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Full Length Research Paper

Anti-inflammatory activities of ethyl acetate extract of *Polygonum jucundum* and its phytochemical study

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***Polygonum* spp. (Polygonaceae) has long been used as folk medicines in China to treat bacterial infection, blood coagulation and cancer and inflammatory diseases. In this study, we aimed to demonstrate the ethnopharmacological activity of *Polygonum jucundum*. Its ethyl acetate extract (PJE, 200, 500 mg/kg, intraperitoneal (p.o)) produced a dose-related anti-inflammatory effects (*P*<0.05 to 0.001) of xylene - induced ear oedema in mice and acetic acid - induced vascular permeability models in mice and did not present acute toxicity at the dose of 3000 mg/kg. We also found that PJE dose-dependently diminished the production of nitric oxide (NO), release of tumour necrosis factor TNF-α and IL-6 in lipopolysaccharide (LPS) - activated RAW264.7 cells. These data suggested that the ethnopharmacological action of *P. jucundum* may be due to its negative modulation of macrophage-mediated inflammatory responses by suppressing NO, TNF-α and IL-6 production. Two new sesquiterpenoids, 2α, 3β-dihydroxycinnamolide (1) and 2α-hydroxyl-3β-angeloylcinnamolide (2), and five known flavonoids isolated from the active extract are speculated to account for the observed anti - inflammatory properties of this species. *In vivo* anti - inflammatory activities of compound 2 at doses of 50 to 200 mg/kg were also evaluated using xylene - induced ear oedema and acetic acid-induced vascular permeability models in mice. Thus, this plant may be developed as a new therapeutic remedy for various inflammatory diseases such as arthritis.**

Key words: *Polygonum jucundum,* anti-inflammatory activities, sesquiterpenolides.

INTRODUCTION

Polygonum jucundum Lindx. (Polygonaceae) is an annual herb (50 to 90 cm), which is widely distributed in China and Japan (Li et al., 1998). The dry herb of *P. jucundum* is frequently used in traditional Chinese medicine with actions to remove heat, counteract toxicity and it can also be externally used for carbuncles (Li et al., 1998). The plants from genus *Polygonum* are well known for producing active compounds in oriental traditional medicine systems (Wang et al., 2006;), for example, the ethanolic extract, sesquiterpenes and flavonoid glycosides from *Polygonum viscosum* are known to have anti-inflammatory properties (Datta et al., 2004), extracts and constituents from *Polygonum spectabile Mart.* showed antimicrobial, antiviral activity (Brand et al., 2010).

As part of our ongoing efforts in discovering active components in medicinal plants of genus *Polygonum* (Qi et al., 2005a, b; Lin et al., 2009), the present paper reports on the investigation of the anti-inflammatory activity of ethylacetate extract (PJE) from *P. jucundum.* In two acute animal models relevant to the inflammatory process.

Preliminary phytochemical process indicated that the active extract of PJE contain mainly two sesquiterpenolides: 2,3-dihydroxycinnamolide (1) and 2-hydroxyl-3-angeloylcinnamolide (2), along with five known flavonoids: isorhamnetin (3), luteolin - 3′ - O - glucoside (4), apigenin - 7 - O - glucoside (5), quercetin - 3 - O -rhamoside (6) and kampferol (7). Furthermore, the *in vivo* anti-inflammatory effects of compound 2 were also investigated to analyze the possible anti-inflammatory

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properties of *P. jucundum*.

MATERIALS AND METHODS

Preparation of the extract

The aerial parts of *P. jucundum* Lindx. was collected in Ju-rong country, Najing, Jiangsu province, China, in December 2007. The herb was authenticated by Prof. Mian Zhang (Department of Pharmacognosy, China Pharmaceutical University). A voucher specimen (No. PJL - 0710) was stored at this Department.

