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Topical anti-inflammatory activity of extracts and compounds from *Hypericum perforatum* L.

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Abstract

Three preparations of *Hypericum perforatum* L. (a hydroalcoholic extract, a lipophilic extract and an ethylacetic fraction) and the pure compounds hypericin, adhyperforin, amentoflavone, hyperoside, isoquercitrin, hyperforin dicyclohexylammonium (DHCA) salt and dicyclohexylamine were evaluated for their topical anti-inflammatory activity. *H. perforatum* preparations provoked a dose-dependent reduction of Croton-oil-induced ear oedema in mice, showing the following rank order of activity: lipophilic extract > ethylacetic fraction > hydroalcoholic extract (ID50 (dose that inhibited oedema by 50%) 220, 267 and >1000 μg cm^{-2}, respectively). Amentoflavone (ID50 0.16 μmol cm^{-2}), hypericin (ID50 0.25 μmol cm^{-2}), hyperforin DHCA salt (ID50 0.25 μmol cm^{-2}) and adhyperofrin (ID50 0.30 μmol cm^{-2}) had anti-inflammatory activity that was more potent or comparable to that of indometacin (ID50 0.26 μmol cm^{-2}), whereas isoquercitrin and hyperoside were less active (ID50 about 1 μmol cm^{-2}). As dicyclohexylamine alone was inactive, the effect of hyperforin DHCA salt can be attributed completely to the phloroglucinol moiety. The pharmacological activity and phytochemical profile of the tested extracts and fraction suggest that different constituents are involved in the topical antiphlogistic property of *H. perforatum* in-vivo.

Introduction

Hypericum perforatum L., commonly called St John's wort, is a perennial herb of the Hypericaceae family, widely distributed in Europe, Asia and Africa, and naturalized in America (Bombardelli and Morazzoni 1995). Its flowering tops contain different compounds, including the phloroglucinols hyperforin and adhyperforin, the naphthodianthrones hypericin and pseudohypericin, the flavonoids hyperoside, quercetin, rutin, quercitrin and isoquercitrin, and the dimeric flavonoids amentoflavone and I3,II8-biapigenin (Bombardelli and Morazzoni 1995; Greeson et al 2001; Patočka 2003).

St John's wort has been used for centuries as a medicinal plant. More recently it has received considerable attention for its efficacy against mild and moderate depression (Barnes et al 2001; Bilia et al 2002), comparable to that of standard antidepressants but with fewer side-effects (Linde et al 1996; Kasper 2001; Schulz 2002; Müller 2003). *H. perforatum* is also used traditionally for its anti-inflammatory, antimicrobial and wound-healing properties, and is useful in the topical treatment of dermatitis, sores, rashes, burns and wounds (Bombardelli and Morazzoni 1995; Schempp et al 2002). The most popular preparation for topical use is St John's wort oil, obtained by macerating the flowering aerial parts with vegetable oil (Bombardelli and Morazzoni 1995; Schempp et al 2002).

Pharmacological studies on *H. perforatum* have focused mainly on its antidepressant activity, attributed initially to hypericin and subsequently to hyperforin (Chatterjee et al 1998a,b; Laakman et al 1998; Zanoli 2004), whereas only a few studies have documented its in-vivo anti-inflammatory properties following systemic administration (Shipochliev et al 1981; Kumar et al 2001; Raso et al 2002; Abdel-Salam 2005). In-vitro studies have shown that extracts or pure constituents of *H. perforatum* inhibit some events involved in inflammatory reactions. In particular, *H. perforatum* extracts inhibit free-radical production (Hunt et al 2001), myeloperoxidase (Pabuçcuoğlu et al 2003), cyclooxygenase-1, 5-lipoxygenase (Albert et al 2002) and the expression of inducible cyclooxygenase and nitric oxide synthase (Raso et al 2002; Tedeschi et al 2003). Furthermore, hypericin has been

