Available online at SciVarse ScienceDirect

**Chinese Herbal Medicines (CHM)**

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

## Letter

# Immunological Activities of Components from Leaves of *Liriodendron chinensis*

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ARTICLE INFO

*Article history*

Received:

Revised:

Accepted:

Available online:

DOI:

ABSTRACT

**Objectives** To investigate the anti-inflammatory components from the leaves of *Liriodendron chinensis*. **Methods** The 95% alcohol extract from the leaves of *L. chinensis* was isolated by column chromatography, and the structures of purified compounds were determined by spectral methods. The bioassay was performed through the inhibitory effects on the inflammatory cells activated by lipopolysaccharide (LPS). **Results** Nine compounds were isolated, including octacosanoic acid (**1**), stearic acid (**2**), (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1,3,4-tri-hydroxyicos-8-en-2-yl]tetracosanamide (**3**), (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1-*O*-β-*D*-glucopyranosyloxy-3,4-dihydroxy-octadec-8-en-2-yl]eicosanamide (**4**), (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1-*O*-β-*D*-glucopyranosyloxy-3,4-dihydroxyoctadec-8-en-2-yl]hexadecanamide (**5**), dicentrinone (**6**), liriodenine (**7**), daucosterol (**8**), and liriodendritol (**9**) and among which five compounds could significantly lower the content of nitric oxide (NO) from peritoneal macrophages of rats induced by LPS and reduce the splenic lymphocyte proliferation in mice. This is the first report on the occurrence of ceramides and dicentrinone in the plants of *Liriodendron* Linn. **Conclusion** The five compounds are likely to be anti-inflammatory compounds concerning to their pronounced inhibitory action on the activated inflammatory cells. This assessment might provide a basis for searching the potent active compounds used for the treatment of inflammation.

*Key words*

active constituents; immunological activity; *Liriodendron chinensis*



## 1. Introduction

*Liriodendron chinensis* (Hemsl.) Sarg. (Magnoliaceae) is often used for landscape and street planting owing to its high resistance to pest diseases and air contamination. *L. chinensis* also has a long history of medicinal utilization in many countries of Asia, especially China. The roots and barks of *L. chinensis* are often used to treat rheumatoid arthritis in traditional Chinese medicine (TCM) (Li et al, 2001; Zhang et al, 2011) Modern study indicated that the barks of *L. chinensis* mainly consist of active constituents such as alkaloids and phenylpropanoid glycosides (Li et al, 2001; Zhang et al, 2011). Many types of actions can be taken in favor of the conservation and sustainable use of medicinal plants.

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Fund: Priority academic program development of Jiangsu Higher Education Institutions (ysxk-2014)

Probably the most important role for medicinal plants in biological conservation is the comprehensive utilization of plant resources. Chemical and pharmacological studies on the deciduous leaves of *L. chinensis* are important to the top value-added development of this medicinal plant, and only the volatile constituents were once analyzed by GC/MS (Zhang et al, 2011). The involatile constituents from the leaves of *L. chinensis* were investigated in this study, which dealed with the isolation and structural elucidation of the nine compounds and some of their immunocompetence. This is the first report on the immune activities of the components extracted from *L. chinensis* against the inflammatory cells.

## 2. Materials and methods

### *2.1 General*

Infra-red (IR) spectra were taken on a Nicolet IR−100 FT-IR Spectrometer in KBr discs. NMR spectra were measured on a Bruker AV−500 MHz. ESI-MS spectra were obtained on a Micromass Q/TOF Mass Spectrometer. Silica gel (200–300 mesh) for column chromatography (CC) and thin layer chromatography (TLC) plates (10–40 μm) were from Qingdao Marine Chemical Co., Ltd. (China). RPMI 1640 medium was the product of Invitrogen and fetal bovine serum (FBS) was obtained from Minhai Bio-engineering Co., Ltd. (Lanzhou, China). Sulfanilic acid (No. 1401101) was purchased from Chemical Reagent Institute (Tianjin, China), while MTT (No. 13081123) was the product of Ameresco Inc. Lipopoly-saccharide (LPS, No. 145K880) was obtained from Sigma. Dexamethasone Acetate Injection (No. 14080205) was from Tianjin Pharmaceutical Group Co., Ltd. (China). Other chemicals not referred were of analytical reagent grade. Male ICR mice weighing 20–22 g and SD rats weighing 200–240 g were provided by the Laboratory Animal Center in Nanjing Medical University. The protocols of animal experiments were permitted by the University Animal Care Committee.