Phytochemical studies

The dried minced plant (10 kg) was heated and refluxed with 85% ethanol for 2 h, 3 times, evaporating under reduced pressure to yield a dark brown tarry mass (PJM, 1.46 kg, yield 14.6%, w/w) at a temperature below 45℃. PJM was dissolved in water and was successively extracted with petroleum ether to remove chlorophyll, then extracted with acetyl acetate. The ethylacetate fraction (PJE, 285 g) was obtained for pharmacological and phytochemical studies. PJE (200 g) was subjected to column chromatography on silica gel and elute by a mixture of petroleum ether and acetyl acetate with rations changing from 100:0 to 20:80 to yield 5 fractions (Fr. 1 ~ 5). Fr. 4 was further purified over sephadex LH-20 column and developed with $CHCl_3$/MeOH (1:1) to afford compound 2 (2.1 g), Fr. 3 was chromatographed repeatedly on silica gel column using gradient elution from $CHCl_3$ to MeOH (50:1, 20:1, 10:1) to afford compound 3 (8.6 mg). Fr. 5 were purified over D101 macroporous resins (Tianjing, China) and silica gel columns and then on Sephadex LH-20 column to obtain compounds 1 (200 mg), 4 (20 mg), 5 (21 mg), 6 (5 mg) and 7 (15 mg). Their structures were elucidated by means of spectral analysis and literature data. Melting points were taken on a hot apparatus without correction. ^{1}H and ^{13}C-NMR were measured by a JNM-A500 spectrometer in $CDCl_3$. Chemical shifts are given in δ value, relative to internal tetramethylsilane. HR-ESI-MS were recorded on a JEOL HX-110 spectrometer. Optical rotations are measured on a JASCO-DIP 360 polarimeter.

Anti-inflammatory activity

In vivo studies

Male Impaired cytokine response (ICR) mice, weighing 18 to 22 g, were obtained from the experimental animal center of China Pharmaceutical University. They were kept on standard laboratory chow with tap water *ad libitum* and the husbandry room was maintained at 22℃ with a 12 h light and 12 h dark cycle.

Xylene-induced ear oedema in mice

The method was used according to previously described by Cao et al. (2010). After 15 min treatment, each animal received 50 µl of xylene on the anterior and posterior surfaces of the right ear lobe, the left ear was considered as control. One hour later, animals were sacrificed by cervical dislocation, and a diameter of 8 mm circular sections was taken from both ears with a cork borer and weighed. The degree of ear swelling was calculated based on the weight of left ear without applying xylene.

Acetic acid-induced vascular permeability in mice

This test was performed by the method described by Li et al. (2010). Male ICR mice were divided into four groups. The vehicle and drugs were administered orally to individual groups of mice, at a dose of 200, 500 mg/kg, once a day for 3 days. Group A received the same volume of normal saline orally as a vehicle control. 1h after the last treatment of drug, 0.2% Evan's blue in normal saline was injected intravenously into the tail vein at a dose of 0.1 ml/10 g body weight. And immediately each mouse was injected intraperitoneally with 0.2 ml of 0.6% acetic acid in normal saline. 30 min after intraperitoneal injection, the mice were killed by dislocating the neck and the abdominal wall was cut to expose the intestine. The abdominal cavity was washed using 5 ml of normal saline to collect pigments in a test tube. After centrifuging the contents of the tube to eliminate contaminants, the solution was subjected to colorimetry using a spectrophotometer at a wavelength of 590 nm. Control mice were treated similarly. The vascular permeability effects were expressed by the absorbance (A) of the total dye amount that leaked into the intraperitoneal cavity.

Acute toxicity

In this study, the acute o.p. toxicity of PJE was assessed using the limit test in the mice (Hayes, 1989). The limit dose (3000 mg/kg) for acute toxicity was used. In the test, male and female mice weighing 18 to 22 g each were used (n = 5 for each group).