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shown to inhibit the transcription factor NF-κB, which up-regulates genes that encode inflammatory macromolecules (Bork et al 1999). Hypericin also possesses antioxidant activity, acts as a free-radical scavenger (Cakir et al 2003), inhibits the formation of interleukin (IL)-1α and IL-12 (Panossian et al 1996; Kang et al 2001) and inhibits the release of arachidonic acid from phospholipids and its metabolism through 5- and 12-lipoxygenase pathways (Panoissian et al 1996; Bezáková et al 1999). The phloroglucinol derivative hyperforin has been shown to possess antioxidant properties (Heilman et al 2003) and to inhibit the formation of reactive oxygen species, the release of elastase from leucocytes (Feisst and Werz 2004), IL-6 (Gobbi et al 2004) and cyclooxygenase-1 and 5-lipoxygenase (Albert et al 2002). Furthermore, some flavonoids and biflavones have been shown to act as antioxidants and free radical scavengers (Cakir et al 2003), to inhibit nitric oxide synthase (Luo et al 2004) and neutrophil elastase (Melzig et al 2001a,b), and to have effective anti-inflammatory activity in-vivo (Della Loggia et al 1986, 1996; Kim et al 1998a; Middleton, 1998).

Despite the traditional use of *H. perforatum* as a topical anti-inflammatory, no pharmacological studies have documented its topical antiphlogistic activity, and the anti-inflammatory constituents of this species are still under discussion. Only one study has shown an immunomodulatory effect for a *H. perforatum* ointment, which reduced stimulation of T lymphocytes by epidermal cells after application to skin (Schempp et al 2000). Clinical studies have shown that St John's wort is effective against bedsores (Lomagno and Lomagno 1979) and atopic dermatitis (Schempp et al 2003).

To verify the topical anti-inflammatory properties of *H. perforatum*, we have characterized three extracts from its flowering tops (a hydroalcoholic extract, a lipophilic extract and a partially purified hydroalcoholic extract (ethylacetic fraction)) and evaluated their ability to inhibit Croton-oil-induced ear oedema in mice. The activity of these extracts was compared with that of some pure compounds from *H. perforatum* (hypericin, adhyperforin, amentoflavone, hyperoside and isoquercitrin). Because of the instability of pure hyperforin, the activity of this compound was evaluated by comparing the effect of its dicyclohexylammonium (DCHA) salt (1:1) to that of dicyclohexylamine alone. The non-steroidal anti-inflammatory drug (NSAID) indometacin was used as reference.

Materials and Methods

Materials

Croton oil and indometacin were purchased from Sigma-Aldrich (Milan, Italy). Ketamine hydrochloride was purchased from Virbac S.r.l. (Milan, Italy). The hydroalcoholic extract (Batch 26838/ml, BA 59916/1), the lipophilic extract (Batch 507/39/C, BA 98/415/LR), the ethylacetic fraction (IDN 5311; Batch 38810, BA 98/750/LR), hypericin (Batch 517/47), hyperforin DHCA salt (1:1; IDN 5522; Batch 38929), dicyclohexylamine (Batch 424112/141601), adhyperforin (Batch 38897/2), amentoflavone (Batch 597/41), hyperoside (Batch 121/18) and isoquercitrin (Batch 03031711) were prepared by Indena S.p.A. (Milan, Italy). The other reagents, of analytical grade, were from Carlo Erba (Milan, Italy).

Preparation of extracts and purified fractions

Flowering tops from *H. perforatum* were extracted by percolation with 80% aqueous methanol (v/v). The percolate was filtered, concentrated under vacuum and homogenized. The soft extract obtained was then dried (maximum temperature 130 ± 10°C) and ground to obtain the dry hydroalcoholic extract. The lipophilic extract was prepared by supercritical carbon dioxide extraction of the flowering tops from *H. perforatum*. The purified fraction was prepared by percolation of flowering tops from *H. perforatum* with 70% aqueous ethanol (v/v). The hydroalcoholic solution was concentrated under vacuum and partitioned with ethyl acetate. The ethylacetic phase was filtered, concentrated and dried under vacuum (70°C for 24 h), obtaining the ethylacetic fraction (IDN 5311).

HPLC analysis of the extracts and ethylacetic fraction

HPLC analysis was carried out at 30°C using a 201 TP 54 RP-18 column (250 × 4.6 mm internal diameter, 5 μm, 300 Å; Vydac separation group Hesperia, CA, USA), protected with an Alltech direct-connect universal column prefilter of 2 μm porosity (Deerfield, IL, USA). Separation was carried out using three solvents: A, water:85% phosphoric acid (99.7:0.3 v/v), B, acetonitrile; C, methanol in a linear gradient programme, as previously reported (Brolis et al 1998). The flow rate was 1.0 mL min^{-1}, the injection volume was 10 μL and the dwell volume was 1.5 mL. Peaks were detected at 270 nm (Brolis et al 1998).

Quantitative analysis of the extracts and fraction was carried out by HPLC using rutin as external standard, as reported by Brolis et al (1998).

