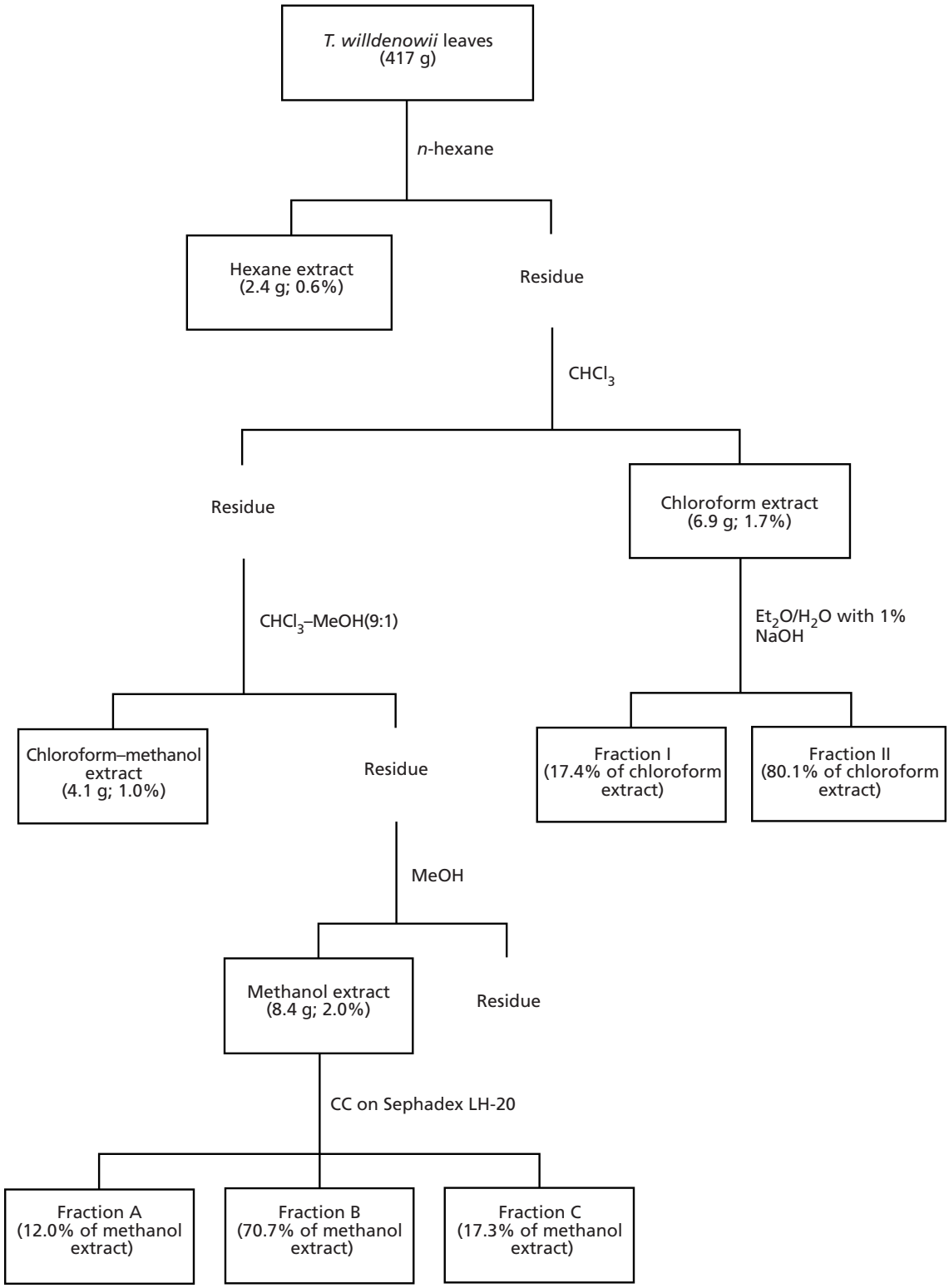

Figure 1 Fractionation procedure for *T. willdenowii* leaves.

