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## Screening of Soluble Epoxide Hydrolase Inhibitory Ingredients from Traditional Chinese Medicines for Anti-inflammatory Use

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### Abstract

**Ethnopharmacological relevance**—Inhibition of soluble epoxide hydrolase (sEH) has been extensively reported to be anti-inflammatory in multiple animal models. Some anti-inflammatory traditional Chinese medicines (TCMs) and a few natural compounds were also found to be inhibitory to sEH *in vitro*.

**Aim of the study**—To determine whether the active ingredient (AI) against sEH of anti-inflammatory TCMs *in vitro* is anti-inflammatory *in vivo* and the sEH inhibitory action of the AI contributes to its anti-inflammatory effect *in vivo*.

**Materials and Methods**—*In vitro* inhibition assay of the sEH was conducted for the methanol and ethanol extracts of 27 anti-inflammatory TCMs. Two potent extracts were subject to further separation guided by bioassay to afford promising AI against sEH *in vitro* [Fr.5 of the crude ethanol extract of *Rhizoma coptidis* (FFCERC)]. Finally, the *in vivo* anti-inflammatory effect and sEH inhibitory potency of FFCERC was evaluated in a lipopolysaccharide (LPS)-challenged murine model of acute systemic inflammation. The inflammatory status was characterized by the inflammatory cytokines TNF- $\alpha$  and interleukine-6 (IL-6) and sEH inhibitory function was evaluated by the plasma levels of epoxyeicosantrienoic acids (EETs) and dihydroxyeicosantrienoic acids (DHETs), which are the sEH mediated substrates and products, respectively.

**Results**—At the concentration of 25 $\mu$ g/mL, the crude ethanol extracts of 6 TCMs including *Herba Asari*, *Radix Polygalae*, *Fructus Amomi*, *Radix Astragali*, *Radix Scutellariae*, and *Rhizoma Coptidis* were potent against sEH. The crude extracts of *Herba Asari* and *Rhizoma Coptidis* were selected for further separation to afford FFCERC as the most promising AI for *in vivo* evaluation.

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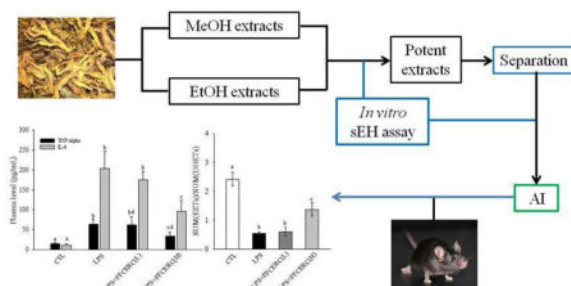
#### Author contribution

JYL (jyliu@tongji.edu.cn), HZH (hzhzhuang@yahoo.com) and BDH (bdhammock@ucdavis.edu) designed the study. JYL, CM (chmorisseau@ucdavis.edu), and HZH conducted the research and analyzed the data. JYL, CM and BDH wrote the paper.

Oral administration of FFCERC attenuated the significant increase in TNF- $\alpha$  and IL-6 caused by LPS challenge in a dose-dependent manner. In parallel, oral administration of FFCERC shifted the changes in plasma levels of EETs and DHETs caused by LPS-challenge like a synthetic sEH inhibitor.

**Conclusions**—A sEH inhibitory AI from *Rhizoma Coptidis* is anti-inflammatory and the inhibition of sEH contributes to this biological effect, indicating that sEH may be at least one of multiple therapeutic targets for relevant TCMs.

## Graphic abstract



## Keywords

*Rhizoma Coptidis*; soluble epoxide hydrolase (sEH); anti-inflammation; eicosanoids; cytokines

## 1. Introduction

Epoxide hydrolases (sEH) are hydrolytic enzymes that widely present in living organisms including plants and animals, mediating the metabolism of epoxides to form vicinal diols by the addition of a water molecule (Newman et al., 2005). The inhibitors of soluble EH (sEH) in mammals have been extensively reported to be anti-inflammatory (Hung et al., 2015; Yang et al., 2015a), analgesic (Hammock et al., 2011), anti-hypertensive (Charles et al., 2014; Honetschlagerova et al., 2013; Varcabova et al., 2013), anti-hypertrophy (Ai et al., 2009; Althurwi et al., 2013; Xu et al., 2006), anti-diabetic (Zuloaga et al., 2014), anti-fibrosis (Sirish et al., 2013) and reno-protective (Honetschlagerova et al., 2013; Kim et al., 2015) mainly through the stabilization of the endogenous epoxyeicosatrienoic acids (EETs) that are vasodilators, platelet aggregation inhibitors, and anti-inflammatory and analgesic mediators involved in the modulation of NF $\kappa$ B and I $\kappa$ B kinase system (Liu et al., 2012; Xu et al., 2006). Numerous sEH inhibitors were synthesized in laboratory for the pharmacological use in multiple diseases. However, as so far, no compound is clinically used specifically/selectively targeting sEH inhibition although both the multikinase inhibitor sorafenib and antimicrobial triclocarban were reported to be potent sEHIs (Liu et al., 2009a; Liu et al., 2011).

In contrast to the synthesized sEH inhibitors, natural products such as isolated compounds or extract formula from traditional Chinese medicines (TCMs) represent valuable pools for the development of sEH inhibitors for clinical use. However, only a few studies were reported regarding the discovery and development of sEH inhibitors from natural materials. For

example, Kitamura et al a potent sEH inhibitor (1, 3-bis (4-methoxybenzyl) urea) from the root of the plant *Pentadiplandra brazzeana* with a  $IC_{50}$  of 92 nM against human sEH (Kitamura et al., 2015). Shi et al reported that the ethanol extract of *Sophora flavescens* root (Fabaceae) exhibits sEH inhibition with a  $IC_{50}$  of 2  $\mu\text{g/mL}$  (Shi et al., 2008). Bai et al found seven compounds from the methanol/water (v/v, 1/1) extract of the leaves of *Eucommia ulmoides* Oliver with  $IC_{50}$ s against human recombinant sEH less than 100  $\mu\text{M}$  (Bai et al., 2015). The lack of the extensive studies conducted to discover natural sEH inhibitors mainly account for two facts: first, no compound has been clinically used to specifically/selectively target sEH inhibition; and second, only few natural medicines have validated their pharmacological effects with sEH inhibition *in vivo*. Here, we present evidences that some extracts of anti-inflammatory TCMs have sEH inhibitory potency, which contributes to the anti-inflammatory effect of the plant active ingredient (AI).