Animals and experimental conditions

Male albino Swiss mice (28–32 g) were purchased from Harlan Italy (Udine, Italy) and were kept for at least 1 week before the experiments at controlled temperature (21 ± 1°C) and humidity (60–70%), with a fixed artificial light cycle (07.00–19.00). Inflammation was induced from 10.00 to 12.00 AM in order to avoid variations in the phlogistic response due to circadian fluctuations in endogenous corticosteroid levels (House et al 1997).

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) relating to animal welfare.

Topical anti-inflammatory activity

Topical anti-inflammatory activity was evaluated as inhibition of Croton-oil-induced ear oedema in mice (Tubaro et al 1985). Mice were anaesthetized with ketamine hydrochloride (145 mg kg^{-1} i.p.). Inflammation was induced in the right ear

by applying Croton oil, 80 μg dissolved in 15 μL vehicle, to the inner surface of the ear (about 1 cm^2). The left ear remained untreated. Control animals were treated only with Croton oil; other animals were treated with both Croton oil and the substances being tested. The following vehicles were used: acetone for extracts, the ethylacetic fraction, hypericin, hyperforin DHCA salt, dicyclohexylamine and the relevant controls; ethanol:acetone (3:1, v/v) for hyperoside and its controls; ethanol:acetone (1:1, v/v) for adhyperforin, amentoflavone, isoquercitrin and the relevant controls. Preliminary experiments showed that none of the vehicles affected the inflammatory response or induced irritation. After 6 h, mice were sacrificed and a diskette (6 mm diameter) was removed from both the treated and the untreated ears. Oedema was measured as the difference in weight between the two diskettes; anti-inflammatory activity was expressed as percentage inhibition of oedema in treated mice compared with control mice. At least two experimental groups of five animals were tested for each dose.

Statistical analysis

Oedema was expressed as mean ± s.d. Oedema values were analysed by one-way analysis of variance followed by the Dunnett's test for multiple comparisons of unpaired data. A P value of less than 0.05 was considered significant. ID50 values (the dose that inhibited oedema by 50%) were calculated by interpolation of dose–effect curves.

Results

HPLC profile of the extracts and ethylacetic fraction

Table 1 shows the main components of the extracts and the ethylacetic fraction of *H. perforatum* analysed by HPLC. The hydroalcoholic extract contained relatively high concentrations of hyperoside (4.88%) and hyperforin (4.50%) but low concentrations of hypericin and pseudohypericin (0.09% and 0.18%, respectively). The lipophilic extract contained no hyperoside, hypericin or pseudohypericin but contained high concentrations of hyperforin and adhyperforin (27.02% and 5.23%, respectively). The ethylacetic fraction contained relatively high concentrations of hyperforin (10.27%), hyperoside (9.29%) and adhyperforin (0.80%) but low concentrations of hypericin and pseudohypericin (0.06% and 0.21%, respectively).

Table 1 Main components of the extracts and ethylacetic fraction of *H. perforatum*

Compound	Hydroalcoholic extract	Lipophilic extract	Ethylacetic fraction
Chlorogenic acid	3.39%	n.d.	0.05%
Rutin	3.42%	n.d.	5.40%
Hyperoside	4.88%	n.d.	9.29%
Isoquercitrin	2.29%	n.d.	3.84%
Quercitrin	0.56%	n.d.	0.95%
Quercetin	0.29%	n.d.	3.20%
I3,II8-biapigenin	0.36%	n.d.	1.18%
Amentoflavone	n.d.	n.d.	n.d.
Pseudohypericin	0.18%	n.d.	0.21%
Hypericin	0.09%	n.d.	0.06%
Hyperforin	4.50%	27.02%	10.27%
Adhyperforin	0.38%	5.23%	0.80%

n.d. = not detected (< 0.05%, practical limit of detection).

Topical anti-inflammatory activity of the extracts and fraction

The topical anti-inflammatory activities of *H. perforatum* extracts and ethylacetic fraction are summarized in Table 2. At doses ranging from 100 to 1000 $\mu g\,cm^{-2}$, the hydroalcoholic extract induced 12–46% reduction of oedema; the lipophilic extract was more active, inhibiting oedema by 27% at a dose of 30 $\mu g\,cm^{-2}$. The ethylacetic fraction (30–1000 $\mu g\,cm^{-2}$) inhibited oedema by 16–77%. The reference drug indometacin, administered at doses ranging from 30 to 300 $\mu g\,cm^{-2}$, inhibited oedema by 18–84%.