Male CD-1 mice, 28–32 g (Harlan-Italy, Udine, Italy), were anaesthetised with ketamine hydrochloride (145 mg kg^{-1}, i.p.). Cutaneous inflammation was induced on the inner surface of the right ear (surface: about 1 cm^2) of anaesthetised mice by application of 80 μg of croton oil dissolved in an appropriate vehicle, as reported below. Control mice received only the irritant solution, whereas the other mice received both the irritant and the test substances. The following vehicles were used: acetone (for hexane, chloroform and chloroform–methanol extracts, fractions I and II, pure compounds and the relevant controls), acetone–ethanol (1 : 1 v/v) (for total extract and its controls), 42 % aqueous ethanol (v/v) (for methanol and its controls). At the maximum oedematous response, 6 h later, mice were sacrificed and a plug (6 mm Ø) was removed from both the treated (right) and the untreated (left) ears. The oedematous response was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as percentage of the oedema reduction in treated mice compared with the control mice. As a reference, the non steroidal anti-inflammatory drug (NSAID) indometacin was used.

Statistical analysis

Pharmacological data were analysed by Student's *t*-test, and a probability level lower than 0.05 was considered as statistically significant. ID50 values (dose giving 50 % oedema inhibition) were calculated by graphic interpolation of the dose–effect curves.

Results

Bioassay-oriented fractionation

The leaves of *T. willdenowii* were successively extracted with solvents of increasing polarity as reported in Figure 1, to give hexane, chloroform, chloroform–methanol (9 : 1) and methanol extracts in the amounts of 2.4, 6.9, 4.1 and 8.4 g (0.2, 1.7, 1.0 and 2.0 % with respect to dry plant material), respectively. Each extract was submitted to the croton oil ear test for a preliminary screening of its anti-inflammatory activity.

Screening of the anti-inflammatory activity

The anti-oedematous effect of the extracts, administered at a dose of 300 μg cm^{-2}, is reported in Table 1. All the extracts exerted some anti-inflammatory activity, inducing oedema inhibition in the range of 20 % (methanol extract) to 92 % (chloroform extract).

Table 1 Anti-inflammatory activity of *T. willdenowii* extracts after 6 h induction of croton oil mouse ear dermatitis.

Substance	Dose ($\mu g\ cm^{-2}$)	No. of mice	Oedema (mg)	Oedema reduction (%)
Control	–	10	7.5 ± 0.3	–
Hexane extract	300	10	3.4 ± 0.3*	55
Chloroform extract	300	11	0.6 ± 0.1*	92
Chloroform–methanol extract	300	10	5.0 ± 0.4*	33
Control	–	10	6.6 ± 0.4	–
Methanol extract	300	10	5.3 ± 0.5*	20

Oedema values are expressed as mean ± s.e. *$P < 0.05$, using Student's *t*-test, as compared with the respective controls.

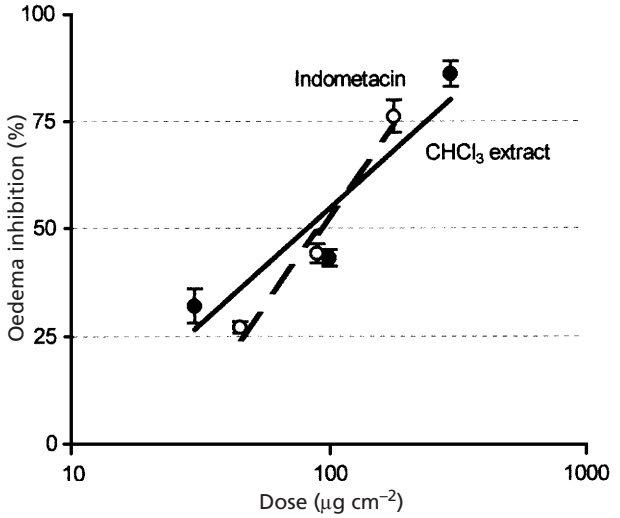

Figure 2 Dose-dependent inhibition, by *T. willdenowii* chloroform extract and indometacin, of oedema induced by croton oil in mouse ear (data represent the mean ± s.e.).

