SUPPLEMENTARY MATERIAL

Chemical constituents from *Melastoma dodecandrum* and their inhibitoryactivity on interleukin 8 production in HT-29 cells

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In search of anti-inflammatory lead compounds from traditional Chinese medicines, a bioassay-guided phytochemical study on *Melastoma dodecandrum* was carried out. As a result, eighteen compounds have been isolated. Their chemical structures were determined on the basis of their physicochemical properties and spectral data. Among the isolates, three pentacyclic triterpenoids, ursolic acid (**1**), asiatic acid (**3**) and terminolic acid (**6**), together with one tannin casuarinin (**17**), were found to significantly decrease interleukin 8 (IL-8) production in human colon cancer cells. The results imply, at least in part, that the anti-inflammatory effect of *M. dodecandrum* could be due to inhibition of IL-8 production, demonstrated by these naturally occurring compounds described above.

**Keywords:** *Melastoma dodecandrum*; Melastomataceae; Triterpenoids; Tannin; Anti-inflammatory; Interleukin 8

# Experimental

# *General procedure*

Optical rotations were measured on a Rudolph Autopol VI polarimeter (Rudolph Research Analytical, NJ, USA). 1H and 13C NMR were recorded on Varian Mercury Plus 400 MHz (Varian, CA, USA); Chemical shifts were given in ppm with TMS as the internal standard. ESI-MS were measured on a Waters UPLC H Class-SQD or an Agilent 1100 series mass spectrometer. Column chromatography (CC) was carried out by using silica gel (Qingdao Marine chemical factory, Qingdao, China), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), RP-18 (25-40 µm, Merck). TLC separations were performed on pre-coated silica gel GF254 plates (0.25 mm, Kang-Bi-Nuo Silysia Chemical Ltd., Yantai, China).

# *Plant material*

The aerial parts of *M. dodecandrum* were purchased from Tongling Hetian Chinese Medicine Herbal Tablets CO. LTD (Tongling, Anhui Province, P. R. China) and were originally collected in Lishui country, Zhejiang Province of China in 2010. The plant was authenticated by Professor Bao-Kang Huang (Department of Pharmacognosy, the Second Military Medical University of China). A voucher specimen (No.100609) was deposited at the herbarium of Department of Natural Products Chemistry, School of Pharmacy, Fudan University.

# *Isolation and Identification*

Dried and powdered aerial parts of *M. dodecandrum* (5 kg) were extracted with 95% ethanol (25 L) at room temperature for 5 times. The combined extract was evaporated under reduced pressure to yield a dark residue (650 g). Afterwards, the dark residue was suspended in water and successively partitioned with petroleum ether, chloroform and ethyl acetate to afford petroleum ether (40 g), chloroform (32 g) and ethyl acetate (41 g) soluble extracts.

Each of these extracts was tested for their potential on IL-8 production induced by IL-1*β* in human colon adenocarcinoma HT-29 cells. The ethyl acetate extract exhibited the most potent activity so further isolation was performed. It was firstly subjected to silica gel CC with gradient petroleum ether/ethyl acetate and ethyl acetate/methanol to afford 9 fractions (F1-F9). F3 (1.2 g) was then purified by repeated silica gel with CHCl3 and Sephadex LH-20 with CH2Cl2/MeOH (2:1) CC to give compounds **1** (29 mg) and **2** (62 mg). Further fractionation of F6 (4.2 g) by silica gel CC with gradient CH2Cl2/MeOH (50:1-20:1) obtained **3** (30 mg), **4** (80 mg), and **5** (2 mg). F7 (3.5 g) was chromatographed on RP-18 with MeOH/H2O (48:52) and Sephadex LH-20 with MeOH/H2O (8:2) to yield **6** (3 mg), **7** (30 mg), **8** (27 mg), **9** (60 mg), **10** (5 mg), **11** (4 mg), **12** (12 mg), and **13** (3 mg). F8 (3.4 g) was further purified on RP-18 eluting with a gradient of MeOH/H2O (4:6-1:1) and Sephadex LH-20 with MeOH/H2O (8:2) repeatedly to get **14** (6 mg), **15** (17 mg), **16** (5 mg), **17** (26 mg), and **18** (4 mg) respectively.