## 2. Material and methods

### 2.1. TCMs materials, chemicals and reagents

The TCM materials used in present study were purchased from a certificated TCM store in Shanghai, China. The voucher specimens were deposited in the herbarium of Department of TCM, Shanghai Tenth People's Hospital. The voucher specimen numbers for all deposited voucher specimen were presented in Table 2. Methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), dichloromethane (DCM) and water were purchased from Fisher Scientific (Pittsburgh, PA). Formic acid, acetic acid, and lipopolysacchride (LPS, *Escherichia coli* serotype 0111:B4) were purchased from Sigma-Aldrich (St. Louis, NJ). The HyperSep C18 SPE columns for fractionation of crude extracts were purchased from the local distributor of Thermo Fisher Scientific (Pittsburgh, PA). Mice were purchased from Shanghai Lab. Animal Research Center (Shanghai, China) and experiments were performed according to protocols approved by the Animal Use and Care Committee of Shanghai Tenth People's Hospital, Tongji University School of Medicine.

### 2.2. Preparation of the crude extracts for *in vitro* sEH inhibitory assay

Dried TCM materials were chopped or pulverized into small pieces with the length in any direction no longer than 5 mm. The chopped or pulverized material (2 g) was mixed with 95% EtOH (10 mL) and sit under room temperature over night. Then the mixture was filtered under vacuum. The residue was extracted a 2<sup>nd</sup> time with 10 mL 95% EtOH. The filtrate was combined and then evaporated under vacuum at room temperature to give the EtOH extract. The second chopped or pulverized material (2 g) was then extracted with MeOH (10 mL $\times$ 2) using the same procedure as the extraction with EtOH. The filtrate from 2 times of extraction with MeOH was combined and then evaporated under vacuum at room temperature to give the MeOH extract. Each extract (2.5 mg) was added into a clean 2 mL eppendorf tube and then mixed with DMSO (1.0 mL). Under room temperature, the mixture was mixed on a Vortex mixer at 1500 rpm for 4 h. The mixture was then centrifuged at 15000 rpm for 5 min and the supernatant (500  $\mu\text{L}$ ) was transferred into a clean tube for *in vitro* sEH inhibitory assay.

### 2.3. Preparation of fraction of the crude extract for *in vitro* sEH inhibitory assay

The crude EtOH extract of *Rhizoma Coptidis*, the most potent one were subject to further fractionation by using a SPE column. The crude extract (2 g) was loaded to a SPE column mixed with MeOH/water (v/v, 2/8, 4 mL). After eluted under vacuum, the eluent was dried on a Speedvac under vacuum to give Fr.1. Then a series solution [MeOH/water (v/v, 4/6), MeOH/water (v/v, 6/4), MeOH/water (v/v, 2/8), MeOH, and MeOH/DCM (v/v, 1/1), each 4 mL] was used to elute the column sequentially. The eluents were evaporated on a Speedvac under vacuum to give Fr.2, Fr.3, Fr.4, Fr.5, and Fr. 6, respectively. Each fractionation (0.250 mg) was formulated in DMSO (1 mL) following the procedure mentioned in 2.2 for *in vitro* sEH inhibitory assay.

### 2.4. *In vitro* sEH Inhibitory assay

The *in vitro* sEH inhibition and IC<sub>50</sub> values were determined by using a fluorescent assay method reported previously (Jones et al., 2005; Liu et al., 2009b).

### 2.5. *In vivo* pharmacological effect of the active intergradient (AI)

Fr.5 of the crude ethanol extract of *Rhizoma Coptidis* (FFCERC) was grounded carefully using a pestle and mortal and then suspended into saline (50 mg/mL). Mice (C57BL/6, male, 8-week old) were assigned at random into 4 groups, each group containing 4 or 5 animals receiving respective treatments. Specifically, the animals in group 1 received saline orally (p.o.) and intraperitoneally (i.p.) as negative control. The animals in group 2 received saline (p.o.) and LPS (10 mg/mL, i.p.) as positive control. The animals in the group 3 received LPS (10 mg/mL, i.p.) and FFCERC (100 mg/kg, p.o.). FFCERC was administered immediately and 12 hr after LPS injection, respectively. The animals in the group 4 received saline (i.p.) and FFCERC (250 mg/kg, p.o.). FFCERC was administered as described in group 3. Mice were sacrificed 24 hours after treatment. The blood was collected to separate plasma for further analysis as previously reported protocol (Liu et al., 2009b). In addition, the chemical fingerprints of the EtOH extract of *Rhizoma Coptidis* and its resulted fraction FFCERC were presented as Figure 1 by using an Agilent 1260 HPLC-AB SCIEX QTrap6500 system.

### 2.6. Quantitative analysis of sEH-mediated lipid signaling mediators

Plasma samples were prepared for quantitative analysis according to the previously reported method (Liu et al., 2010a). The separation of sEH-mediated lipid signaling mediators, such as EETs and DHETs was conducted on Agilent 1260 equipped with an Agilent Eclipse plus C18 RRHT column (1.8  $\mu$ , 2.1 $\times$ 150 mm) according to previous method (Yang et al., 2009) while the LSMs were monitored by an AB SCIEX QTrap6500 mass spectrometer. The detailed MS parameters were presented in Table 1.

### 2.7. ELISA assay of TNF- $\alpha$ and IL-6

Plasma levels of TNF- $\alpha$  and IL-6 were measured by using ELISA kits according to the previously reported method (Liu et al., 2010b).