The dose–activity relationship of the most active extract (chloroform) was compared with that of the reference drug indometacin. The extract provoked a dose-dependent oedema inhibition similar to that induced by indometacin (ID50 (dose inducing 50 % oedema inhibition) = 82 and 93 $\mu g\ cm^{-2}$, respectively (Figure 2)).

The effect of the chloroform extract was compared with that of a virtual total extract, prepared as the pool of hexane (11.0 %), chloroform (31.7 %), chloroform–methanol (18.8 %) and methanol (38.5 %) extracts, according to the respective extraction yields. As reported

Table 2 Anti-inflammatory activity of the total and the chloroform *T. willdenowii* leaf extracts after 6 h induction of croton oil mouse ear dermatitis.

Substance	Dose (μg cm^{-2})	No. of mice	Oedema (mg)	Oedema reduction (%)
Control	–	10	7.1 ± 0.4	–
Total extract	300	9	3.7 ± 0.5*	48
Control	–	11	7.6 ± 0.2	–
Chloroform extract	100	11	3.8 ± 0.1*	50

Oedema values are expressed as mean ± s.e. $*P < 0.005$, using Student's *t*-test, as compared with the respective controls.

Table 3 Anti-inflammatory activity of fractions I and II from the *T. willdenowii* leaf chloroform extract after 6 h induction of croton oil mouse ear dermatitis.

Substance	Dose (μg cm^{-2})	No. of mice	Oedema (mg)	Oedema reduction (%)
Control	–	20	7.6 ± 0.3	–
Chloroform extract	300	10	1.3 ± 0.3*	83
Fraction I	52[a]	11	5.3 ± 0.4*	30
Fraction II	240[a]	10	2.4 ± 0.5*	68

Oedema values are expressed as mean ± s.e. $*P < 0.001$, using Student's *t*-test, as compared with controls; [a]dose of the fraction equivalent to 300 μg of the chloroform extract.

in Table 2, 300 μg cm^{-2} of total extract or a corresponding amount of chloroform extract (100 μg cm^{-2}) exhibited similar activity (48 and 50% oedema inhibition, respectively). Therefore, the anti-inflammatory effect of the chloroform extract is greater than the activity of the hexane, chloroform–methanol and methanol extracts, and these extracts act without synergistic effects.

Phytochemical analysis of the chloroform extract

TLC analysis of the chloroform extract, being the most active, 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–water containing 1% NaOH, two main fractions were obtained: fractions I (0.14 g) and II (0.67 g), which represented 17.4% and 80.1% of the parent extract, respectively (Figure 1).

Separation of fraction II by reverse-phase HPLC followed by ^{1}H NMR analysis revealed ursolic and oleanolic acids as its only components, in the ratio 35:65 (Doddrell et al 1974; Seo et al 1975).

Anti-inflammatory activity of the partially purified fractions I and II

Fractions I and II were evaluated for their anti-inflammatory activity at the respective doses of 52 and 240 μg cm^{-2}, calculated on the basis of the fractionation yield and corresponding to 300 μg of the parent chloroform extract. Fraction II reduced the oedematous response by 68%, showing an activity greater than that of fraction I, which provoked only 30% oedema reduction (Table 3). Therefore, fraction II, containing ursolic and oleanolic acids and representing 80% of the parent chloroform extract, accounts for almost all its activity, and for that of the whole plant.