The chemical structures were mainly determined by a direct comparison of their spectroscopic data and physical properties with those reported in the literature, including four triterpenes: ursolic acid (**1**), betulinic acid (**2**), asiatic acid (**3**), and terminolic acid (**6**); three steroids: daucosterol 6′-*O*-eicosanoate (**4**) (Voutquenne et al. 1999), daucosterol (**5**) (identified by a direct TLC analysis with authentic sample, which was purified and identifiedfrom fermented mycelium of *Paecilomyces hepiali* by us (Hong et al. 2013)), and cellobiosylsterol (**14**) (Yang et al. 2010); eight flavonoid glycosides: vitexin (**7**) (Zheng et al. 2013), kaempferol-3-*O*-*β*-D-glucopyranoside (**8**) (Lu & Foo 1999), isovitexin (**9**) (Abd-Alla et al. 2009), quercetin-3-*O*-*β*-D-(6′′-galloyl) glucopyranoside (**11**) (Smolarz 2002), quercetin-3-*O*-*β*-D-glucopyranoside (**12**) (Lu & Foo 1999), luteolin-6-*C*-*β*-glucopyranoside (**13**) (Rayyan et al. 2010), quercetin-3-*O*-*β*-robinobioside (**15**) (Yang et al. 2010), and kaempferol-3-*O-β*-robinobioside (**16**) (Pistelli et al. 1993); one phenolic glucoside gallate, 4-hydroxy-3-methoxyphenyl 1-*O*-(6′-*O*-galloyl)-*β*-D-glucopyranoside (**10**) (Ishimaru et al. 1987), one tannin, casuarinin (**17**), together with a cerebroside:dracontioside B (**18**) (Tuntiwachwuttikul et al. 2004; Napolitano et al. 2011).

## Biological investigations

### Enzyme linked immunosorbent assay for IL-8 production

The human colon adenocarcinoma HT-29 cell line was purchased from Shanghai Institutes for Biological Sciences, Shanghai, China. IL-1*β* induced IL-8 production in HT-29 cells was determined by using an enzyme-linked immunosorbent assay (ELISA). In brief, confluent cells grown in 96-well plates were serum starved for 24 h and then stimulated with IL-1*β* (10 ng/ml) in the presence or absence of dilutions of isolates. After 12 h incubation, the cell conditioned media were collected and IL-8 protein levels were measured by ELISA using a human IL-8 kit (eBioscience) according to the manufacturer′s instruction.

### Cell viability assay

Cell viability was determined by mitochondrial-dependent reduction of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) to formazan. Briefly, HT-29 cells were plated in 96-well cell microtiter plates at a density of 3.5×104 cells/well. After a 24 h incubation, cells were treated with concentrations of isolates for 24 h. Following a 24 h incubation, growth medium was changed to 180 μL serum free medium, then 20 μL (10% volume of the culture media) of 5 mg/mL MTT was added. After 4 h incubation, the medium was removed and 150 μL DMSO was added to dissolve the formazan. Finally, the optical densities of dissolutions were measured using a universal microplate reader (FLX 800, Bio-TEK instruments, INC. USA) at a wavelength of 570 nm. The viability of HT-29 was expressed as a percentage of control cells (0.1% DMSO medium).

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**Figure S1** Chemical constituents isolated from *Melastoma dodecandrum* Lour.

**Figure S2.**The effect of compounds **1**, **3**, **6**, **17** on cell viability of HT-29. Confluent cells were incubated for 24 h with **1** (94 µM), **3** (96 µM), **6** (95 µM), **17** (42 µM), respectively. Cell viability was assessed by MTT assay and expressed as a percentage of control. Values are means± S.E.M. of four independent experiments.