In vitro studies

Raw 264.7 cell line was from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The mammalian cells were cultured in Dulbecco's-modified Eagle's medium with 10% heat-inactivated fetal bovine serum (FBS), 25 mM HEPES (pH7.5), 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were plated at a density of 1×10^{6} and preincubated for 24 h at 37℃, and maintained in a humidified atmosphere containing 5% CO_2. For all experiments, the cells were grown to 80 to 90% confluence, and subjected to no more than 20 cell passages. The RAW 264.7 cells were incubated with LPS (1 µg/ml) in the presence or absence of PJE (30 to 300 µg/ml) for 24 h and then washed twice with ice-cold phosphatebuffered saline (PBS). The cells were lysed in a buffer containing 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.9), 0.1 M KCl, 0.3 M NaCl, 10 mM ethylene diamine tetraacetic acid (EDTA), 1% sodium dodecyl sulphate (SDS), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 µg/ml leupeptin and 1 µg/ml pepstatin.

Nitric oxide measurement

The production of NO was measured by the accumulation of nitrite in the culture supernatants, using a colorimetric reaction with the Griess reagent (Cruz et al., 2001; Zhou et al., 2006). The cells were plated at 0.3×10^{6} cells/well in 96-well culture plates and then incubated with culture medium (control), or stimulated with 5 µg/ml LPS in the presence of the different samples, for 24 h. Briefly, 170 µl of culture supernatants were collected and diluted with equal volumes of the Griess reagent [0.1% (w/v) N- (1-naphthyl) ethylenediamine dihydrochloride and 1% (w/v) sulphanilamide containing 5% (w/v) H_3PO_4], during 10 min, in the dark. The absorbance at 550 nm was measured in an automated plate reader (SLT, Austria). Nitrite concentration was determined from a sodium nitrite standard curve.

Table 1. Effect of PJE on xylene-induced ear edema and acetic acid-induced vascular permeability in mice ($\bar{X}$±S, n = 10).

Groups	Dose (mg/kg/d)	Difference (mg)	Inhibition (%)	Absorbance (A)	Inhibition (%)
Control	-	18.32±5.12	-	1.441±0.140	-
Aspirin	300	9.48±3.01**	48.25	1.135±0.015**	30.19
PJE	500	10.32±2.21**	43.65	1.006±0.003**	29.91
	200	12.12±2.32**	33.84	1.010±0.025*	29.08

Values are expressed in mean ± S.E.M.,*$P<0.05$, **P < 0.01 when compared with control group.

Table 2. The influence of PJE from *P. jucundum* on inflammatory factors NO, IL-6 and TNF-α.

Section	Dose (μg/ml)	NO (μmol/L)	TNF-α (pg/ml)	IL-6 (pg/ml)
Blank	-	7.28±0.01	75.91±9.72	2.58±0.80
LPS	5	59.48±0.03***	277.51±23.19***	15.51±2.09***
PJE	30	17.44±0.03▲▲	206.74±10.07▲▲	10.01±2.43▲
	100	8.65±0.02▲▲	174.96±7.14▲▲▲	7.46±0.97▲▲
	300	7.43±0.02▲▲	144.97±9.16▲▲▲	5.85±2.46▲▲

*P<0.05, **P<0.01, ***P<0.001 compared with control group; ▲P<0.05, ▲▲P<0.01, ▲▲▲P<0.001 compared with model.

Inhibitory effects on LPS-induced TNF - α and IL-6 releases from RAW264.7 cells

The RAW 264.7 cells were seeded in 96-well plates with 1.0×10^5 cells/well and allowed to adhere for 1 h at 37℃ in a humidified atmosphere containing 5% CO_2. After that the medium was replaced with a fresh medium containing 500 mg/ml of LPS together with the test samples at various concentrations and was then incubated for 48 h. The supernatant was transferred into 96-well plate and then TNF-α and IL-6 concentrations were determined using commercial enzyme immunoassay kits (BD PharMingen) according to the manufacturer's protocol. The test sample was dissolved in dimethyl sulfoxide (DMSO) and the solution was added to RPMI. The inhibition on TNF-α and IL-6 releases was calculated.

