Research Article

Selected Extracts of Chinese Herbal Medicines: Their Effect on NF-κB, PPARα and PPARγ and the Respective Bioactive Compounds

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Abstract

Chinese herbal medicinal (CHM) extracts from fourteen plants were investigated in cell-based in vitro assays for their effect on NF-κB (NF-κB), a key regulator of inflammation, as well as on peroxisome proliferator-activated receptors (PPARs) being key regulators of genes involved in lipid and glucose metabolism. 43% of the investigated CHMs showed NF-κB inhibitory and 50% PPARα and PPARγ activating effects. Apolar extracts from cortex and flos of Albizia julibrissin Durazz. and processed rhizomes of Arisaema sp. and Pinellia ternata (Thunb.) Breit. that effectively inhibited TNF-α-induced NF-κB activation and dose-dependently activated PPARα and PPARγ were further investigated. Bioassay-guided fractionation and analysis by GC-MS led to the identification of fatty acids as PPAR agonists, including linoleic and palmitic acid.

1. Introduction

Herbal medicines are an important part of Traditional Chinese Medicine (TCM) of which the medical use and processing methods are well documented. Traditional processing methods (pao zhi) are important to enhance the efficacy and/or to reduce the toxicity of crude herbal products [1]. Chinese herbal medicine (CHM) encompasses over 11,000 species of medicinal plants and is a valuable source [2] for the identification of biologically active natural products. Investigation of the molecular targets and mechanistic action of CHMs and their single compounds is currently a central task in TCM research [3]. Thanks to advances in molecular biology, refined bioassays are now available which enable rapid screening of natural products for bioactivity towards specific targets [4]. In this study, extracts of different polarity from CHMs of fourteen plant species were tested for a potential inhibition of TNF-α-induced NF-κB activation and an agonistic activity towards PPARα and PPARγ. The CHMs were selected in cooperation with Chinese partners [5]. This in-house collection of CHMs (Table 1) was already examined earlier based on their traditional use against insomnia and anxiety regarding a putative modulation of the GABA_A receptor [6]. The listed CHMs are, however, also traditionally prescribed as single herbs as well as in formulations for clearing heat and drying dampness, among others. Therefore, to gain insight into possible multi-target effects of the individual CHMs, this study examined their influence on the nuclear factor κB (NF-κB) pathway and peroxisome proliferator-activated receptors (PPARs), which are important drug targets with regard to inflammation and metabolic dysfunction.

The NF-κB signaling pathway is a key regulator of inflammation. Inflammatory stimuli such as tumor necrosis factor alpha (TNF-α), infectious agents (lipopolysaccharide (LPS)), injury, and other stressful conditions activate the NF-κB signal transduction pathway. Thereby, activation of the IKK complex leads to phosphorylation and subsequent proteasomal degradation of 1κB proteins. NF-κB dimers are translocated to the nucleus and bind to κB promoter
sites resulting in the transcription of proinflammatory target genes [7, 8]. Inhibitors of NF-κB signaling are promising candidates for both the prevention and therapy of (chronic) inflammation. Terpenoids, such as parthenolide, are known to be effective natural NF-κB inhibitors [9].

PPARs regulate the expression of genes involved in lipid and glucose metabolism and homeostasis in a ligand-dependent manner [10]. In the activated form, PPARα promotes mainly fatty acid (FA) catabolism, and PPARγ enhances insulin sensitivity and lipid storage [11, 12]. PPARα and PPARγ agonism is also linked to the suppression of proinflammatory genes, for example, via an interference with the NF-κB signaling pathway [13, 14]. Synthetic PPAR agonists (fibrates and thiazolidinediones) bind PPARs with high affinity. They, however, show significant dose limiting adverse side effects. Natural products were revealed to be a promising source of safer PPARα and PPARγ dual agonists or partial PPAR modulators [15–18].