## 2.8. Statistical analysis

Data are presented as mean  $\pm$  SD. Statistical analyses were conducted by ANOVA followed with Tukey's (variance homogeneity) or Games-Howell's (variance heterogeneity) post ad hoc comparison test using the software SPSS 22.0 (SPSS Inc., Chicago, IL) with  $P < 0.05$  as the significant level.

## 3. Results

### 3.1. The yields and sEH inhibitory potency of crude extracts of TCMs

As shown in Table 2, the ethanol and methanol crude extracts yields of raw materials were at the range between 0.2 and 50.7%. Among tested TCMs, the ethanol crude extracts yields were usually lower than those of methanol crude extracts except the extracts of *Fructus Amomi*, *Fructus Forsythiae*, *Fructus Gardeniae* and *Fructus Schisandrae chinensis*.

At the tested concentration of 0.025 mg/mL, the ethanol extracts of most tested TCMs usually yield the higher inhibition of the human sEH than methanol extracts. In addition, the crude ethanol extracts of 6 TCMs, including *Herba Asari*, *Radix Polygalae*, *Fructus Amomi*, *Radix Astragali*, *Radix Scutellariae*, and *Rhizoma Coptidis* had the sEH inhibitory potency of more than 100% at the tested concentration of 0.025 mg/mL.

### 3.2 sEH inhibitory potency of fractions of promising extracts of TCMs

As shown in Table 2, the ethanol crude extracts of *Rhizoma Coptidis* and *Herba Asari* were the most promising ones among all the extracts. Therefore, they were further fractioned by flash chromatography over SPE columns, each to give 6 fractions as presented in Table 3. At the tested concentration of 2.5  $\mu$ g/mL, the Fr.5 of the ethanol crude extract of *Rhizoma Coptidis* (FFCERC) was the most potent against sEH, with inhibition over 80%. Unexpectedly, the six fractions of the ethanol crude extract of *Herba Asari* did not show any strong inhibition of the human sEH ( $< 40\%$ ).

### 3.3 Anti-inflammatory effect of FFCERC

To test the anti-inflammatory effect of FFCERC, we administered FFCERC suspension to a murine model of acute systemic inflammation caused by LPS. As expected, in the animals received LPS only, the plasma levels of pro-inflammatory cytokines TNF- $\alpha$  and IL-6 were significantly increased. In the test animals, this inflamed state was significantly attenuated by the oral administration of FFCERC in a dose dependent manner (Table 4 and Figure 2A and 2B).

### 3.4 FFCERC mediated epoxide lipids like sEH inhibitors

To test whether FFCERC can inhibit sEH *in vivo*, we measure the plasma levels of anti-inflammatory lipid signaling mediator EETs, which are hydrolyzed by sEH to their corresponding metabolites DHETs. The plasma levels of four EETs and four corresponding DHETs are presented in Table 4. As illustrated in Figure 2C, LPS-challenge significantly decreased plasma level of EETs while significantly increased plasma level of DHETs, resulting in a significant decrease in the ratio of EETs to DHETs (Figure 2D). High dose of FFCERC significantly shifted the changes caused by LPS in plasma levels of EETs and

DHETs, as well as the corresponding change in EETs/DHETs ratio while low dose of FFCERC slightly changed plasma levels of EETs and DHETs.

#### 4. Discussion

The sEH has been documented to be a potential intervention target for many inflammation-associated morbid conditions like pain (Pillarisetti and Khanna, 2012; Wagner et al., 2011), cardiovascular and renal diseases (Fleming, 2014; Imig and Hammock, 2009; Kim et al., 2015). In contrast to the data available for synthetic sEH inhibitors, only a few studies were reported regarding natural sEH inhibitors and sEH inhibition of natural materials (Bai et al., 2015; Kitamura et al., 2015; Lee et al., 2014; Li et al., 2015b; Shi et al., 2008; Sun et al., 2014). We also note that, in most studies the natural sEH inhibitors and sEH inhibition of natural materials were only evaluated *in vitro* except for 1, 3-bis (4-methoxybenzyl) urea, a compound from the root of the plant *Pentadiplandra brazzeana*, whose analgesic effect as a sEH inhibitor was evaluated in a rodent model of nociceptive pain (Kitamura et al., 2015). Different from most previous studies, we tested the *in vitro* sEH inhibitory activity of crude extracts of 27 TCMs first. And then we performed fractionation of two promising extracts and selected the most promising fraction guided by the bio-assay for further *in vivo* anti-inflammatory investigation.

This study evaluated the *in vitro* inhibitory potency against human sEH of MeOH and EtOH extracts of 27 anti-inflammatory TCMs, respectively. The major traditional use of these TCMs relating to anti-inflammation was presented in Table 2. Usually, the EtOH extract is more potent than the MeOH extract of the same TCM, probably due to the former contains less polar components than the latter, which is also supported by the structurally similar synthetic sEH inhibitors, showing the less polar one with stronger potency against sEH (Kim et al., 2007). Previously reported active pure natural compounds or natural component mixture against sEH usually yields IC<sub>50</sub> values in the μM range (Bai et al., 2015; Lee et al., 2014; Li et al., 2015b; Shi et al., 2008; Sun et al., 2014). In this study, we found the EtOH extracts from eight TCMs, including *Radix Glycyrrhizae*, *Herba Asari*, *Fructus Amomi*, *Salvia prionitis*, *Herba Houttuyniae*, *Radix Scutellariae*, *Herba Taraxaci* and *Rhizoma Coptidis*, have potent inhibitory activity against sEH, suggesting AIs with IC<sub>50</sub> values in the low nM range (Table 2). Among them, *Herba Asari* is the only one whose MeOH and EtOH were very potent both with the IC<sub>50</sub> values below 10 μg/ml (Table 2). Therefore, *Herba Asari* and *Rhizoma Coptidis*, which EtOH extract was the most potent against sEH, were selected for further purification.