Anti-inflammatory activity of ursolic acid and oleanolic acid

The two constituents of fraction II, ursolic and oleanolic acids, were submitted to the anti-inflammatory activity assay, and a significant dose-dependent inhibition of the oedematous response was induced by both the compounds (Figure 3). Ursolic acid was more potent than oleanolic acid (ID50 = 56 and 132 μg cm^{-2}, correspond-

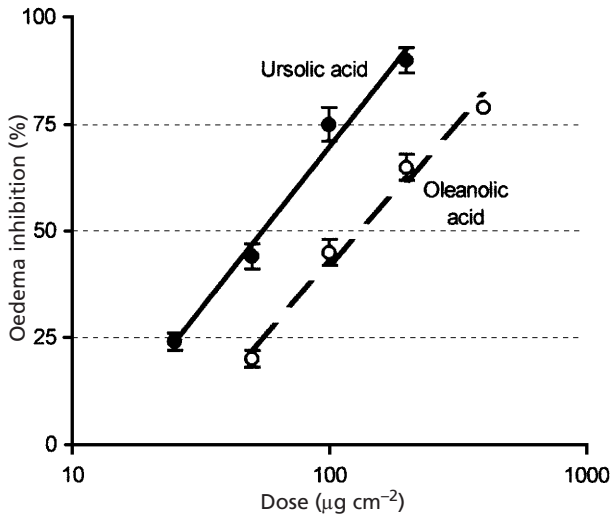

Figure 3 Dose-dependent inhibition, by ursolic and oleanolic acids, of oedema induced by croton oil in mouse ear (data represent the mean ± s.e.).

ing to 0.12 and 0.29 μmol cm^{-2}, respectively), as well as indometacin (ID50 = 93 μg cm^{-2} corresponding to 0.26 μmol cm^{-2}). The activity shown by these triterpenic acids accounts for the oedema inhibition observed with fraction II, containing oleanolic and ursolic acids (65:35).

Phytochemical analysis of the methanol extract

TLC analysis of the *T. willdenowii* leaf methanol extract, the most abundant one, revealed the presence of flavonoids as its major constituents. This extract was subjected to a preliminary fractionation on Sephadex LH-20 column to give fractions A, B and C (Figure 1). The most representative fractions were fraction B (70.7 %) containing flavonoid glycosides, and fraction C (18.1 %) containing flavonoid aglycones. In particular, RP-HPLC separation of fraction B gave luteolin-3′-*O*-glucuronide (R_t = 15 min; isolated amount, 8 mg), luteolin-7-*O*-glucoside (R_t = 19 min; isolated amount, 15 mg) and eriodictyol-7-*O*-glucoside (R_t = 25 min; isolated amount, 9.1 mg) as its major constituents, besides rosmarinic acid (R_t = 12 min; isolated amount, 4 mg). The main components of fraction C were luteolin (R_t = 15.5 min; isolated amount, 3.7 mg), eriodictyol (R_t = 20.1 min; isolated amount, 2.5 mg) and thymonin (R_t = 30 min; isolated amount, 1.8 mg). Among these compounds, the flavonoids luteolin-3′-*O*-glucuronide and eriodictyol-7-*O*-glucoside were found for the first time in the genus *Thymus*.

Rosmarinic acid (Mahmood et al 1993), luteolin-3′-*O*-glucuronide (Heitz et al 2000), luteolin-7-*O*-glucoside (Hernandez et al 1987), eriodictyol-7-*O*-glucoside (Tomas-Barberan & Wollenweber 1990), luteolin (Hernandez et al 1987; Corticchiato et al 1995), eriodictyol (Corticchiato et al 1995; Encarnacion et al 1999) and thymonin (Hernandez et al 1987; Tomas-Barberan & Wollenweber 1990) were identified by their experimentally derived NMR, MS and UV spectra in comparison with literature data.

Discussion

Although the leaves of *Thymus willdenowii* are empirically used in Moroccan folk medicine, this is the first study on their pharmacological property as well their chemical composition. In particular, following a bioassay-oriented fractionation, the topical anti-inflammatory property of some extracts obtained from *T. willdenowii* leaves were demonstrated. The most active extract was the chloroform, which antiphlogistic potency was slightly higher than that of the NSAID indometacin (ID50 = 82 and 93 μg cm^{-2}, respectively). Moreover, the antiphlogistic effect of the *T. willdenowii* leaf chloroform extract apparently accounts for the anti-inflammatory activity of the whole herbal drug.