In our study, extracts of selected CHMs that exhibited a significant activity towards PPARα or NF-κB were further subjected to dose-response studies and bioassay-guided fractionation. These selected CHMs were cortex and flos of *Albizia julibrissin* Durazz. as well as processed and crude rhizomes of *Arisaema* sp. and *Pinellia ternata* (Thunb.) Breit. Traditionally, *A. julibrissin* cortex and flos are used in CHM to treat insomnia and injuries and to calm the mind. The rhizomes of *Arisaema* sp. and *P. ternata* are toxic in their crude form and therefore traditionally processed (Table 1). Processed rhizomes of *Arisaema* sp. and *P. ternata* CHMs are effective in removing damp-phlegm such as in convulsions and spasms in the intestines [19, 20]. The dried, pulverized crude rhizomes from *P. ternata* are used externally to treat swellings. Bioactive compounds were isolated, analyzed, and characterized by mass spectrometry and NMR.

### 2. Materials and Methods

#### 2.1. Materials

Human embryonic kidney (HEK) 293 cells stably transfected with an NF-κB-responsive luciferase reporter were purchased from Lonza Group AG (Basel, Switzerland) and 3T3-L1 preadipocytes and HEK293 cells from the American Type Culture Collection (Manassas, VA, USA). The PPAR luciferase reporter construct (tk-PPREx3-luc) and the expression plasmids for PPARα and PPARγ (pCMX-mPPARα and pCMX-mPPARγ) were a gift from Professor Ronald M. Evans (Howard Hughes Medical Institute, La Jolla, CA, USA). The plasmid encoding enhanced green fluorescent proteins (pEGFP-N1) was obtained from Clontech (Mountain View, CA, USA). Dulbecco’s modified Eagle’s medium (DMEM) containing 4.5 g/L glucose was purchased from Lonza Group AG (Basel, Switzerland) and fetal bovine serum (FBS) from Invitrogen (Lofer, Austria). GW7647 and troglitazone were purchased from Cayman Europe (Tallinn, Estonia) and 2-deoxy-D-(1H3)-glucose from Perkin Elmer (Waltham, MA, USA). For extraction, fractionation, and isolation by silica column chromatography (CC), solvents of highest available purity were used (VWR, Vienna, Austria). All other chemicals were obtained from Sigma-Aldrich (Vienna, Austria).

#### 2.2. Plant Materials

Material of the CHMs no. 1–14 were purchased from Plantasia (Oberndorf, Austria) (see Table 1) [19, 20], except of no. 12a which was purchased from Sichuan Neautus Traditional Chinese Medicine Co., Ltd (Chengdu, China).
2.3. Extraction and Fractionation. 50 g of each of the CHMs nos. 1a, 1b, 2a, 3–11, 12b, 13, and 14 was soaked in 500 mL petroleum ether (PE) for 10 min, and extracted under reflux for 30 min. The obtained extracts were filtered and evaporated to dryness. The remaining drug material was air-dried overnight and extracted each with 500 mL of ethyl acetate (EtOAc), methanol (MeOH), and distilled water likewise, whereas the water extract was lyophilized. All following extractions were performed at 150 bar and 40°C using an ASE 200 accelerated solvent extractor and a solvent controller ( Dionex, Sunnyvale, CA, USA). With the ASE every ca. 10 g of pulverized plant material in a capsule was extracted three times with 22 mL of solvent (66 mL/ca. 10 g). The scheme of each run of extraction was as follows: 5 min heating, 2 min static at 150 bar and 40°C, 10 s flushing and 60 s purging. For CHMs nos. 2b, 12a, and 12b out of 50 g each DCM dry extract yields of 0.4–0.7% were obtained and for no. 1b a yield of 2.9% (See Schemes 1 and 2 in Supplementary Material available online at doi:10.1155/2012/983023).