Interestingly, the further fractionation of the EtOH extract of *Herba Asari* did not yield any fraction with high potency (table 3). On the other end, the EtOH extract of *Rhizoma Coptidis* provided two fractions with high sEH inhibitory potency; with fraction 5 (FFCERC) showing higher potency than fraction 2 against sEH. Thus FFCERC was selected for further *in vivo* test.

We used a murine model of acute systemic inflammation that was previously used to evaluate synthetic sEH inhibitors (Liu et al., 2013; Liu et al., 2009a; Liu et al., 2011; Liu et al., 2009b), to test the biological property of FFCERC. It should be noted that the TCM



extract is much different than the synthetic sEHs. For the synthetic sEHs with the IC<sub>50</sub> values of 1–10 nM *in vitro*, for example, AUDA-BE, *t*-AUCB, and TPPU, the *in vivo* effective oral doses in a murine model of inflammation were of 0.3–10 mg/kg depending on the oral bioavailability of these sEHs (Liu et al., 2013; Liu et al., 2009a; Liu et al., 2011; Liu et al., 2009b). However, in this study the FFCERC is much different. The FFCERC has a similar *in vitro* IC<sub>50</sub> value to the synthetic sEHs *t*-AUCB, but in the pilot study, even 10 mg/kg oral dose of FFCERC didn't work on the inflamed animals. The favorite effects (TNF- $\alpha$  and IL-6 plasma levels) weren't observed in the inflamed animals until the oral dose of FFCERC was elevated to 250 mg/kg. Specifically, after LPS challenge, the plasma levels of pro-inflammatory TNF- $\alpha$  and pleiotropic IL-6 significantly increased in comparison to the control group. Oral administration of FFCERC significantly decreased these markers of inflammation in a dose-dependent manner (Figure 2A and 2B). In response to the LPS-challenge, when compared to the control groups, the plasma level of EETs significantly decreased while the corresponding DHETs increased significantly, resulting in a significant decrease in plasma ratio of EETs to DHETs (Figure 2C and 2D). Oral administration of FFCERC revoked some of the changes in plasma levels of EETs and DHETs caused by LPS-challenge, in a similar way observed previously with synthetic sEH inhibitor (Liu et al., 2013; Liu et al., 2009a). These data suggest the anti-inflammatory effect of FFCERC in the tested animal model is in part, if not totally, due to its *in vivo* inhibition of sEH.

*Rhizoma Coptidis*, well known as “Huanglian” in China, is the dried rhizomes of *C. chinensis* Franch, *C. deltoidea* C. Y. Cheng et Hsiao, and *C. teeta* Wall. *Rhizoma Coptidis* has been widely reported to be anti-inflammatory as both a single drug and an ingredient of a TCM formula (Choi et al., 2013; Friedemann et al., 2014; Li et al., 2015a; Lower-Nedza et al., 2013; Wu et al., 2012; Yang et al., 2015b). Previous studies showed that Huang-Lian-Jie-Du-Tang, a formula of TCMs containing *Rhizoma Coptidis*, as well as some natural compounds obtained from *Rhizoma Coptidis* like coptisine, inhibits the production of arachidonic acid-derived eicosanoids by directly inhibiting 5-lipoxygenase and down-regulation of cyclooxygenase-2 (Li et al., 2012; Zeng et al., 2011). This study reported the sEH inhibitory activity of *Rhizoma Coptidis* for the first time, resulting in a better understanding of the pharmacological effects of *Rhizoma Coptidis* including but not limited to anti-inflammation, cardiovascular protection, lowering glycemia and hyperlipidemia, which are associated with and contributed, at least in part, by inhibition of sEH evidenced by synthetic sEH inhibitors (Lorthioir et al., 2012; Morisseau and Hammock, 2013; Pillarisetti and Khanna, 2012). However, an obvious limitation for this study is the active compounds against sEH have not been identified yet from the EtOH extract of *Rhizoma Coptidis*. The metabolites from the extract of *Rhizoma Coptidis* were previously reported (Jiang et al., 2012; Wang et al., 2014), in particular, the MS data of some metabolites were included in the online databases like hmdb.ca and foodb.ca. These published data facilitate the tentative structural identification of the chemicals in FFCREC. Based on the comparing the molecular mass and the key fragments with the previously reported data, 10 molecules in FFCREC were tentatively identified as in Table S1. However, it should be noted that these identification need further confirmation, such as comparison with authentic reference and isolation of the pure compounds for <sup>1</sup>H- and <sup>13</sup>C-NMR analysis. Berberine, a well-known

active compound of the TCM *Rhizoma Coptidis*, is a quaternary ammonium salt with the similar structure to coptisine, both belonging to a class of isoquinoline alkaloid (Tang et al., 2009). Berberine has been clinically used in China to treat gastroenteritis, and bacterial dysentery intestinal infection, eye conjunctivitis, suppurative otitis media and cardiovascular diseases like arrhythmia, hypertension and congestive heart failure. Berberine was also found to be anti-inflammatory in many *in vitro* and *in vivo* models, perhaps involved in AMPK/mTOR signaling pathway (Fan et al., 2015; Mo et al., 2014). In current study, berberine was obtained from FFCERC. However, berberine is not the active ingredient against sEH in EtOH extract of *Rhizoma Coptidis* because the IC<sub>50</sub> value of berberine against sEH is more than 100  $\mu$ M, much weaker than FFCERC when co-assayed with the fluorescent method. And unfortunately, the activity of other sub-fractions of FFCERC became much weaker than FFCERC, suggesting a profound synergism among the chemicals in FFCERC. In addition, as shown in Table S1, most compounds have the similar structures to berberine. All these information indicate meaningless to isolate the pure active compounds from FFCERC.