Statistical analysis

All the experiments were performed in duplicate. Results are presented as mean±standard error (SE) of the indicated number of experiments, and the means were statistically compared using the one-way ANOVA test, with a Dunnett's post-test. The significance level was *p<0.05, **p<0.01, ***p<0.001.

RESULT AND DISCUSSION

In vivo and *in vitro* anti-inflammatory effects of PJE

As shown in Table 1, ethyl acetate-soluble portion (PJE) of 85% ethanol extract from *P. jucundum* at a dose of 200 to 500 mg/kg exhibited significant anti-inflammatory. Significantly inhibitory effect recorded with PJE at a dose of 500 mg/kg was comparable to that of aspirin. Xylenen cause instant irritation of the mouse ear, which led to fluid accumulation and edema characteristic of the acute inflammatory response. Suppression of this response was a likely indication of antiphlogistic effect. Similar result was obtained against another inflammatory model based on acetic acid-induced vascular permeability in mice (Table 1). Vascular permeability was induced by acetic acid, which can cause the increase of chemical mediators such as prostaglandin E_2 (PGE_2), histamine in peritoneal fluids, leading to the increase in vascular permeability. These results suggest that *P. jucundum* exerts an anti-acute inflammatory effect. The mechanism may be due to inhibiting the inflammatory mediators. RAW264.7 cells, amurine macrophage cell line, are widely used to establish inflammatorymodel *in vitro* (Kang et al., 2006). In this study, we investigated *in vitro* the anti-inflammatory effects of PJE on the generation of several chemokines and cytokines, involved in the inflammatory process, such as NO, TNF-α and IL-6 in LPS-induced murine RAW264.7 macrophages. PJE significantly decreased the production of NO, tumor necrosis factor TNF-α and IL-6 production in a dose-dependent manner (Table 2).

Spectral data of two new sesquiterpenoids and its structural elucidation

2α,3β -dihydroxycinnamolide *(*3aS,4aS,6R,7R)-6,7-dihydroxy-8a-methyl-3a,4,4a,5, 6, 7,8,8a -octahydronaphtho [2, 3-c] furan- 1(3H)-one, 1) White powder ($CHCl_3$). Mp.172-174℃ ultraviolet (UV)

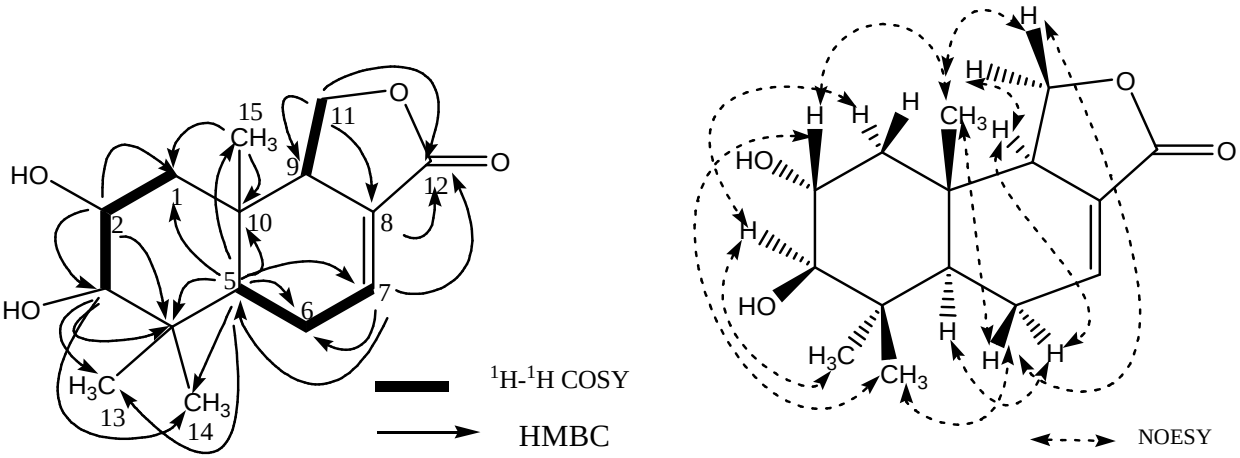

Figure 1. Main correlations in 2D-NMR spectra of 1.