For bioassay-guided fractionation, 1.4 kg of CHMs no. 1a and 1.9 kg of no. 2a were pulverized and extracted by DCM and subsequently by MeOH. 68 g DCM extract (4.7% yield) and 92 g MeOH extract (6.6% yield) were obtained of no. 1a. 12.1 g DCM (0.6% yield) and 10.7 g MeOH (0.8% yield), out of 1.4 kg of the material already extracted by DCM, were obtained of no. 2a. The dried extracts of no. 2a were subjected to silica CC with Silica Gel 60 (particle size of 0.063–0.200 mm, Merck, Darmstadt, Germany). The column with DCM extract was eluted with CHCl3:MeOH:H2O 98:2:1 to 60:38:8.5 to obtain eight fractions (D1–D8). From fractions D8, cerebrosidea were isolated by silica CC eluting with CHCl3:MeOH:H2O 65:25:4. Cerebrosidea were also detected in CHMs no. 12a and b from P. ternata. 15 g of DCM extract of no. 1a was fractionated using VLC (Vacuum Liquid Chromatography) using Silica Gel 60 (particle size of 0.063–0.200 mm, Merck, Darmstadt, Germany) as solid phase. The silica column was eluted subsequently with PE, DCM (twice), EtOAc, MeOH, and 70% MeOH (3 L per solvent) under reduced pressure. Thereby 12 g PE extract, 963 mg extract from the first 3L eluted with DCM (DCM-I), 776 mg from the next 3L eluted with DCM (DCM-II), 6.90 g EtOAc extract, 4.08 g MeOH extract, and finally 170 mg extract eluted with 90% MeOH were obtained as dry extracts. Betulinic acid (12 mg) was isolated from EtOAc VLC fraction of the DCM extract from no. 1a. A mixture of trihydroxy and epoxy-dihydroxy fatty acid methyl esters (FAMEs) (8 mg) and α-spinasterol-3-O-β-D-glucopyranoside (61 mg) was isolated from the crude MeOH extract by repeated silica CC eluting with CHCl3:MeOH:H2O and n-hexane:EtOAc gradients. The compounds in the mixture could be separated by GC-MS and were identified based on resemblance with the fragmentation in EI MS reported by Hamberg [21] as methyl 9,10,13-trihydroxy-11-octadecenoate, methyl 9,12,13-trihydroxy-11-octadecenoate, and methyl 9,10-epoxy-13-hydroxy-11-octadecenoate. Their hydroxylated FA derivatives were prepared by basic hydrolysis with 0.1 M NaOH (15 min RT) of the hydroxylated FAMEs and acidification with 0.1 M HCl/MeOH. For biological testing from all corresponding samples, always dry material was dissolved in DMSO.

2.4. NMR Analysis. All 1D (1H and 13C) and 2D (COSY, NOESY, HMQC, and HMBD) NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer. Betulinic acid was dissolved in CDCl3 (99.96 atom% D) and α-spinasterol-3-O-β-D-glucopyranoside in d5-pyridine (99.96 atom% D). The 1H and 13C NMR spectra were operated at 500 and 125 MHz, respectively. 13C and 1H NMR data of both were consistent with the literature values for betulinic acid [22, 23] and α-spinasterol-3-O-β-D-glucopyranoside [24, 25].

2.5. Analysis of Active Compounds by GC-MS. The investigated extracts and fractions were dissolved to 5 mg/mL in DCM, and 200 μL of the dissolved sample was transferred to a GC-MS vial, to which 50 μL of TMSH was added. The mixture with a concentration of 4 mg/mL was shaken for 30 s. A GCMS-QP2010 gas chromatograph mass spectrometer was utilized (Shimadzu Scientific Instruments, Columbia, MD, USA). Helium 5.0 was used as carrier gas, and the column flow was set at a constant value of 1.7 mL/min. The column used was a Zebon ZB-5 60 m × 0.25 mm (inner diameter), 0.25 μm (film thickness) (Phenomenex, Torrance, CA, USA). The software used was Lab Solutions GC-MS (version 2.50 SU3, Shimadzu). 1 μL of sample was injected of the prepared solutions with a split 1:10, and analysis was performed in the EI mode (70 eV, 250°C ion source temperature). The injection temperature was 270°C. A method with a temperature gradient from 120°C to 320°C in 40 min and 5 min hold on 320°C was applied for analysis. A mixture of trihydroxy- and epoxy-hydroxy FAMEs from no. 1a was derivatized by BSTFA-TMCS (99:1) for 30 min at 50°C and detected by GC-MS in the same conditions as above. The analyses were performed on an Agilent Technologies 6890 N Network GC equipped with an Agilent Technologies 5973 inert mass selective detector and a Combi PAL autosampler (CTC Analytics). The column used was a DB-5 with dimensions of 30 m × 0.25 mm (inner diameter), 0.23 μm (film thickness) (Agilent Technologies). The software used was MSD Chemstation 2004.