In addition to *Rhizoma Coptidis*, we also found the MeOH and EtOH extracts of several other TCMs display sEH inhibition (Table 2). It suggests the possibility to find active compounds and ingredients with the functions associated with sEH, including but not limited to anti-inflammation, anti-hypertension, anti-hypertrophy, renal-protection. It also cautions us to consider the adverse effects of sEH inhibition when we prescribe TCMs with sEH inhibitory activity for some diseases like cancer (Zhang et al., 2013).

## 5. Conclusions

This study demonstrated that a promising ingredient, FFCREC from *Rhizoma Coptidis*, has some anti-inflammatory activity linked to its ability to inhibit sEH *in vivo*, indicating that sEH may be a therapeutic target, at least one of the multiple targets for relevant TCMs.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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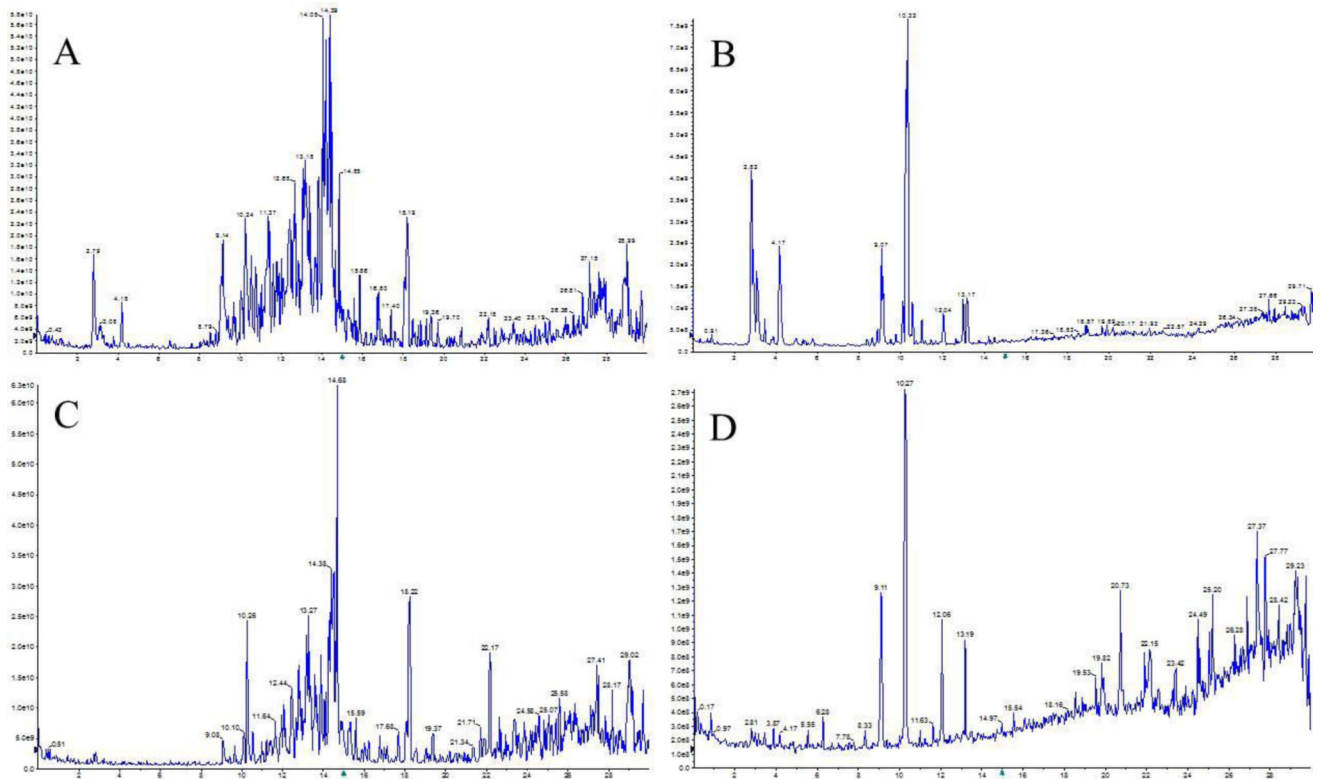