### *2.2 Plant materials*

The deciduous leaves of *Liriodendron chinensis* (Hemsl.) Sarg. were collected in July, 2010 from Nanjing, China, and identified as the dry leaves of *L. chinensis* by associate professor Nian-yun Yang, Nanjing University of Chinese Medicine. A voucher specimen (LLC-20101126) is kept in Herbarium of Nanjing University of Chinese Medicine.

### *2.3 Extraction and isolation*

The air-dried and powdered deciduous leaves of *L. chinensis* (600 g) were extracted with 95% alcohol under reflux. The extract was evaporated under reduced pressure to leave the residue (80 g), which was chromatographed on silica gel (1 kg) eluting with petroleum ether-$CHCl_3$-$CH_3OH$ by a stepwise gradient (100:0:0→0:2:1) to obtain compounds **1** (70 mg), **2** (90 g), **3** (5 mg), **4** (3 mg), **5** (15 mg), **6** (50 mg), **7** (5 mg), **8** (55 mg), and **9** (1400 mg).

### *2.4 Preparation of test samples*

All compounds from the leaves of *L. chinensis* were dissolved respectively, mixed with LPS solution, and then co-cultured with effector cells to evaluate their inhibitory effects. Samples were dissolved in dimethyl sulphoxide (DMSO) prior to adding PBS to yield appropriate concentration. The final concentration of DMSO was less than 0.2% after the addition of all test samples in cell culture medium. Culture medium with 0.2% DMSO was employed as control.

### *2.5 Nitric oxide generation assay*

Peritoneal macrophage cells were harvested from SD rats by washing peritoneal cavity with 10 mL of D-Hanks solution. Macrophages were centrifuged (1000 r/min, 10 min, 4 °C) and resuspended in supplemented culture medium consisting of RPMI 1640 medium. The cell number was adjusted to $1 \times 10^6 - 1.5 \times 10^6$/mL, and 180 μL cell suspension was transferred to each well of 96-well cell culture plate. The cultures were incubated in a humidified 5% $CO_2$ incubator at 37 ºC for 72 h. After incubation, miscellaneous cells were removed by adherent method, and the same volume of RPMI 1640 medium enriched with FBS (10%) was added. The concentration of tested compounds in the mixture of cell culture medium was 1, 10, and 100 μg/mL. The tested compounds and LPS were added to each well of cell cultures and incubated for 14 h with dexamethasone (Dex) solution (5 μg/mL) as a control, 100 μL fresh Griess reagent was added in the same volume of culture medium, and then the plates were shaken for 10 min for sufficient reaction. The absorbance was measured at 570 nm and the results were expressed as the difference between the NO production.

### *2.6 Spleen lymphocyte proliferation assay*

The primary T lymphocytes suspension of mice was aseptically prepared from the spleens of mice. A single-cell suspension of spleen cells was obtained by pushing the spleen through nylon mesh bags in D-Hank's solution. The cells were collected and washed twice with D-Hank's solution, centrifuged at 1000 r/min for 5 min, 4 ºC, and any remaining red blood cells were lysed by Tris-NH4Cl. The cells were suspended in RPMI 1640 medium containing FBS (10%). The cell number was adjusted to $1 \times 10^6 - 1.5 \times 10^6$/mL, and 180 μL cell suspension was transferred to each well of 96-well cell culture plate. The cultures were incubated in a humidified 5% $CO_2$ incubator at 37 ºC. After 1 h culture, the cells of model group were given LPS solution (1 μg/mL), and the cells of medication group were given LPS solution (1 μg/mL) and tested samples simultaneously. After 44 h culture, MTT solution (10 μg/mL) was added to each well of cell cultures and incubated for 4 h, and then 180 μL DMSO was added after removing the supernatant. The plates were slightly shaken and the optical

density (OD) was measured at 570 nm. Proliferation index (PI) (OD of treated cells/OD of untreated cells) was calculated to assess the anti-inflammatory activity of the samples.

### *2.7 Statistical analysis*

Data were presented as $\bar{x} \pm s$. The significance of differences was determined through Student's *t*-test and $P < 0.05$ or 0.01 was taken as statistical significance.