2.6. NF-κB Transactivation Assay. HEK 293 cells, stably transfected with as NF-κB-responsive luciferase reporter (Panomics, Fremont, CA, USA), were seeded in 10 cm dishes and transfected with 5 μg pEGFP-N1 (Clontech, Mountain View, CA, USA). Six hours later, cells were transferred to 96-well plates and incubated overnight (5% CO2, 38°C). On the next day, the medium was exchanged with a serum-free DMEM, and cells were treated with the indicated extracts or fractions. The solvent vehicle (0.1% DMSO) served as negative control and 5 μM parthenolide as positive control. After one hour, cells were stimulated with 2 ng/mL human recombinant TNF-α for 6h, then the medium was removed, and cells were lysed. The luminescence of
Table 2: Screening of Chinese herbal medicines for agonistic activity towards PPARα and PPARγ and inhibition of TNF-α-induced NF-κB activity in vitro. PE = petroleum ether, EtOAc = ethyl acetate, MeOH = methanol. Dry material from all test samples was dissolved in DMSO at a concentration of 10 mg/mL and tested at a final concentration of 10 μg/mL. Regarding PPARs: no activation compared to control (0.1% DMSO): −; 1.5- to 2-fold activity compared to control (P < 0.05): +; more than 2-fold of the control (P < 0.05): ++. Regarding NF-κB: inhibition in the range of 0–50% compared to the vehicle-treated control (0.1% DMSO): −; 50–80% inhibition (P < 0.05): +; over 80% inhibition (P < 0.05): ++. At least three independent experiments were performed with every sample, and statistical analysis was performed by ANOVA.

| Plant species                      | CHM no. | Extract   | PPARα activation | PPARγ activation | NF-κB inhibition |
|------------------------------------|---------|-----------|------------------|------------------|-----------------|
| *Albizia julibrissin* Durazz.      | 1a      | PE        | ++               | ++               | +               |
|                                    |         | EtOAc     | ++               | ++               |                 |
|                                    |         | MeOH      | +                | +                |                 |
|                                    |         | H2O       | −                 | −                 | +               |
|                                    | 1b      | PE        | ++               | ++               | +               |
|                                    |         | EtOAc     | ++               | ++               | +               |
|                                    |         | MeOH      | +                | +                | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Arisaema* sp.                     | 2a      | PE        | ++               | +                |                 |
|                                    |         | EtOAc     | −                 | −                 | −               |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Arnebia/Lithospermum* sp.         | 3       | PE        | −                 | −                 | −               |
|                                    |         | EtOAc     | −                 | −                 | −               |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Atractyloides macrocephala* Koidz.| 4       | PE        | −                 | −                 | −               |
|                                    |         | EtOAc     | −                 | −                 | −               |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Cnidium* monnieri (L.) Cuss.      | 5       | PE        | −                 | −                 | −               |
|                                    |         | EtOAc     | ++               | +                |                 |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Dimocarpus* (Euphoria) longan* Lour. | 6     | PE        | −                 | −                 | −               |
|                                    |         | EtOAc     | −                 | −                 | −               |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Forsythia* suspensa* (Thunb.)* Vahl. | 7     | PE        | −                 | −                 | +               |
|                                    |         | EtOAc     | −                 | −                 | −               |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Juncus* effusus* L.              | 8       | EtOAc     | −                 | −                 | +               |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Lilium* sp.                      | 9       | EtOAc     | +                 | +                 | −               |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Lophatherum gracile* Brongn.      | 10      | PE        | +                 | +                 | −               |
|                                    |         | EtOAc     | −                 | −                 | −               |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
| *Nelumbo nucifera* Gaertn.         | 11      | PE        | −                 | −                 | −               |
|                                    |         | EtOAc     | −                 | −                 | −               |
|                                    |         | MeOH      | −                 | −                 | −               |
|                                    |         | H2O       | −                 | −                 | −               |
the firefly luciferase and the fluorescence of the enhanced green fluorescent protein (EGFP) were quantified on a
GeniosPro plate reader (Tecan, Austria). The luciferase signal derived from the NF-κB reporter was normalized with the
EGFP-derived fluorescence to account for differences in the cell number.