Phytochemical and pharmacological investigation of this extract allowed identification of the triterpenes ursolic and oleanolic acids as its anti-inflammatory principles. Indeed, the strong activity of ursolic and oleanolic acid (ID50 = 0.12 and 0.29 μmol cm^{-2}), about two fold higher or similar to that of indometacin (ID50 = 0.26 μmol cm^{-2}), accounted for the global effect of the parent extract and also for the total extract.

A topical anti-inflammatory activity of these triterpenic compounds has already been reported (Huang et al 1994; Recio et al 1995a, b) and, interestingly, ursolic acid has been found to be the main anti-inflammatory principle of another species belonging to the Labiatae family, *Salvia officinalis* L. (Baricevic et al 2001). Moreover, these compounds are also reported to be active after oral or intraperitoneal administration, as observed in carrageenan-induced rat paw oedema (Kosuge et al 1985; Singh et al 1992; Kapil & Sharma 1995; Recio et al 1995a, b). Therefore, their effect is not only limited to a topical action, but can also be obtained after systemic administration. Consequently, it can be hypothesized that ursolic and oleanolic acids can contribute to the systemic anti-inflammatory properties of *T. willdenowii*. In fact, a decoction of leaves of this plant is traditionally used in Morocco to alleviate some inflammatory-based diseases, like bronchitis, colitis and rheumatisms (Bellakhadar et al 1991; Thiri 1996).

The hydrophilic methanol extract is the most abundant one, representing about 40 % of the total extraction products obtained from *T. willdenowii* leaves. Its phytochemical characterization revealed, besides rosmarinic acid, the presence of the flavonoids luteolin, eriodictyol and thymonin, and, in higher amounts, the flavonoid glycosides luteolin-3′-*O*-glucuronide, luteolin-7-*O*-glucoside and eriodictyol-7-*O*-glucoside.

Flavonoids are known to possess in-vitro biological properties related to an anti-inflammatory effect. In fact, some of them are able to inhibit the enzymes involved in arachidonic acid metabolism and, in particular, luteolin-3′-*O*-glucuronide has been reported to possess antioxidant activity (Okamura et al 1994), to act as a lipid peroxide formation and lipooxygenase inhibitor (Kubo et al 1992a, b), and, like other natural glucuronides, to inhibit β-glucuronidase (Narita et al 1993). Other flavonoids, like apigenin, luteolin and kaempferol derivatives, are reported also to possess topical anti-inflammatory activity (Della Loggia et al 1986; Tubaro et al 1989). Generally, the aglycones of

flavonoids are more potent than the respective glycosides in these in-vivo effects (Della Loggia et al 1986). However, despite these properties of flavonoids, *T. willdenowii* methanol extract, containing such compounds, does not give a significant contribution to the whole topical anti-inflammatory activity of the plant. This could be explained by the relatively high content of flavonoid glycosides in the extract (about 70%), which can be expected to be less active than their aglycones. Moreover, due to the metabolic fate of flavonoids after oral administration (Hollman & Katan 1999), it can be hypothesized that the flavonoidic component of *T. willdenowii* does not significantly contribute to the systemic anti-inflammatory effect of its leaf decoctions.

It has to be emphasized that, among the flavonoid glycosides, luteolin-3′-*O*-glucuronide and eriodictyol-7-*O*-glucoside have been identified for the first time in a *Thymus* species, even though they have been isolated from other species of the Labiatae family, such as *Rosmarinus officinalis* (Okamura et al 1994) and *Mentha spicata* (Nair & Gunasegaran 1981).

In conclusion, the obtained results demonstrate the anti-inflammatory property of *Thymus willdenowii*, attributed to the triterpenes ursolic and oleanolic acids, providing a support to the traditional use of the plant in the folk medicine of Morocco and Tunisia against inflammatory-based disorders.