## 3. Results and discussion

The 95% alcohol extract from the leaves of *L. chinensis* was separated by CC on silica gel to yield nine compounds, which were individually identified as octacosanoic acid (**1**), stearic acid (**2**), (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1,3,4-trihydroxyicos-8-en-2-yl]tetracosanamide (**3**), (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1-*O*-β-*D*-glucopyranosyloxy-3,4-dihydroxy - octadec-8-en-2-yl]eicosanamide (**4**), (2*R*)-2-hydroxy-*N*-[(2*S*, 3*S*,4*R*,8*E*)-1-*O*-β-*D*-glucopyranosyloxy-3,4-dihydroxyoctadec - 8-en-2-yl]hexadecanamide (**5**), dicentrinone (**6**), liriodenine (**7**), daucosterol (**8**), and liriodendritol (**9**) by comparison of their spectral data with those reported in the literatures (Figure 1) (Jones et al, 1987; Cava et al, 1971; Costa et al, 2010; Angyal et al, 1961; Zhang et al, 2006; Yang et al, 2009). This is the first report on the occurrence of ceramides and dicentrinone in the plants of *Liriodendron* Linn.

The NO production of macrophage and the proliferation of spleen cells could be regarded as signs of the activation of those inflammatory cells. In order to discover the active compounds in the leaves of *L. chinensis*, eight compounds were isolated for *in vitro* evaluation for NO production and splenocyte proliferation on LPS activated cell models (Yu et al, 2012). As shown in Table 1, all the compounds could reduce the generation and the release of NO from the peritoneal macrophages of rats induced by LPS. The NO production [(23.79 ± 1.22) μg/mL] in LPS stimulation group was markedly increased compared with that [(13.81 ± 0.62) μg/mL] of the normal control group, while compounds **3**−**5** and **7**−**9** could significantly lower the content increasing induced by LPS at three concentration levels, and the NO production after the administration of these compounds at the highest concentration

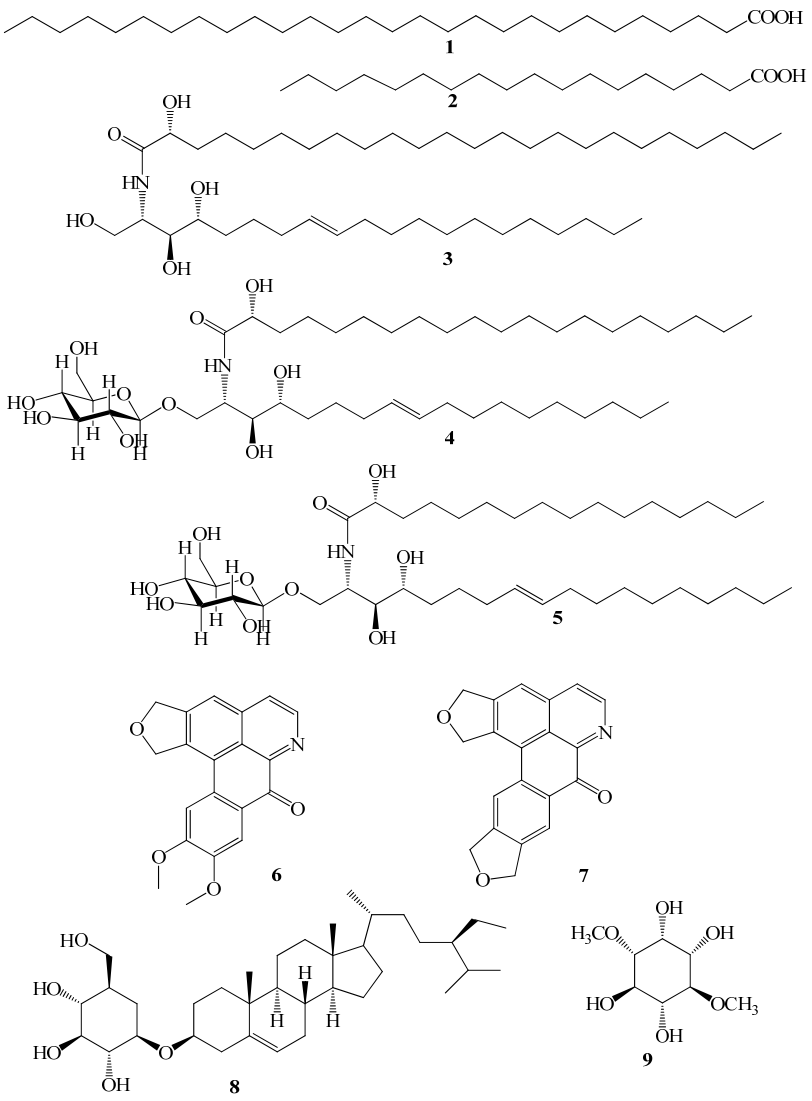


**Figure 1 Chemical structures of compounds 1−9**

**Table 1 Inhibition of compounds from leaves of *L. chinensis* on NO production of macrophages and proliferation of splenocytes stimulated by LPS ( $\bar{x} \pm s$ , $n = 4$ )**