2.7. PPAR Luciferase Reporter Gene Assay. The PPAR-luciferase reporter gene assay was performed as previously de-
scribed [26]. HEK293 cells were transiently transfected with a PPARα or PPARγ receptor expression plasmid, a reporter
plasmid (tk-PPREx3-luc), and a green fluorescent protein plasmid (pEGFP-N1) as an internal control. The cells were
harvested 6 h after the transfection and reseeded in 96-well
plates (5 × 10^4 cells/well). In the initial tests, cells were
measured with 10 μg/mL of the indicated extracts. In the dose-
response experiments, cells were treated with 0, 3, 27 or
81 μg/mL of the DCM extracts of CHMs nos. 1a, 1b, 2a,
2b, 12a, and 12b. To account for potential effects of the
solvent, 0.1% DMSO served as vehicle control. As positive
controls, 50 nM GW7647 and 5 μM troglitazone were used
to activate PPARα and PPARγ, respectively. Treated cells
were incubated for 18 h. After cell lysis, the luminescence
of the firefly luciferase and the fluorescence of EGFP were
quantified on a GeniosPro plate reader (Tecan, Salzburg,
Austria). The luminescence signals were normalized to the
DMSO-treated controls, 50 nM GW7647 and 5 μM troglitazone served as vehicle control. As positive
controls, 50 nM GW7647 and 5 μM troglitazone were used
to activate PPARα and PPARγ, respectively. Treated cells
were incubated for 18 h. After cell lysis, the luminescence
of the firefly luciferase and the fluorescence of EGFP were
quantified on a GeniosPro plate reader (Tecan, Salzburg,
Austria). The luminescence signals were normalized to the
EGFP-derived fluorescence to account for differences in cell
number or transfection efficiency.

2.8. Statistical Analysis. Statistical analysis was performed
using Prism Software (ver. 4.03; GraphPad Software Inc.,
San Diego, CA). Data were normalized to DMSO-treated
control of which the mean value was set as 1.0. The data
shown represent arithmetic mean and standard deviation of
3-4 independent experiments. Statistical significance was
determined by a one-way analysis of variance combined with
a Dunnett’s Multiple Comparison posttest. Results with P <
0.05 were considered significant.

3. Results and Discussion

In this study, extracts of important Chinese herbal medicines from fourteen different plant species (Table 1) were tested for
their potential to inhibit NF-κB and/or to activate PPARα
and PPARγ.

Table 2: Continued.

| Plant species                  | CHM no. | Extract | PPARα activation | PPARγ activation | NF-κB inhibition |
|-------------------------------|---------|---------|------------------|------------------|------------------|
| *Pinellia ternata* (Thunb.) Breit. | 12b     | PE      | ++               | +                | +                |
|                               |         | EtOAc   | -                | -                | -                |
|                               |         | MeOH    | -                | -                | -                |
|                               |         | H2O     | -                | -                | -                |
| *Polygonum multiflorum* Thunb. | 13      | PE      | -                | -                | -                |
|                               |         | EtOAc   | -                | -                | -                |
|                               |         | MeOH    | -                | -                | -                |
|                               |         | H2O     | -                | -                | -                |
| *Tribulus terrestris* L.      | 14      | PE      | -                | -                | -                |
|                               |         | EtOAc   | +                | +                | -                |
|                               |         | MeOH    | -                | -                | -                |
|                               |         | H2O     | -                | -                | -                |