References

Baricevic, D., Sosa, S., Della Loggia, R., Tubaro, A., Simonovska, B., Krasna, A., Zupancic, A. (2001) Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: the relevance of ursolic acid. *J. Ethnopharmacol.* **75**: 125–132

Bellakhadar, J., Claisse, R., Fleurentin, J., Younos, C. (1991) Repertory of standard herbal drugs in the Moroccan pharmacopoea. *J. Ethnopharmacol.* **35**: 123–143

Corticchiato, M., Bernardini, A., Costa, J., Bayet, C., Saunois, A., Voirin, B. (1995) Free flavonoid aglycones from *Thymus* herba barona and its monoterpenoid chemotypes. *Phytochemistry* **40**: 115–120

Della Loggia, R., Tubaro, A., Dri, P., Zilli, C., Del Negro, P. (1986) The role of flavonoids in the anti-inflammatory activity of *Chamomilla recutita*. *Progr. Clin. Biol. Res.* **213**: 481–484

Doddrell, D. M., Khong, P. W., Lewis, K. G. (1974) The stereochemical dependence of ^{13}C chemical shifts in olean-12-enes and urs-12-enes as an aid to structural assignments. *Tetrahedron Lett.* **14**: 2381–2385

Encarnacion, R. D., Nogueiras, C. L., Salinas, V. H. A., Anthoni, U., Nielsen, P. H., Christophersen, C. (1999) Isolation of eriodictyol identical with huazhongilexone from *Solanum hindsianum*. *Acta Chem. Scand.* **53**: 375–377

Heitz, A., Carnat, A., Fraisse, D., Carnat, A. P., Lamaison, J. L. (2000) Luteolin-3′-glucuronide, the major flavonoid from *Melissa officinalis* subsp. *officinalis*. *Fitoterapia* **71**: 201–202

Hernandez, L. M., Tomas-Barberan, F. A., Tomas-Lorente, F. (1987) A chemotaxonomic study of free flavone aglycones from some Iberian *Thymus* species. *Biochem. Syst. Ecol.* **15**: 61–67

Hollman, P. C. H., Katan, M. B. (1999) Dietary flavonoids: intake, health effects and bioavailability. *Food Chem. Toxicol.* **37**: 937–942

Huang, M.-T., Ho, C.-T., Wang, Z. Y., Ferraro, T., Lou, Y.-R., Sta'uber, K., Ma, W., Georgiadis, C., Laskin, D. L., Conney, A. H. (1994) Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. *Cancer Res.* **54**: 701–708

Kapil, A., Sharma, S. (1995) Effect of oleanolic acid on complement in adjuvant- and carrageenan-induced inflammation in rats. *J. Pharm. Pharmacol.* **47**: 585–587

Kosuge, T., Yokota, M., Sugiyama, K., Mure, T., Yamazawa, H., Yamamoto, T. (1985) Studies on bioactive substances in crude drugs used for arthritic diseases in traditional Chinese medicine. III. Isolation and identification of anti-inflammatory and analgesic principles from the whole herb of *Pyrola rotundifolia* L. *Chem. Pharm. Bull.* **33**: 5355–5357

Kubo, M., Yanagisawa, T., Sasaki, H., Kaizu, R. (1992a) Lipoxygenase inhibitors as pharmaceuticals from *Schizonepeta tenuifolia*. Jpn. Kokai Tokkyo Koho, CODEN: JKXXAF JP 04208221 A2 19920729 Heisei

Kubo, M., Yanagisawa, T., Sasaki, H., Sato, S. (1992b) Phenol compounds as lipid peroxide formation inhibitors for therapeutic use. Jpn. Kokai Tokkyo Koho , CODEN: JKXXAF JP 04187631 A2 19920706 Heisei

Lattaoui, N., Tantaoui-Elaraki, A. (1994) Comparative kinetics of microbial destruction by the essential oils of *Thymus broussonettii*, *T. zygis* and *T. satureioides*. *J. Ess. Oil Res.* **6**: 165–171