The anti-inflammatory potency of the extracts and fraction was determined by calculating ID50 values from dose–effect graphs. The lipophilic extract and ethylacetic fraction were the most active preparations of *H. perforatum* (ID50 values 220 and 267 $\mu g\,cm^{-2}$, respectively), and were about four times more potent than the hydroalcoholic extract (ID50 >1000 $\mu g\,cm^{-2}$) and only 2–3-fold less active than the NSAID indometacin (ID50 93 $\mu g\,cm^{-2}$).

Topical anti-inflammatory activity of pure compounds

The compounds isolated from *H. perforatum* induced a dose-dependent reduction in oedema (Table 3). In particular,

Table 2 Topical anti-inflammatory activity of *H. perforatum* extracts and fractions, measured as the percentage reduction of oedema induced by application of Croton oil to mouse ears and the dose that gives 50% inhibition (ID50)

Substance	Dose ($\mu g\,cm^{-2}$)	Oedema (mg) mean ± s.d.	% reduction	ID50 ($\mu g\,cm^{-2}$)
Controls	–	7.4 ± 0.6	–	
Hydroalcoholic extract	100	6.5 ± 0.9*	12	> 1000
	300	4.9 ± 0.7*	34	
	1000	4.0 ± 0.8*	46	
Lipophilic extract	30	5.4 ± 0.9*	27	220
	100	3.7 ± 1.0*	50	
	300	3.8 ± 1.0*	49	
	1000	3.1 ± 1.1*	58	
Ethylacetic fraction	30	6.2 ± 0.9*	16	267
	100	4.4 ± 0.9*	40	
	300	3.8 ± 0.7*	49	
	1000	1.7 ± 0.6*	77	
Indometacin	30	6.1 ± 0.8*	18	93
	100	3.6 ± 0.6*	51	
	300	1.2 ± 0.5*	84	

*$P < 0.05$ compared with controls (one-way analysis of variance).

Table 3 Topical anti-inflammatory activity of pure compounds from *H. perforatum*, measured as the percentage reduction of oedema induced by application of Croton oil to mouse ears and the dose that gives 50% inhibition (ID50)

Substance	Dose (μmol cm^{-2})	Oedema (mg) mean ± s.d.	% reduction	ID50 (μmol cm^{-2})	ID50 (μg cm^{-2})
Controls	–	7.2 ± 0.7	–	–	–
Adhyperforin	0.100	5.4 ± 0.9*	25	0.30	165
	0.300	4.1 ± 0.8*	43		
	1.000	1.1 ± 0.6*	85		
Controls	–	7.8 ± 0.7	–	–	–
Hypericin	0.125	6.0 ± 0.6*	23	0.25	126
	0.250	4.2 ± 0.7*	46		
	0.500	0.9 ± 0.5*	88		
Controls	–	7.2 ± 0.7	–	–	–
Amentoflavone	0.100	4.6 ± 0.7*	36	0.16	86
	0.300	2.2 ± 0.6*	69		
	1.000	0.4 ± 0.2*	94		
Controls	–	7.5 ± 0.6	–	–	–
Hyperoside	0.250	6.4 ± 0.7*	15	1.00	464
	0.500	5.9 ± 0.6*	21		
	1.000	3.6 ± 0.6*	52		
Controls	–	7.3 ± 0.7	–	–	–
Isoquercitrin	0.100	6.2 ± 0.8	14	0.98	455
	0.300	5.4 ± 0.7*	25		
	1.000	3.5 ± 0.6*	51		
Controls	–	7.3 ± 0.8	–	–	–
Indomethacin	0.125	5.1 ± 0.8*	30	0.26	93
	0.250	3.7 ± 0.7*	49		
	0.500	2.2 ± 0.6*	70		

*$P < 0.05$ compared with controls (one-way analysis of variance).