Extracts of CHMs of six out of fourteen plant species
reduced TNF-α-induced NF-κB activity significantly (P <
0.05) (Table 2). In fact, this is the first report of a significant
NF-κB inhibitory activity of extracts from *A. julibrissin,
Arisaema sp.*, *J. effusus*, and *P. ternata*. A moderate (see expla-
nation accompanying Table 2) reduction of TNF-α-induced
NF-κB activity (P < 0.05) was found for the PE, EtOAc, and
MeOH extracts of *A. julibrissin* no. 1a and the EtOAc extract
of no. 1b and *C. monnieri* no. 5, and PE extracts of *Arisaema
sp.* no. 2a and *P. ternata* no. 12b. The EtOAc extracts of
*F. suspensa* no. 7, *J. effusus* no. 8, and the aqueous extract
of *A. julibrissin* no. 1b exhibited an especially strong ability
to inhibit NF-κB activity. Betulinic acid was isolated from the DCM extract of the bark of *A. julibrissin* no. 1a [27]. Many terpenoids,
including betulinic acid, are reported to effectively inhibit
NF-κB signaling [9, 28]. However, we neither detected NF-κB
inhibition nor PPARα and PPARγ activation by betulinic acid
in concentrations up to 30 μM excluding betulinic acid as one
of the main active principles in our cellular models. Osthole
might in part have contributed to the observed activities of
the EtOAc extract of *C. monnieri* no. 5 [29–31].

When testing for a PPARα and PPARγ agonistic activity, a
significant effect (P < 0.05) was observed for apolar extracts
of seven out of the fourteen plants used as CHM (Table 2).
Moderate to strong PPARα and PPARγ activity was observed
for apolar extracts from *A. julibrissin* no. 1a and 1b, *Arisaema
sp.* no. 2a, *C. monnieri* no. 5, *P. ternata* no. 12a and *T. terrestris*
no. 14 (P < 0.05). Aporal extracts of *Lilium* sp. no. 9 and *L.
lophatherum* no. 10 activated PPARα moderately (P < 0.05).
Previously, a saponin from fruits of *T. terrestris* [32, 33] and
aqueous extracts of arillus from *D. longan* and rhizomes of
Figure 1: Dose-response experiments with DCM extracts of CHMs of *A. julibrissin* no. 1a and b, *Arisaema* sp. no. 2a and b, and *P. ternata* no. 12a and b. HEK293 cells were transiently transfected with the expression plasmid for PPARα or PPARγ, the reporter plasmid pPPRE-tk3x-luc, and the internal control plasmid (EGFP). The transiently transfected cells were incubated for 18 h with 3, 9, 27, and 81 μg/mL of each indicated extract. Furthermore, the cells were similarly incubated with 0.1% DMSO (negative control), 50 nM GW7647 (PPARα agonist, activated PPARα 2.5–3.5 fold but not PPARγ), or 5 μM troglitazone (PPARγ agonist, activated 3.5–6.5 fold PPARγ but not PPARα) as positive controls (not shown). Luciferase activity and fluorescence intensity were measured. Results are presented as mean ± SD, (n = 4). Significantly different from the negative control (ANOVA), *P < 0.05; **P < 0.01.

*P. ternata* [34–36] were reported to increase PPARα and PPARγ gene expression levels.

A selection of CHMs with strong activity, namely, *A. julibrissin* no. 1a and b, *Arisaema* sp. no. 2a and b and of *P. ternata* no. 12a, and b were further studied. The DCM extracts of no. 1a, 1b, 2a, 2b, 12a, and 12b showed a significant dose-dependent increase of PPARα and PPARγ activity (*P < 0.01*) (Figure 1), whereby those of nos. 1a and 1b from
(5) octadecanoic acid (3.3; 9.4; 2.2%), (6) docosanoic acid (—; 4.5; 3.0%).