(MeOH,λ_{max}):222 nm. $[\alpha]^{25}_{D}$ -9.6°(c = 0.1, MeOH). IR (KBr): 3279.6, 2967, 2923, 1775, 1697, 1257, 1178 cm^{-1}. ^{1}H-NMR (CH_3OD_3, 300 MHz): 1.90 (1H, dd, *J* = 9.2, 5.3Hz, H - 1β), 1.32 (1H, t, *J* = 8.9Hz, H - 1α, 3.67 (1H, m, H - 2), 3.02 (1H, d, *J* = 10Hz, H - 3), 1.53 (1H, dd, *J* = 5.5, 12.1Hz, H – 5), 2.32 (1H, m, H - 6β), 2.49 (1H, m, H - 6α), 2.96 (1H, m, H - 9), 6.88 (1H, dd, *J* = 9.3, 3.3Hz, H-7), 4.46 (1H, dd, *J* = 9.2, 9.2Hz, H - 11α), 4.13 (1H, dd, *J* = 9.2, 9.1Hz, H - 11β), 1.07 (3H, s, H-13), 0.95 (3H, s, H-14), 0.90 (3H, s, H-15)。 ^{13}C-nuclear magnetic resonance (NMR) (CH_3OD, 500 MHz): 46.9 (C - 1), 68.9 (C - 2), 84.4 (C - 3), 40.8 (C – 4), 50.7(C - 5), 26.5 (C - 6), 52.4 (C -7), 128.8 (C - 8), 138.3 (C - 9), 36.5 (C - 10), 69.2 (C - 11), 172.8 (C - 12), 17.0 (C - 13), 29.3 (C - 14), 15.0 (C - 15). HR - TOF - MS *m/z*: 289.1412 $[M+Na]^{+}$ (calcd. 289.1410 for $C_{15}H_{22}O_4Na$). Compound 1, white amorphous powder, showed positive reaction with a 10% vanillin-sulfuric acid solution in thin layer chromatography. The molecular formula $C_{15}H_{22}O_4$ was determined by HR electrospray ionization mass spectrometry (ESI-MS) and 1D – NMR analysis, and with 5 degrees of unsaturation.

The UV spectrum showed absorption at 222 nm, meaning the presentation of a conjugated system. The Infrared (IR) spectrum revealed the absorptions of one carbonyl group at 1775 cm^{-1} and double bonds at 1697 cm^{-1}. The ^{1}H-NMR spectrum showed one olefinic proton at δ 6.88 (1H, dd, *J* = 9.3, 3.3Hz, H-7) and three methyl groups at δ 1.07(3H, s), 0.95 (3H, s) and 0.90 (3H, s). The ^{13}C-NMR, DEPT and HSQC spectra revealed the presence of 15 carbon atoms, including 3×CH_3, 3×CH_2, 5×CH and four quaternary carbons. The five methine carbon are including one double bond at δ 138.3, two oxygen - substituted methine carbon at δ 68.9 (C-2) and 84.4 (C-3), and two others carbon at δ 50.7 (C-5) and 52.4 (C-9); the four quaternary carbons including one carbonyl at δ 172.8 (C-12); one double bond at δ 128.8 (C-8); and other signals at δ 40.8 (C-4) and 36.5(C-10). The ^{1}H-^{1}H COSY spectrum exhibited the correlations between H_2 – 6 / H - 7, H_2-1 / H-2 / H-3 and H-9 / H_2-11. Based on above analysis, compound 1 was indicated with a tricyclic drimane sesquiterpenoid skeleton (Ayer et al., 1992).