Table 4 Anti-inflammatory activity of hyperforin dicyclohexylammonium (DCHA) salt, measured as the percentage reduction of oedema induced by application of Croton oil to mouse ears

Substance	Dose (μmol cm^{-2})	Oedema (mg) mean ± s.d.	% reduction vs control	% reduction vs dicyclohexylamine
Controls	–	7.0 ± 0.8	–	–
Dicyclohexylamine	0.1	7.1 ± 0.9	−1	–
	0.3	6.4 ± 0.8	9	–
	1.0	6.3 ± 0.9	11	–
Hyperforin DCHA salt	0.1	5.1 ± 0.7	27*	28**
	0.3	3.4 ± 0.7	51*	47**
	1.0	0.8 ± 0.3	89*	87**

*$P < 0.05$ compared with controls; **$P < 0.05$ compared with the corresponding doses of dicyclohexylamine (one-way analysis of variance).

adhyperforin inhibited oedema by 25–85% at doses of 0.1–1.0 μmol cm^{-2}. Similarly, hypericin inhibited oedema by 23–88% at doses of 0.125–0.5 μmol cm^{-2}. Amentoflavone was more active, reducing oedema by 36% and 94% at doses of 0.1 and 1.0 μmol cm^{-2}, respectively. The flavonoidic glycosides hyperoside and isoquercitrin were less active, inhibiting oedema by about 50% at the highest dose tested (1.0 μmol cm^{-2}).

The activity of pure hyperforin could not be verified because of its instability; instead, the stable hyperforin DHCA salt was tested and its effect compared with that of dicyclohexylamine (Table 4). The hyperforin DHCA salt inhibited the oedematous response in a dose-dependent manner: 0.1–1 μmol cm^{-2} of the salt inhibited oedema by 27–89% whereas the same doses of dicyclohexylamine were inactive, indicating that the effect of the salt can be completely attributed to the hyperforin moiety. The hyperforin DHCA salt, as compared with dicyclohexylamine, inhibited oedema by 28–87%.

The ID50 values of the pure compounds show that amentoflavone was the most active compound (ID50 0.16 μmol cm^{-2}), and was about two fold more potent than the reference drug indometacin (ID50 0.26 μmol cm^{-2}). Hypericin (ID50 0.25 μmol cm^{-2}), adhyperforin (ID50 0.30 μmol cm^{-2})

and hyperforin DHCA (ID50 0.25 μmol cm^{-2}) possessed strong anti-inflammatory activity, similar to that of indometacin. An ID50 value of 0.27 μmol cm^{-2} for hyperforin was estimated by comparing the activity of the hyperforin DHCA salt with that of dicyclohexylamine alone. Hyperoside (ID50 1.0 μmol cm^{-2}) and isoquercitrin (ID50 0.98 μmol cm^{-2}) were the least active compounds.

Discussion

This study describes the topical anti-inflammatory activity of hydroalcoholic and lipophilic extracts and a partially purified hydroalcoholic extract (ethylacetic fraction) of the flowering tops of *H. perforatum*. These preparations inhibited Croton-oil-induced ear oedema in mice in a dose-dependent manner, and had the following rank order of activity: lipophilic extract > ethylacetic fraction > hydroalcoholic extract (ID50: 220, 267 and >1000 μg cm^{-2}, respectively). The activity of the lipophilic extract supports the effectiveness of oily preparations of *H. perforatum* that are traditionally used in folk medicine for the topical treatment of inflammatory disorders (Bombardelli and Morazzoni 1995; Schempp et al 2002).

The lipophilic extract was free of the naphthodianthrones hypericin, pseudohypericin and of the main flavonoid derivatives. It was characterized by a relatively high concentration of the phloroglucinol derivatives hyperforin and adhyperforin (27.02% and 5.23%, respectively), suggesting that these compounds make a significant contribution to its anti-inflammatory activity. The ethylacetic fraction, which had activity comparable to that of the lipophilic extract, contained a high concentration of hyperforin (10.27%) but lower than that of the lipophilic extract. It also contained hyperoside as the main flavonoidic constituent (9.29%) and low concentrations of hypericin and pseudohypericin (0.06% and 0.21%, respectively). The hydroalcoholic extract was the least active and had the lowest concentration of hyperforin (4.50%) and adhyperforin (0.38%); concentrations of hypericin and pseudohypericin were 0.09% and 0.18%, respectively. This extract also contained different flavonoid derivatives, among which hyperoside was the most abundant (4.88%).