(2) linoleic acid (4.5; 1.0; 47%), (3) 9-octadecenoic acid (3.3; 5.1; 4.4%), (4) oleic acid (—; 3.8; 5.8%), (5) octadecanoic acid (3.3; 9.4; 2.2%), (6) docosanoic acid (—; 4.5; 1.7%), and (7) tetracosanoic acid (—; 2.3; 3.0%).

A. julibrissin and no. 2a from Arisaema sp. were most potent. Loss of activity at 81 μg/mL of these extracts was possibly due to toxicity or unspecific inhibitory action.

Bioassay-guided fractionation showed that the fraction “DCM-II” (see Section 2) of the DCM extract from A. julibrissin no. 1a significantly activated PPARα by 3.5-fold (±0.31, P < 0.01) and PPARγ by 2.8-fold (±0.28, P < 0.01) (compared to DMSO as solvent control). The EtOAc VLC fraction significantly activated PPARα by 2.9-fold (±0.52, P < 0.01) and PPARγ by 2.5-fold (±0.19, P < 0.01). The apolar fraction “D6” from Arisaema sp. no. 2a significantly activated PPARα by 2.2-fold (±0.10, P < 0.01) and PPARγ by 1.5-fold (±0.06, P < 0.01) (Figure 2). GC-MS analysis after derivatisation revealed that the VLC fractions of A. julibrissin no. 1a (DCM) and fraction “D6” from Arisaema sp. no. 2a contained mainly fatty acids (FAs), such as palmitic acid (16:0), stearic acid (18:0), and linoleic acid (18:2) (Figures 3 and 4) that are known as PPAR agonists [11]. 13-Phenyltridecanoic acid (13:0) was found as a compound specific for aroids [37]. Especially polysaturated FAs activate PPARs potently [38]. Wolfrum et al. reported palmitic acid (16:0), linoleic acid (18:2), oleic acid (18:1), and stearic acid (18:0) to activate PPARα by 2.5-, 4.6-, 3.5-, and 2-fold, respectively [39]. The processing might have influenced availability of FA from rhizomes of P. ternata. The concentration of FAs detected was higher in the DCM extract of processed rhizomes of P. ternata (no. 12b) than in that of the crude rhizomes (no. 12a) (Figure 4). This was in line with the observation that the DCM extract of the processed 12b was PPARα and PPARγ active at slightly lower concentration than that of the crude rhizomes (no. 12a) (Figure 1). The activation of PPARα by fraction “D6” may be attributed largely to palmitic acid, which is highly enriched in fraction “D6” reaching 61% of the total content (Figure 4).

\[ \alpha \text{-Spinasterol-3-O-β-D-glucopyranoside from } A. \text{julibrissin}\]

and cerebrosides isolated from the DCM extract of rhizomes.

Figure 2: Comparison of the PPARα- and PPARγ-activating potential of the VLC fractions petroleum ether (PE), DCM-I and -II, and ethyl acetate extract (EtOAc) of the DCM extract from A. julibrissin no. 1a (a) and the DCM fractions D1-8 from Arisaema sp. no. 2a. (b). HEK293 cells, transiently transfected with expression plasmids for PPARα or PPARγ, luciferase reporter (tk-PPREx3-luc), and EGFP as internal control, were incubated for 18 h with 10 μg/mL of the indicated fraction, solvent vehicle (0.1% DMSO, negative control), and 50 nM GW7647 (PPARα agonist) or 5 μM troglitazone (PPARγ agonist) as positive control. Luciferase activity and fluorescence intensity were measured. Results are presented as mean ± SD (n = 4). Significantly different from the negative control (ANOVA), *P < 0.05; **P < 0.01.