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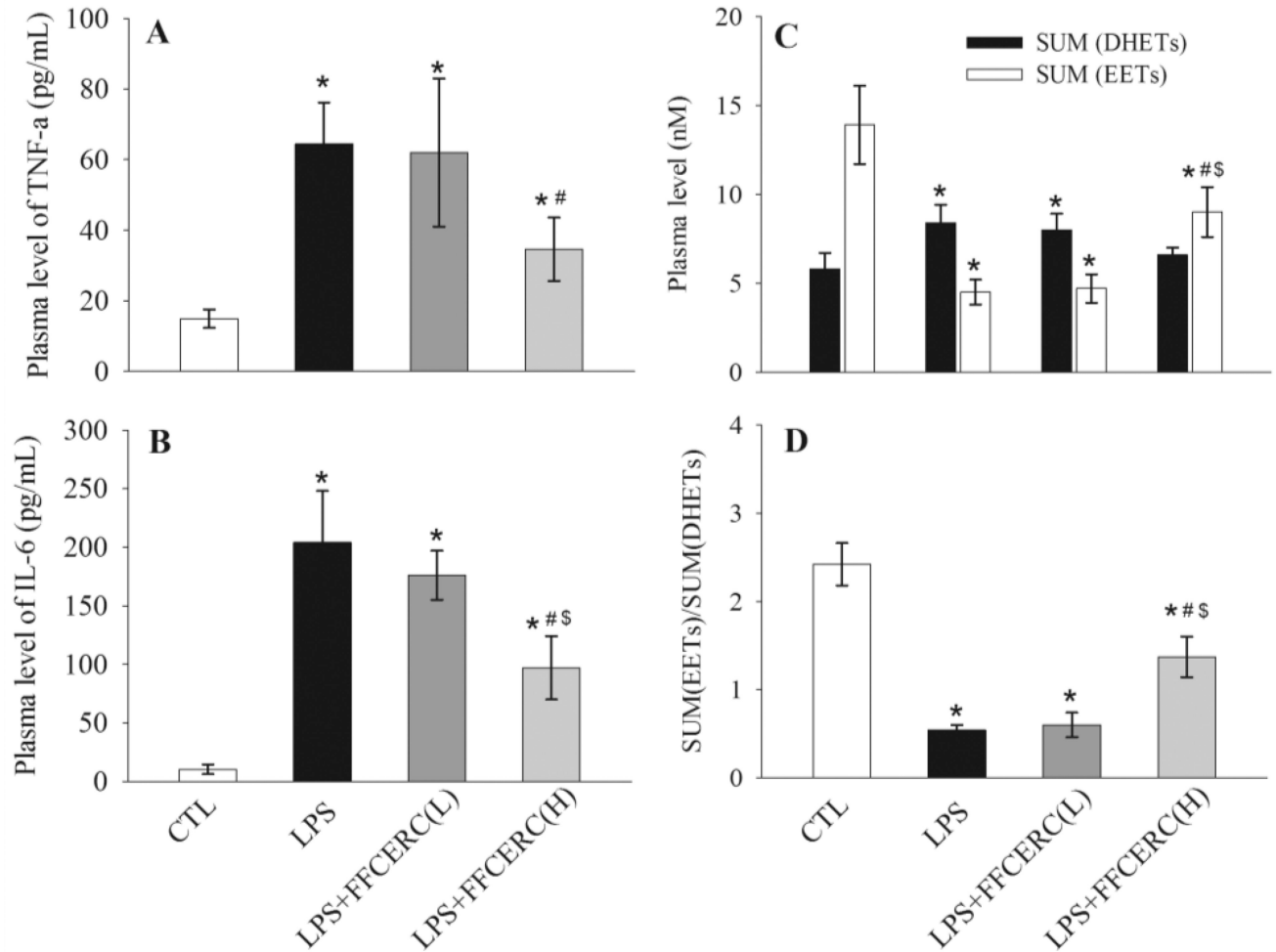
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**Figure 1.** The chemical fingerprints of the EtOH extract of *Rhizoma Coptidis* (A and B) and its resulted fraction FFCERC (C and D) recorded by positive (A and C) and negative modes (B and D), respectively.



**Figure 2.**

Administration of FFCERC dose-dependently inhibited the release of proinflammatory cytokines TNF- $\alpha$  (A) and IL-6 (B), and altered the plasma levels of EETs (C, white bar) and DHETs (C, black bar), resulting in an alteration in the ratio of EETs to DHETs (D). LPS (10 mg/kg) and FFCERC (100 and 250 mg/kg) were administered to male C57BL/6 mice (8-week old) with i.p. injection and oral gavage, respectively. Animals were sacrificed 24 h after treatment. Data represent mean  $\pm$  SD of 4–5 mice. The total EETs column sums 14, 15-, 11, 12-, 8, 9- and 5, 6-EETs and their corresponding diols compose the total DHETs column. The detailed EET and DHET data are presented in Table 4. \*significantly different ( $P < 0.05$ ) from control group (CTL), # significantly different ( $P < 0.05$ ) from LPS group, \$ significantly different ( $P < 0.05$ ) from LPS + FFCERC (low dose) group determined by ANOVA followed with Tukey's or Games-Howell's test.



Table 1

MS Optimum tandem mass conditions of SHE-mediated lipid signaling mediators

Analytes	Precursor ions $m/z$ [M-H] <sup>-</sup>	Predominant product ion $m/z$	Declustering potential (V)	Collision Energy (V)	Retention time (Min)	Detection range (nM)
CUDA <sup>a</sup>	338.8	214.3	-24	-28	8.35	
14(15)-EET-d11 <sup>b</sup>	330.5	232.7	-10	-19	15.92	0.2-500
11,12-DHET-d11 <sup>b</sup>	348.4	166.9	-14.0	-24.5	10.35	0.5-1000
14,15-DHET	336.8	207.1	-17.0	-22.0	9.72	0.5-1000
11,12-DHET	336.8	166.9	-13.0	-24.0	10.40	0.5-1000
8,9-DHET	336.8	268.9	-14.0	-17.0	11.16	0.5-1000
5,6-DHET	336.9	268.9	-17.0	-17.0	12.34	0.5-1000
14(15)-EET	319.1	219.0	-30.0	-16.0	15.96	0.2-500
11(12)-EET	318.8	167.0	-28.0	-20.0	16.60	0.2-500
8(9)-EET	319.0	274.8	-14.0	-17.0	16.69	0.2-500
5(6)-EET	319.0	191.0	-25	-20	16.82	0.2-500

<sup>a</sup> 12-(3-cyclohexan-1-yl-ureido) dodecanoic acid (CUDA) is the internal standard with a fixed concentration of 100 nM;<sup>b</sup> 14(15)-EET-d11 and 11,12-DHET-d11 are the surrogates for epoxides and diols, respectively. The surrogates are used to examine the extraction efficiency of analytes and other errors in sample processing. The recovery of surrogates was validated to be more than 80%. The LQT and accuracy of analytes are the similar to or better than those in the literature (Yang et al., 2009).