The results obtained suggest that hypericin and pesudohypericin, the characteristic naphthodianthrones of *H. perforatum*, are not essential for the anti-inflammatory activity of this species, as the most active extract (the lipophilic one) did not contain these compounds. Indeed, despite the high anti-inflammatory activity of hypericin (ID50 126 μg cm^{-2}), its concentration in the alcoholic extract (0.09%) and ethylacetic fraction (0.06%) was too low to support this activity. Similarly, despite its high antiphlogistic action, amentoflavone (ID50 86 μg cm^{-2}) does not significantly contribute to the topical anti-inflammatory activity of the *H. perforatum* preparations tested, as its concentration was below the limit of detection. The flavonoidic glycosides hyperoside and isoquercitrin showed a low activity (ID50 464 and 455 μg cm^{-2}, respectively) that does not account for the effect of the hydroalcoholic extract or the ethylacetic fraction. Furthermore, since the two compounds were not detected in the most active extract, it seems likely that they do not play a significant role in the anti-inflammatory properties of *H. perforatum*. Thus, we hypothesize that other compounds, such as the phloroglucinols hyperforin and/or adhyperforin, are involved in the antiphlogistic activity of the *Hypericum* preparations tested. Among them, adhyperforin showed a potent effect (ID50 165 μg cm^{-2}) but the concentration of this compound in the preparations tested (0.38–5.23%) was too low to support this activity. The most concentrated compound in the highly active extracts was hyperforin. Because of its instability, this compound was tested as the DHCA salt. The effect of the salt was completely due to hyperforin and the ID50 value for the phloroglucinol equivalent was estimated to be 145 μg cm^{-2} (0.27 μmol cm^{-2}). The anti-inflammatory potency of this compound does not fully support the effect of the tested *H. perforatum* preparations. Moreover, although the concentration of hyperforin in the lipophilic extract (27.02%) was about three-fold higher than that in the ethylacetic fraction (10.27%), the two preparations had similar anti-inflammatory activity (ID50 220 and 267 μg cm^{-2}, respectively), suggesting that other compounds are involved in the antiphlogistic effect of the extracts.

Taken together, these observations suggest that the topical anti-inflammatory property of *H. perforatum* derives from various compounds, which may be acting in a synergistic way, rather than to a single constituent. These compounds could act by different mechanisms, as several in-vitro anti-inflammatory activities have been reported for *H. perforatum* constituents. The pure compounds have shown in-vitro activities related to an in-vivo anti-inflammatory effect, as described in the Introduction. In particular, hypericin has been shown to possess antioxidant properties and is a free-radical scavenger (Cakir et al 2003), inhibits release of arachidonic acid from phospholipids and its metabolism through 5- and 12-lipoxygenase pathways (Panossian et al 1996; Bezáková et al 1999), and inhibits formation of IL-1α and IL-12 formation (Panossian et al 1996; Kang et al 2001). Moreover, hypericin was reported to inhibit NF-κB, a messenger that regulates expression of inflammatory-involved macromolecules (Bork et al 1999). Hyperforin has been shown to possess antioxidant properties (Heilman et al 2003) and inhibits formation of reactive oxygen species, release of elastase from leucocytes (Feisst and Werz 2004), cyclooxygenase-1, 5-lipoxygenase (Albert et al., 2002) and release of IL-6 (Gobbi et al 2004). The flavonoids hyperoside and isoquercitrin have been shown to inhibit neutrophil elastase, an enzyme involved in the pathogenesis of inflammatory reactions (Melzig et al 2001a, b), while hyperoside also inhibits nitric oxide synthase (Luo et al 2004) and isoquercitrin inhibits the biosynthesis and release of prostaglandins (Chanh et al 1986). A variety of actions have been reported for amentoflavone, including antioxidant activity (Mora et al 1990), inhibition of degranulation and arachidoinic acid release from neutrophils (Tordera et al 1994), and inhibition of phospholipase A2 (Kim et al 2001), cyclooxygenases (Kim et al 1998b; Banerjee et al 2002a), cyclooxygenase-2 expression (Banerjee et al 2002b) and inducible nitric oxide synthase (Banerjee et al 2002a).

Conclusion

The hydroalcoholic, lipophilic and partially purified hydroalcoholic extracts of *H. perforatum* possessed different chemical

profiles and had significant topical anti-inflammatory activity, justiying the traditional use of this species in the treatment of inflammatory skin disorders. The potent activity of the lipophilic extract supports the use of oily preparations of *H. perforatum* as topical anti-inflammatory remedies in the traditional medicine of different countries. Moreover, the pure compounds hypericin, hyperforin dicyclohexylammonium, adhyperforin, amentoflavone and, to a less extent, hyperoside and isoquercitrin, showed topical antiphlogistic activity. As none of these compounds was present in sufficient concentration to support the anti-inflammatory activity of the tested extracts, we hypothesize that different constituents are involved in the topical antiphlogistic properties of *H. perforatum*.

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