The HMBC spectrum displayed the cross-peaks from one olefinic proton at δ 6.88 (1H, dd, *J* = 9.3, 3.3Hz, H-7) to carbonyl signal at 172.8 (C-12), a methylene carbon signal at δ 26.5 (C-6) and a methine carbon signal at δ 50.7 (C-5), from two singlets methyl signals at δ 1.07 (3H, s, H-13), 0.95 (3H, s, H-14) to carbon signals including one quaternary carbon signal at δ 40.8 (C-4), one oxygen-substituted methine carbon signal at δ 84.4(C-3) and methine carbon signal at δ 50.7 (C-5). The long-range correlations from H-15 to C-10, C-1 and C-5, from H-5 to C-15, C-10, C-6, C-4 and C-1 were also be seen in HMBC spectrum, which were in accordance with the assignment of compound 1 (Figure 1). The relative configuration can be determined by NOESY spectral analysis, in which there were clear correlations between H-2/ H_3-14 and H_3-15, H-3 / H_3-13, H-6β/H_3-15, H-6a/H-9, indicating a *cis*-orientation at H-2, H_3-14 and H_3-15; while there are two cross peaks between H-5/H-6β and H_3-15/H-6α, there was not correlations between H-5 and H_3-15, indicating a *trans*-orientation at H_3-15 and H-5 (Figure 1). Thus, the relative configuration of compound 1 was therefore identified as a derivative of 3β, 9α-dihydroxycinnamolide (Kioy et al., 1990) and named as 2α, 3β- dihydroxycinnamolide.

2α, 3β-dihydroxycinnamolide ((Z) - ((3aS, 4aS, 6R, 7R) - 7 - hydroxy - 8a – methyl – 1 - oxo - 1, 3, 3a, 4, 4a, 5, 6, 7, 8, 8a - decahydronaphtho [2, 3-c] furan – 6 - yl) 2 – methylbut - 2 - enoate,*2*) White crystal ($CHCl_3$). mp172-174℃. UV (MeOH, λ_{max}): 220 nm. $[\alpha]^{20}_{D}$+2.4°(*c* 0.01,

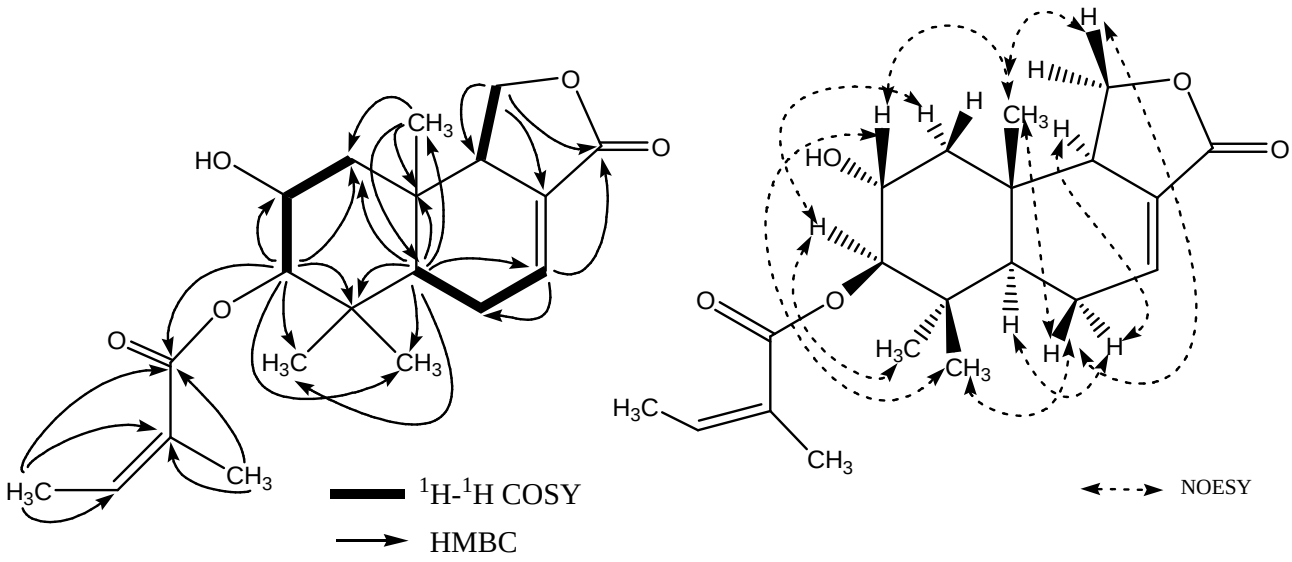

Figure 2. Main correlations in 2D-NMR spectra of 2.

