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ANTI-INFLAMMATORY ACTIVITIES OF FLAVONOIDS FROM *Luxemburgia octandra* FLOWERS

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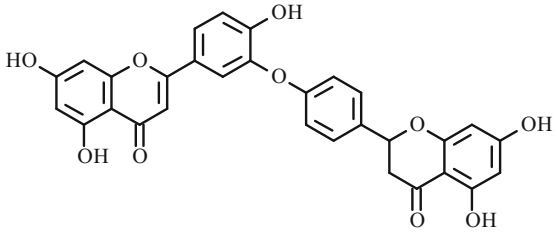
Flavonoids are a class of natural products widely distributed in the plant kingdom. It has been reported that the major bioactive constituents of many medicinal herbs were flavonoids for their antioxidant, anti-inflammatory, and anti-carcinogenic activities [1]. Among them *C*-glycosylflavonoids also have various activities, such as antioxidant, antimolluscidal, antimicrobial, antinociceptive, antispasmodic, antihepatotoxic, anti-inflammatory, antidiabetic, antihypertensive, and others [2]. *Luxemburgia octandra* St. Hil (Ochnaceae) is a shrub or small tree distributed throughout the Brazilian Southeastern region. Ochnaceae is a large tropical family including 40 genera and 600 species with the greatest concentration in tropical South America [3]. In the course of our phytochemical and pharmacological investigations of Brazilian plants, we have studied species of both *Ouratea* and *Luxemburgia* genera, in which the presence of biflavonoids was observed [4–7], along with their antitumor activities [8–10]. In the present study we investigated the anti-inflammatory activity of the flavonoids 2″,3″-dihydro-ochnaflavone (**1**), 7-*O*-methylorientin (**2**), orientin (**3**), orientin 2″-*O*-glucoside (**4**), and catechin (**5**) using the mouse model of ear edema.

The structures of the flavonoids 2″,3″-dihydro-ochnaflavone (**1**), 7-*O*-methylorientin (**2**), orientin (**3**), orientin 2″-*O*-glucoside (**4**), and catechin (**5**) were identified by EI-MS, ^{1}H NMR, ^{13}C NMR, and comparison of those with literature data [11, 12].

Flavonoids **1**–**5** were tested *in vivo* for their ability to reduce the inflammatory response in the TPA-induced ear edema in mice (1.0 mg/ear). Compounds **2** and **3** showed activity in the ear topical test, inducing an edema inhibition over 50%, which was comparable to that of indomethacin at a dose of 0.5 mg/ear (Table 1). Compound **2** showed excellent anti-inflammatory activity and was relatively more effective than compounds **3** and **4** and indomethacin. However, these flavonoids **2**–**4** reduced the edematous response, with compound **2** the most active one. Note that the presence of the methoxy group may be responsible for the large increase in activity. Moreover, it increases the liposolubility and absorption, leading to more pronounced activity [13].



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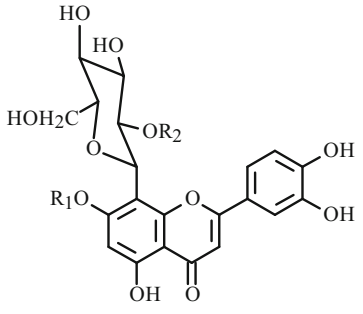

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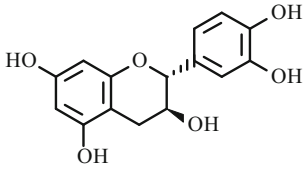

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TABLE 1. Topical Anti-inflammatory Activity of Compounds **1–5** Isolated from Flowers of *L. octandra* on TPA-induced Ear Edema in Mice

Groups	Dose, mg/ear	Weight[a], mg	Inhibition, %
Baseline control (acetone)	–	9.7 ± 0.6[b]	
Control (TPA)	–	20.7 ± 3.6	
2″,3″-Dihydroochnaflavone (**1**)	1.0	18.8 ± 2.1[b]	33.2
7-*O*-Methylorientin (**2**)	1.0	15.4 ± 2.6[b]	59.8
Orientin (**3**)	1.0	15.9 ± 3.2[b]	55.6
Orientin 2″-*O*-glucoside (**4**)	1.0	16.2 ± 2.3[b]	48.2
Catechin (**5**)	1.0	18.5 ± 3.1[b]	31.8
Indomethacin	0.5	15.8 ± 2.6[b]	56.0

[a]Values are mean ± S.D., $n = 5$; [b]$P < 0.05$ vs. TPA group.