Table 2

Yields and sEH inhibitory potency of extracts of 27 anti-inflammatory TCMs

TCMs (voucher specimen number)	Source materials with full scientific name	Yields (%)		Inhibition of hsEH at 25 µg/ml (%) <sup>*</sup>		IC <sub>50</sub> against hsEH (µg/mL)		Major traditional use relating to anti-inflammation
		MeOH Extract	EtOH Extract	MeOH Extract	EtOH Extract	MeOH Extract	EtOH Extract	
<i>Herba Sarcandra</i> (SY-CNCM-2015-SG-01)	Aerial parts of <i>Sarcandra glabra</i> (Thunb.) Nakai	3.9	0.8	27.5	57.3	- <sup>#</sup>	-	Anti-stress, antioxidant, anti-inflammatory, detoxifying and blood activating (Yu, 1982a)
<i>Radix Paeoniae alba</i> (SY-CNCM-2015-CO-02)	Rhizomes of <i>Cynanchum otophyllum</i>	9.5	0.7	38.4	48.0	-	-	Analgesia (Yu, 1977a)
<i>Radix Glycyrrhizae</i> (SY-CNCM-2015-GU-01)	Rhizomes of <i>Glycyrrhiza uralensis</i> Fisch.	14.8	3.5	47.6	95.3	-	5.6	Analgesia (Chen and Chen, 2004)
<i>Radix Bupleuri</i> (SY-CNCM-2015-BC-01)	Rhizomes of <i>Bupleurum chinense</i> DC.	5.6	3.0	36.0	79.0	-	12.7	Anti-inflammatory, antipyretic, analgesia (Yu, 1979d)
<i>Herba Asari</i> (SY-CNCM-2015-AS-01)	Aerial parts of <i>Asarum sieboldii</i> Miq	7.0	2.5	85.3	114.6	6.9	4.6	Anti-inflammatory, antipyretic (Yu, 1988a)
<i>Rhizoma Cimicifugae</i> (SY-CNCM-2015-CF-01)	Rhizomes of <i>Cimicifuga foetida</i> L.	20.0	3.1	30.0	59.8	-	-	Relieving swelling and pain (Yu, 1979a)
<i>Radix Polygalae</i> (SY-CNCM-2015-PT-01)	Root of <i>Polygala tenuifolia</i>	22.9	5.2	44.3	110.1	-	11.6	Treating pain, cough and bronchitis (Wu, 1997a)
<i>Fructus Amomi</i> (SY-CNCM-2015-AV-01)	Fruits of <i>Amomum villosum</i> Lour. Var. <i>xanthioides</i> (Wu et al.) (Wu et al., 2015) (Wu et al., 2015)	1.4	1.6	54.9	103.1	-	6.2	Treating diarrhea and vomiting (Yu, 1981)
<i>Fructus Forsythiae</i> (SY-CNCM-2015-FS-01)	Fruits of <i>Forsythia suspensa</i>	6.6	10.2	76.8	55.5	30.0	-	Antipyretic, anti-hypertension (Yu, 1992)
<i>Radix Notoginseng</i> (SY-CNCM-2015-PN-01)	Root of <i>Panax notoginseng</i> (Bulk.) F. H. Chen	50.7	5.5	19.2	35.6	-	-	Relieving swelling and pain (Yu, 1978)
<i>Radix Platycodonis</i> (SY-CNCM-2015-PG-01)	Root of <i>Platycodon grandiflorus</i>	15.6	1.2	26.4	33.0	-	-	Anti-inflammation, treating cough (Yu, 1983)
<i>Radix Pseudostellariae</i> (SY-CNCM-2015-PH-01)	Root of <i>Pseudostellaria heterophylla</i> (Miq.) Pax et Hoffm	1.7	0.2	51.2	22.5	-	-	Tonifying Qi, nourishing blood and saliva (Wu, 1996c)
<i>Radix Astragali</i> (SY-CNCM-2015-AM-01)	Root of <i>Astragalus membranaceus</i> (Fisch.) Bunge.	10.1	2.8	19.6	104.0	-	13.0	Antihypertension, anti-oxidant (Wu, 1993)
<i>Radix Scrophulariae</i> (SY-CNCM-2015-SN-01)	Root of <i>Scrophularia ningpoensis</i> Hemsl	28.8	1.3	29.2	15.4	-	-	Relieving swelling and detoxicating (Yu, 1979b)

TCMs (voucher specimen number)	Source materials with full scientific name	Yields (%)		Inhibition of hSEH at 25 µg/ml (%) <sup>*</sup>			IC <sub>50</sub> against hSEH (µg/mL)		Major traditional use relating to anti-inflammation
		MeOH Extract	EtOH Extract	MeOH Extract	EtOH Extract	MeOH Extract	EtOH Extract		
<i>Semen Cassiae</i> (SY-CNCM-2015-SO-01)	Seeds of <i>Senna obtusifolia</i>	7.2	4.3	35.8	18.1	-	-	-	Live detoxicating and vision improving (Yu, 1988b)
<i>Radix Isatidis</i> (SY-CNCM-2015-BC-01)	Roots of <i>Baphicacanthus cusia</i> (Nees) Brenek	1.9	1.0	25.9	23.0	-	-	-	antipyretic, detoxicating, analgesic (Yu, 1987)
<i>Salvia prionitis</i> (SY-CNCM-2015-SP-01)	Entire plant of <i>Salvia Prionitis</i> Hance	4.0	2.8	68.9	86.4	10.2	9.6	-	Treating cold and Abdominal pain (Yu, 1977c)
<i>Flos Ionicerae</i> (SY-CNCM-2015-LJ-01)	Flowers of <i>Lonicera japonica</i>	21.1	3.7	60.8	85.8	-	13.2	-	Anti-inflammatory, antipyretic, detoxicating, analgesic (Yu, 1988c)
<i>Herba Houttuyniae</i> (SY-CNCM-2015-HC-01)	Aerial parts of <i>Houttuynia cordata</i> Thunb.	4.5	2.3	72.8	90.4	13.1	6.9	-	Anti-inflammatory, antipyretic (Yu, 1982b)
<i>Fructus Aurantii</i> (SY-CNCM-2015-CA-01)	Fruits of <i>Citrus aurantium</i> L. or <i>C. sinensis</i> Osbeck	14.1	3.3	5.0	57.4	-	-	-	Relieving arthritic pain (Wu, 1997b)
<i>Radix Scutellariae</i> (SY-CNCM-2015-SB-01)	Roots of <i>Scutellaria baicalensis</i>	7.6	4.5	46.9	109.0	-	7.6	-	Anti-inflammatory (Yu, 1977b)
<i>Herba Taraxaci</i> (SY-CNCM-2015-TM-01)	Entire plants of <i>Taraxacum mongolicum</i> Hand	6.1	3.4	41.9	94.2	-	4.1	-	Antipyretic, relieving swelling (Wu, 1999a)
<i>Rhodiola dumulosa</i> (SY-CNCM-2015-RD-01)	Rhizomes of <i>Rhodiola dumulosa</i> (Franch.) S. H. Fu	19.0	18.0	54.6	36.0	-	-	-	Relieving swelling (Yu, 1984)
<i>Fructus Gardeniae</i> (SY-CNCM-2015-GJ-01)	Fruits of <i>Gardenia jasminoides</i> Ellis	8.7	10.5	45.6	28.8	-	-	-	Antipyretic (Wu, 1999b)
<i>Rhizoma Coptidis</i> (SY-CNCM-2015-CC-01)	Rhizomes of <i>Coptis chinensis</i> Franch	15.7	2.4	60.9	127.4	-	4.7	-	Treating acute conjunctivitis (Yu, 1979c)
<i>Radix Stephaniae Tetrandra</i> (SY-CNCM-2015-ST-01)	Roots of <i>Stephania tetrandra</i> S. Moore	3.0	0.2	28.0	20.6	-	-	-	Treating arthritis and hypertension (Wu, 1996b)
<i>Fructus Schisandrae</i> (SY-CNCM-2015-SC-01)	The dried mature fruits of <i>Schisandra chinensis</i> (Turcz.) Baill. or <i>S. sphenanthera</i> Rehd. et Wils	9.9	25.9	15.2	63.8	-	-	-	Treating cough (Wu, 1996a)