MeOH). IR(KBr): 3475, 3008, 2972, 2918, 1763, 1697,1654, 1257, 1178, 955 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz): 2.05 (1H, dd, *J* = 12.3, 1.0 Hz, H-1α), 1.40 (1H, dd, *J* = 12.3, 3.6 Hz, H-1β), 3.87(1H, m, H-2), 4.65(1H, d, *J* = 10.0 Hz, H-3), 1.56 (1H, dd, *J* = 12.0, 3,3Hz, H-5), 2.43 (1H, ddd, *J* = 16.0, 3.9, 8.9 Hz, H-6α), 2.23 (1H, ddd, *J* = 16.0, 12.0, 3.3 Hz, H-6β), 2.89 (1H, m, H-7), 2.90 (1H, m, H-9), 4.41 (1H, dd, *J* = 9.2, 9.2 Hz, H-11α), 4.05 (1H, dd, *J* = 9.2, 9.3 Hz, H-11β), 1.03 (3H, s, H-13), 0.95 (3H, s, H-14), 0.88 (3H, s, H-15), 6.12 (1H, ddd, *J* = 1.4, 1.9, 14.5Hz, H-3´), 2.02 (3H, dd, *J* = 1.4, 7.2Hz, 3´-CH_3), 1.93 (3H, dd, *J* = 1.9, 1.5 Hz, 2´-CH_3). ^{13}C-NMR ($CDCl_3$, 500 MHz): δ 46.2 (C - 1), 66.6 (C - 2), 83.6(C-3), 39.1(C - 4), 48.9 (C - 5), 24.6 (C - 6), 135.6 (C-7), 126.9 (C-8), 50.6 (C - 9), 35.0 (C-10), 66.7 (C-11), 169 (C-12), 17.1(C-13), 28.2 (C-14), 14.3 (C-15), 168.7 (C-1´), 127.5 (C-2´), 139.1 (C-3´), 15.8 (3´-CH_3), 20.6 (2´-CH_3). HR-TOF-MS *m/z*: 371.1829 $[M+Na]^+$ (*calcd* 371.1829 for $C_{20}H_{28}O_5Na$).

Compound 2 has molecular formula $C_{20}H_{28}O_5$ as established by HR-ESI/MS. IR spectrum showed strong absorptions of an unsaturated γ-lactone (1763 cm^{-1}) and OH group (3475 cm^{-1}). The ^{1}H-NMR spectrum showed three methyl groups as singlets at δ 0.88 (3H, s, H-15), 0.95 (3H, s, H-14) and 1.03 (3H, s, H-13), which together with a high field signal at δ 1.56(1H, dd, *J* = 12.0, 3,3 Hz, H-5) suggested the same skeleton as compound 1. The angeloyl fragment was indicated by its characteristic chemical shifts, two doublet methyl groups and one olefinic proton signal at δ 6.12 (1H, ddd, *J* = 1.4, 1.9, 14.5Hz, H-3´) in the ^{1}H-NMR spectrum. Location of the angeloyl group at C-3 is based on the long-range correlation in HMBC experiment from proton signal at δ 4.65 (1H, d, *J* = 10.0Hz, H-3) to carbonyl signal at δ 168.7 (C-1´). The ^{1}H - ^{1}H COSY and especially the HMBC spectrum confirm the structure of 2 (Figure 2). It relative stereo-structure can also be confirmed by NOESY analysis, in which cross-peaks can be seen from H-2 to H_3-14, 15, from H-3 to H_3-13, from H-6β to H_3-15, from H-6α to H-9. Thus, the structure of compound 2 was confirmed and named as 2α-hydroxyl-3β-angeloylcinnamolide.