Figure 3: Comparative analysis by GC-MS of the DCM-I and -II and EtOAc VLC fractions (1 μL of 4 mg/mL in DCM) from the mother DCM extract of CHMs nos. 1a of A. julibrissin. FAMEs formed from the present FAs after derivatisation by TMSH were detected by GC-MS on a Zebron ZB-5 column (60 m × 0.25 mm, 0.25 μm) (Phenomenex) with a temperature gradient of 120°C-320°C in 40 min and 5 min hold; carrier gas: helium; flow rate: 1.7 mL/min. Ion source: EI 70 eV, 250°C. The following FAs were identified by GC-MS with a relative content (%) in each enriched extract given in the order DCMI; DCMII and EtOAc: (1) palmitic acid (2.9; 37; 23%), (2) linoleic acid (4.5; 1.0; 47%), (3) 9-octadecenoic acid (3.3; 5.1; 4.4%), (4) oleic acid (—; 3.8; 5.8%), (5) octadecanoic acid (3.3; 9.4; 2.2%), (6) docosanoic acid (—; 4.5; 1.7%), and (7) tetracosanoic acid (—; 2.3; 3.0%).
of Arisaema sp. and P. terna were tested; however, did not show agonistic activity towards PPARα- and PPARγ-activity in vitro.

It became further evident that the content of the FAs present in “D6” was also high in the DCM extracts from CHMs from Arisaema sp. and P. terna (Figure 4), especially of palmitic acid (30%–74%). The CHMs of T. terrestris and C. monnieri were previously described to contain FAs including palmitic acid, stearic acid, oleic acid, and linoleic acid [19, 32]. Besides common FAs, trihydroxy- and epoxy-hydroxy FAMEs and α-spinasterol-3-O-β-D-glucopyranoside were isolated from the MeOH extract of A. julibrissin no. 1a. Although mono- and dihydroxylated FAs are natural PPARγ agonists [40], trihydroxy- and epoxy-hydroxy FAMEs as well as their FA derivatives, identified by GC-MS [21], lacked PPARα and PPARγ activity in vitro in this study. Interestingly, chronic exposure to high levels of palmitic acid and stearic acid, identified as one of the main active principles in this study, are associated with lipotoxicity or insulin resistance, the main causative factor of type 2 diabetes and the metabolic syndrome [33, 39]. However, unsaturated free FAs, such as linoleic acid and oleic acid, do not promote insulin resistance [41]. In contrast, even a protective effect of oleic acid against palmitate-induced insulin resistance in L6 rat myotubes has been demonstrated [42]. Moreover, FAs have recently been identified as rather potent inhibitors of PTP1B, the main negative regulator of insulin and leptin signaling [43]. Thus, the impact of FAs on health and disease seems to highly depend on duration of exposure, concentration of the FA, or the degree of saturation.

4. Conclusion

In this study, extracts from CHMs were tested for a putative PPARα- or PPARγ-activating and NF-κB-inhibiting effect. Out of the fourteen plant species of which extracts were tested, 43% exhibited NF-κB inhibitory and 50% PPAR activating effects. The apolar PPAR active extracts and enriched fractions from flos and cortex of A. julibrissin and rhizomes of Arisaema sp. and P. terna mainly contained fatty acids as PPAR-agonists, including palmitic acid, linoleic acid, oleic acid, and stearic acid. The outcome of this study contributes to the molecular understanding and explanation of some effects elicited by extracts of these three traditional Chinese herbs by revealing PPAR activation due to the present fatty acids.

Abbreviations

ASE: Accelerated solvent extractor
BSTFA-TMCS: N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane
CC: Column chromatography
CHM: Chinese herbal medicine
DCM: Dichloromethane
DMEM: Dulbecco’s modified Eagle’s medium
DMSO: Dimethylsulfoxide
EGFP: Enhanced green fluorescent protein
El MS: Electron ionization mass spectrometry
EtOAc: Ethylacetate
FA: Fatty acid
FAME: Fatty acid methyl ester
FBS: Fetal bovine serum
GC-MS: Gas chromatography mass spectrometry
LPS: Lipopolysaccharide
MeOH: Methanol
NF-κB: Nuclear factor κB
NMR: Nuclear magnetic spin resonance
PE: Petroleum ether
PPAR: Peroxisome proliferator-activated receptor
TNF: Tumor necrosis factor
TMSH: Trimethylsulphoniumhydroxide
VLC: Vacuum liquid chromatography.

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