The skin inflammation produced by a single application of the protein kinase C activator, TPA, is characterized by erythema, edema, and polymorphonuclear leukocyte infiltration. In the model of acute edema induced by TPA, an efficient reference drug is indomethacin, a cyclooxygenase inhibitor [14]. In conclusion, the flavonoids **2**–**4** may possess anti-inflammatory activity mediated via either inhibition of phospholipase A_2 (PLA_2) activity or cyclooxygenase pathway and by blocking the release of vasoactive substances (histamine, serotonin, and kinins).

These flavonoids are reported for the first time in the flowers of this species, and the results obtained in our studies suggest that *C*-glycosylflavonoids may possibly be the active ingredient effective in the acute TPA model, reducing edema. These findings can facilitate the discovery of essential components that possess therapeutic effects.

Further studies are needed to evaluate the clinical value of the flavonoids of this plant.

Methods. ^{1}H NMR and ^{13}C NMR (1D and 2D) spectra were run in DMSO-d_6 on a JEOL JNM-GX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C). 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Plant Materials. *Luxemburgia octandra* St. Hil (Ochnaceae) flowers were collected in Morro de Sao Sebastiao, Ouro Preto, Minas Gerais, Brazil. A voucher specimen (No. 26197) is deposited at the OUPR-UFOP Herbarium, Instituto de Ciencias Exatas e Biologicas of the Universidade Federal de Ouro Preto-MG, Brazil.

Extraction and Isolation. The dried flowers of *L. octandra* (160.0 g) were powdered and extracted with ethanol by maceration at room temperature. The macerate was concentrated under reduced pressure to yield an ethanolic crude extract (32.0 g). The extract was suspended in MeOH–water (9:1) and partitioned with ethyl acetate. The ethyl acetate residue (8.0 g) was chromatographed on a silica gel column in a gradient system from acetate and methanol as mobile phase. The 76 collected fractions were analyzed by TLC and combined. Fractions 23–25 (406.0 mg) were recrystallized with acetone, furnishing **1** (65 mg). Fractions 30–42 (102 mg) were subjected to preparative TLC deactivated with sodium acetate (5%) and eluting with $CHCl_3$–HCO_2H–MeOH (8.5:0.5:1), yielding **2** (12.0 mg) and **3** (20.0 mg). Fractions 48–55 (96.0 mg) were dissolved in methanol and successively submitted to column chromatography on Sephadex LH-20 using methanol as mobile phase to give **4** (65.0 mg). Fraction 65 (125.0 mg) was chromatographed under the same conditions as the previous fraction, furnishing **5** (10.0 mg).

2″,3″-Dihydroochnaflavone (1). Yellow powder. IR (KBr, ν_{max}, cm^{-1}): 3357, 1646, 1609, 1503, 1440. ^{1}H NMR (400 MHz, acetone-d_6, δ, ppm, J/Hz): 2.8 (dd, J = 17.2, 2.9, H-3″), 3.19 (dd, J = 17.2, 12.4, H-3″), 5.55 (dd, J = 12.4, 2.9, H-2″), 5.96 (s, H-6″), 5.99 (s, H-8″), 6.25 (s, H-6), 6.53 (s, H-8), 6.68 (s, H-3), 7.03 (d, J = 8.8, H-3‴, H-5‴), 7.23 (d, J = 8.4, H-5′), 7.55 (d, J = 8.8, H-2‴, H-6‴), 7.79 (d, J = 2.2, H-2′), 7.86 (dd, J = 8.3, 2.2, H-6′), 12.13 and 12.93 (s, HO-5,5″). ^{13}C NMR, see [16].