To assess if the large amount of new compound 2 (3.5 g) is active component, *in vivo* anti-inflammatory experiments were conducted using xylene - induced ear oedema and acetic acid-induced vascular permeability in mice. Compound 2 exhibited significant anti – inflammatory activities at doses of 50 and 200 mg/kg after administration ($p<0.01$), respectively. Additionally, compound 2 showed significantly better activity at doses of 200 mg/kg than aspirin at the dose of 300 mg/kg ($p<0.01$) (Table 3). NO is produced by activated macrophages as a result of induction by several stimuli, including TNF-a, IFN-r and LPS and may contribute to the pathological process in various acute and chronic inflammatory conditions (Kilbourn and Belloni, 1990). Therefore, the reduction of NO production may present a useful strategy for the treatment of a variety of inflammatory diseases, including some neurological disorders (Albina and Reichner, 1998; Boucher et al., 1999; Lee et al., 2006). In the present study, first we carried out the *in vivo* and *in vitro* anti-inflammatory effects of the ethyl acetate extract (PJE) from *P. jucundum*.

It is concluded that the present study may support the use in China traditional medicine of *P. jucundum* for treatment of inflammatory-related diseases through the inhibition of NO, TNF-α and IL-6 production or releases. In this study, we demonstrate that PJE significantly inhibited NO production, TNF-α, and IL-6 release from LPS-stimulated macrophages in dose-dependent manner.

Table 3. Effect of compound 2 on xylene-induced ear edema and acetic acid-induced vascular permeability in mice ($\bar{X}$±S, n = 10).

Groups	Dose (mg/kg/d)	Difference (mg)	Inhibition (%)	Absorbance (A)	Inhibition (%)
Control	-	18.1±2.31	-	0.52±0.190	-
Aspirin	300	9.2±2.32**	49.1	0.22±0.014**	57.65
PJE	500	11.7±2.50**	35.6		
	50	10.0±1.90**	44.8	0.36±0.1100**	46.3
Compound 2	100	9.9±3.01**	45.4	0.25±0.090**	51.68
	200	9.2±2.00**	49.1	0.23±0.006**	55.75

Values are expressed in mean ± S.E.M., **P < 0.01 compared with control.

Then we investigated phytochemical study of PJE. Two new sesquiterpenoids, 2α, 3β-dihydroxycinnamolide (1) and 2α-hydroxyl-3β-angeloylcinnamolide (2), and five known flavonoids were obtained. Flavonoids are known to possess various biological activities, including anti-inflammatory effects (Kim et al., 1999; Matsuda et al., 2003; Takada and Aggarwal, 2004; Yerra et al., 2005). Some sesquiterpene lactones isolated from other plants also have been shown to possess significant anti-inflammatory properties (Hall et al., 1980) by suppressing LPS-induced NO production (Jin et al., 2000) or TNF-α release (Cho et al., 2000).

As related with these data, it is suggested that flavonoids and sesquiterpene lactones in this species may be partly responsible for the anti-inflamatory effect of *P. jucundum*. So, in order to assess anti-inflammatory properties of new compounds, *in vivo* anti- inflammatory effects of 2 were evaluated using xylene - induced ear oedema and acetic acid - induced vascular permeability models (Table 3). Compound 2 showed better activity at doses of 200 mg/kg comparable to aspirin at the dose of 300 mg/kg ($p<0.01$). Therefore, it is concluded that *P. jucundum* are capable of inhibiting inflammatory reactions, the isolated flavonoids and sesequiterpenes from the ethyl acetate extract (PJE) could partially explain the anti-inflammatory effect of this species. The results provided experimental evidence for its traditional use in treating various diseases associated with inflammation, new compounds 1 and 2 may be served as the lead compounds for development of anti-inflammatory agents.

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