| Groups | Concentration / (μg mL$^{-1}$) | NO / (μg mL$^{-1}$) | PI |
|---|---|---|---|
| Control | − | 13.81 ± 0.62$^{**}$ | |
| LPS | 1 | 23.79 ± 1.22 | 1.559 ± 0.130 |
| LPS+Dex | 5 | 16.50 ± 0.98$^{**}$ | 0.932 ± 0.018$^{**}$ |
| LPS+**1** | 1 | 21.94 ± 0.70$^{*}$ | 1.525 ± 0.200 |
| | 10 | 20.95 ± 0.31$^{*}$ | 1.135 ± 0.120 |
| | 100 | 19.74 ± 0.54$^{**}$ | 0.966 ± 0.098$^{**}$ |
| LPS+**2** | 1 | 22.12 ± 0.75$^{*}$ | 1.542 ± 0.260 |
| | 10 | 21.05 ± 0.37$^{*}$ | 1.186 ± 0.081 |
| | 100 | 20.14 ± 0.53$^{**}$ | 1.034 ± 0.101$^{*}$ |
| LPS+**3** | 1 | 20.01 ± 0.50$^{**}$ | 1.305 ± 0.091 |
| | 10 | 19.64 ± 1.41$^{**}$ | 1.153 ± 0.028 |
| | 100 | 16.56 ± 0.50$^{**}$ | 0.881 ± 0.089$^{**}$ |
| LPS+**4** | 1 | 19.38 ± 0.42$^{**}$ | 1.203 ± 0.080 |
| | 10 | 18.89 ± 0.39$^{**}$ | 1.068 ± 0.031 |
| | 100 | 16.01 ± 0.48$^{**}$ | 0.847 ± 0.073$^{**}$ |
| LPS+**5** | 1 | 20.27 ± 0.56$^{**}$ | 1.339 ± 0.107 |
| | 10 | 19.18 ± 1.03$^{**}$ | 1.186 ± 0.112 |
| | 100 | 17.46 ± 0.55$^{**}$ | 0.983 ± 0.068$^{**}$ |
| LPS+**6** | 1 | 20.48 ± 1.01$^{*}$ | 1.475 ± 0.119 |
| | 10 | 21.58 ± 1.08 | 1.186 ± 0.095 |
| | 100 | 17.55 ± 0.84$^{**}$ | 1.915 ± 0.501 |
| LPS+**7** | 1 | 20.01 ± 0.17$^{**}$ | 1.542 ± 0.114 |
| | 10 | 19.09 ± 0.64$^{**}$ | 2.322 ± 0.248 |
| | 100 | 17.58 ± 0.34$^{**}$ | 3.779 ± 0.465 |
| LPS+**8** | 1 | 18.93 ± 1.08$^{**}$ | 1.356 ± 0.089 |
| | 10 | 17.85 ± 0.24$^{**}$ | 1.271 ± 0.075 |
| | 100 | 16.50 ± 1.04$^{**}$ | 0.983 ± 0.080$^{**}$ |
| LPS+**9** | 1 | 20.00 ± 0.78$^{**}$ | 1.457 ± 0.051 |
| | 10 | 18.54 ± 0.77$^{**}$ | 1.457 ± 0.052 |
| | 100 | 17.69 ± 0.60$^{**}$ | 1.475 ± 0.068 |

$^{*}P < 0.05$ $^{**}P < 0.01$ *vs* LPS group

was decreased to (16.56 ± 0.50), (16.01 ± 0.48), (17.46 ± 0.55), (17.58 ± 0.34), (16.50 ± 1.04), and (17.69 ± 0.60) μg/mL, respectively. Splenic lymphocyte proliferation induced by LPS was reduced after the administration of compounds **1**, **3**, **8**, and **9** in a dose-dependent way, while compounds **6** and **7** could increase the LPS-induced proliferation. Compounds **6** and **7** thus have dual-directional immunoregulatory effects on inflammatory cells.

## 4. Conclusion

All the compounds shows the significant inhibitory effects on the NO production of macrophage, so the deciduous leaves of *L. chinensis* potentially possess the same anti-inflammatory activity as its roots and barks (Li et al, 2001) , and the observed anti-inflammatory activity might be attributable to the compounds **3**−**5**, **8**, and **9**. Some long-chain aliphatics and daucosterol were reported to have anti-inflammatory actions (Spitzer, 1997; Lina et al, 2006; Lee et al, 2007). This assessment might provide a basis for searching the potential active compounds used in the treatment of inflammation, and this study is important to the high value-added development of the deciduous leaves of *L. chinensis*.

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