7-*O*-Methylorientin (2). Yellow powder. IR (KBr, ν_{max}, cm^{-1}): 3386, 1653, 1618, 1565, 1514, 1439, 1184. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.73 (m, H-5″), 3.28 (m, H-3″), 3.28 (m, H-4″), 3.35 (m, H-6″), 3.88 (s, O$\underline{CH}_3$), 4.52 (m, H-2″), 4.71 (d, J = 9.9, H-1″), 6.52 (s, H-3), 6.68 (s, H-6), 6.84 (d, J = 8.4, H-5′), 7.48 (d, J = 2.0, H-2′), 7.54 (dd, J = 8.4, 2.0, H-6′). ^{13}C NMR, see [16].

Orientin (3). Yellow powder. IR (KBr, ν_{max}, cm^{-1}): 3307, 1675, 1630, 1562, 1515, 1445. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.72 (m, H-5″), 3.25 (m, H-4″), 3.30 (m, H-3″), 3.33 (m, H-6″), 3.98 (m, H-2″), 4.71 (d, J = 9.0, H-1″), 6.22 (s, H-6), 6.62 (s, H-3), 6.80 (d, J = 8.5, H-5′), 7.46 (d, J = 2.0, H-2′), 7.55 (dd, J = 8.5, 2.0, H-6′). ^{13}C NMR, see [16].

Orientin 2″-*O*-glucoside (4). Yellow powder. IR (KBr, ν_{max}, cm^{-1}): 3346, 1636, 1610, 1515, 1450. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.80 (m, H-5″), 3.42 (m, H-6″), 3.61 (m, H-4″), 3.76 (m, H-4‴), 3.80 (m, H-5‴), 3.85 (m, H-2‴), 3.89 (m, H-3″), 3.95 (m, H-2″), 4.06 (m, H-3‴), 4.15 (m, H-6‴), 4.45 (d, J = 7.5, H-1‴), 4.65 (d, J = 9.5, H-1″), 6.25 (s, H-6), 6.65 (s, H-3), 6.85 (d, J = 8.5, H-5′), 7.53 (d, J = 2.0, H-2′), 7.57 (dd, J = 8.5, 2.0, H-6′). ^{13}C NMR, see [16].

Catechin (5). Yellow powder. IR (KBr, ν_{max}, cm^{-1}): 3360, 1563, 1524, 1440. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.52 (dd, J = 16.0, 8.6, H-4β), 2.90 (dd, J = 16.0, 5.3, H-4α), 4.0 (td, J = 8.0, 8.8, 6.0, H-3), 4.55 (d, J = 7.74, H-2), 5.86 (d, J = 2.4, H-6), 6.01 (d, J = 2.4, H-8), 6.76 (m, H-5′, H-6′), 6.88 (d, J = 2.0, H-2′). ^{13}C NMR, see [16].

Animals. Rats ICR, males and females 8–10 weeks old, weighing 25–35 g. The animals were maintained under controlled conditions of temperature and light (between the 06:00 and 18:00 hours), with *ad libitum* access to water and food. During the experiments the animals were treated according to ethical limits for care of laboratory animals.

Mouse Acute Ear Edema Induced by TPA. Groups of 5 animals each were used. Edema was induced on the right ear by topical application of 2.5 μg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as 0.125 μg/μL acetone solution (10 μL to each side of the ear) [19]. Compounds **1**–**4** and **5** (1.0 mg/ear) were dissolved in the same volume of acetone and applied topically immediately after TPA. The left ear, used as a control, received the vehicle. Indomethacin (0.5 mg/ear) was used as reference. Animals were killed 6 h after TPA application, and disks of 6 mm diameter were removed from each ear and their weight determined. The swelling was measured as the difference in weight between the punches from right and lefts ears, and expressed as an increase in ear thickness.

Statistical Analysis. Inhibition percentages are calculated from the differences between treated and untreated ears and are referred to the control treated with TPA. One-way analysis of variance (ANOVA) followed by Dunnett′s *t*-test for multiple comparisons of unpaired data was used for statistical evaluation.

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