Lattaoui, N., Tantaoui-Elaraki, A., Errifi, A. (1993) Composition and antimicrobial activity of the essential oils of *Thymus broussonettii*, *T. zygis* and *T. satureioides J. Ess. Oil Res.* **5**: 45–53

Mahmood, N., Pizza, C., Aquino, R., De Tommasi, N., Piacente, S., Colman, S., Burke, A., Hay, A. J. (1993) Inhibition of HIV infection by flavonoids. *J. Antiviral Res.* **22**: 189–199

Miura, K., Nakatami, N. (1989) Antioxidative activity of flavonoids from thyme (*Thymus vulgaris* L.). *Agric. Biol. Chem.* **53**: 3043–3045

Nair, A. G. R., Gunasegaran, R. (1981) Rare flavone glucuronides from *Mentha spicata*. *Curr. Sci.* **50**: 181

Narita, M., Nagai, E., Hagiwara, H., Aburada, M., Yokoi, T., Kamataki, T. (1993) Inhibition of β-glucuronidase by natural glucuronides of Kampo medicines using glucuronide of SN-38 (7-ethyl-10-hydroxycamptothecin) as substrate. *Xenobiotica* **23**: 5–10

Okamura, N., Haraguchi, H., Hashimoto, K., Yagi, A. (1994) Flavonoids in *Rosmarinus officinalis* leaves. *Phytochemistry* **37**: 1463–1466

Recio, M. D. C., Giner, R. M., Máñez, S., Gueho, J., Julien, H. R., Hostettmann, K., Ríos, J. L. (1995a) Investigation of the steroidal anti-inflammatory activity of triterpenoids from *Diospyros leucomelas*. *Planta Med.* **61**: 9–12

Recio, M. D. C., Giner, R. M., Máñez, S., Ríos, J. L. (1995b) Structural requirements for the anti-inflammatory activity of natural triterpenoids. *Planta Med.* **61**: 182–185

Saturnino, P., Capasso, A., Saturnino, C., De Martino, G., Lozano Reyes, N., Aquino, R. (1997) Flavonol glycosides from *Aristeguetia discolor* and their inhibitory activity on electrically-stimulated guinea pig ileum. *Int. J. Pharmacog.* **35**: 305–312

Seo, S., Tomita, S., Tori, K. (1975) Carbon-13 NMR spectra of urs-12-enes and application to structural assignments of components of *Isoton japonicus* Hara tissue cultures. *Tetrahedron Lett.* **15**: 7–10

Singh, G. B., Singh, S., Bani, S., Gupta, B. D., Banerjee, S. K. (1992) Anti-inflammatory activity of oleanolic acid in rats and mice. *J. Pharm. Pharmacol.* **44**: 456–458

Thiri, B. (1996) *Contribution a l'étude biosystématique et chimique du genre* Thymus (*Labiatae*) *du Maroc*. Thèse de Doctorat de 3° cycle, Université Mohamed V, Faculté des Sciences, Rabat, Maroc, N. 1441

Tomas-Barberan, F. A., Wollenweber, E. (1990) Flavonoid aglycones from the leaf surfaces of some Labiatae species. *Pl. Syst. Evol.* **173**: 109–118

Tubaro, A., Dri, P., Delbello, G., Zilli, C., Della Loggia, R. (1985) The Croton oil ear test revisited. *Agents Actions* **17**: 347–349

Tubaro, A., Del Negro, P., Bianchi, P., Romussi, G., Della Loggia, R. (1989) Topical anti-inflammatory activity of a new acylated flavonoid. *Agents Actions* **26**: 229–230

Van Den Broucke, C. O., Lemli, J. A. (1981) Pharmacological and chemical investigation of Thyme liquid extract. *Planta Med.* **41**: 123–127

Van Den Broucke, C. O., Lemli, J. A., Lamy, J. (1982) Action spasmolytique des flavones de différentes espèces de *Thymus*. *Plantes Médicinales et Phytothérapie* **XVI**: 310–317.