Note:

<sup>\*</sup> sEH inhibitory potency was determined at the final concentration of 25 µg/mL.

<sup>#</sup> The crude extracts yielding more than 70% inhibition of hSEH at the final concentration of 25 µg/mL were subject to IC<sub>50</sub> measurement. “-” indicates that the IC<sub>50</sub> was not determined.

<sup>§</sup> Peer reviewed scientific papers reported the anti-inflammatory effect of target TCMs.

sEH inhibitory potency of six fractions of crude ethanol extracts of *Rhizoma Coptidis* and *Herba Asari***Table 3**

TCMs	Fr.1	Fr.2	Fr.3	Fr.4	Fr.5	Fr.6	
<i>Rhizoma Coptidis</i>	Yield (mg/g dried TCM)	2.0	3.5	4.4	1.5	4.3	2.2
	sEH inhibition (%) <sup>*</sup>	26.2	36.0	59.9	14.2	82.2	43.5
<i>Herba Asari</i>	Yield (mg/g dried TCM)	5.4	1.8	0.4	0.8	4.9	7.0
	sEH inhibition (%) <sup>*</sup>	35.5	< 5	< 5	< 5	< 5	< 5

Note:

<sup>\*</sup> sEH inhibition was determined at the final concentration of 2.5 µg/mL.

**Table 4**

Murine plasma levels of key lipid signal mediators and cytokines

Group	1 (N = 4)	2 (N = 4)	3 (N = 5)	4 (N = 5)
LPS (mg/kg)	-	10	10	10
FFCERC (mg/kg)	-	-	100	250
TNF- $\alpha$ (pg/mL)	14.9 $\pm$ 2.7 <sup>b</sup>	64.5 $\pm$ 11.7 <sup>a</sup>	62.0 $\pm$ 21.7 <sup>a</sup>	34.6 $\pm$ 9.0 <sup>a, b</sup>
IL-6 (pg/mL)	10.4 $\pm$ 4.2 <sup>b</sup>	203.8 $\pm$ 43.6 <sup>a</sup>	175.6 $\pm$ 21.2 <sup>a</sup>	97.4 $\pm$ 26.7 <sup>a, b, c</sup>
14,15-EET (nM)	2.12 $\pm$ 0.17 <sup>b</sup>	0.88 $\pm$ 0.26 <sup>a</sup>	0.85 $\pm$ 0.23 <sup>a</sup>	1.49 $\pm$ 0.27 <sup>a, b, c</sup>
11,12-EET (nM)	2.34 $\pm$ 0.29 <sup>b</sup>	0.76 $\pm$ 0.21 <sup>a</sup>	0.84 $\pm$ 0.17 <sup>a</sup>	1.55 $\pm$ 0.26 <sup>a, b, c</sup>
8,9-EET (nM)	1.88 $\pm$ 0.56 <sup>b</sup>	0.46 $\pm$ 0.21 <sup>a</sup>	0.53 $\pm$ 0.32 <sup>a</sup>	0.80 $\pm$ 0.23
5,6-EET (nM)	7.56 $\pm$ 2.0 <sup>b</sup>	2.45 $\pm$ 0.64 <sup>a</sup>	2.48 $\pm$ 0.54 <sup>a</sup>	5.2 $\pm$ 0.84 <sup>b, c</sup>
14,15-DHET (nM)	1.65 $\pm$ 0.73	2.18 $\pm$ 0.66	1.98 $\pm$ 0.58	1.73 $\pm$ 0.49
11,12-DHET (nM)	1.29 $\pm$ 0.38	2.00 $\pm$ 0.50	1.83 $\pm$ 0.32	1.43 $\pm$ 0.22
8,9-DHET (nM)	2.04 $\pm$ 0.38	2.22 $\pm$ 0.43	2.13 $\pm$ 0.42	2.02 $\pm$ 0.39
5,6-DHET (nM)	0.80 $\pm$ 0.14 <sup>b</sup>	2.02 $\pm$ 0.28 <sup>a</sup>	2.02 $\pm$ 0.30 <sup>a</sup>	1.46 $\pm$ 0.20 <sup>a, b, c</sup>

<sup>a</sup>, Significantly different ( $p < 0.05$ ) from group 1;<sup>b</sup>, significantly different ( $p < 0.05$ ) from group 2;<sup>c</sup>, significantly different ( $p < 0.05$ ) from group 3 determined by ANOVA followed with Tukey's (variance homogeneity) or Games-Howell's (variance heterogeneity) post ad hoc comparison test using the software SPSS 22.0.