Identification of Compounds in *Coleus forskohlii* Extract Involved in the Induction of Hepatic CYP and Fatty Liver in Mice

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Abstract: *Coleus forskohlii* extract (CFE), a popular weight-loss herbal product, induces hepatic cytochrome P450 (CYP) and fatty liver in mice; however, its main bioactive ingredient, forskolin, does not show such effects. To ensure the safety of CFE as a dietary supplement, identification of the compounds implicated in the induction of hepatic CYP and fatty liver is required. In this study, we separated a crude CFE extract into 5 fractions (Fr.) by column chromatography and administered the fractions to mice for one week to assess their ability to induce CYP and fatty liver. CYP induction was detected for all fractions, indicating that many compounds may be involved in CYP induction, while fatty liver was only detected for Fr. 2. Further isolation and purification of Fr. 2 by column chromatography identified 14-deoxycoleon U as a major compound and crocetin dialdehyde as a pigment compound. An *in vivo* mouse study revealed that crocetin dialdehyde had no effect on the liver and, as 14-deoxycoleon U was the major compound in Fr. 2, it is likely that the active compound inducing fatty liver in CFE is 14-deoxycoleon U. These findings will facilitate the preparation of standardized safe CFE ingredients for dietary supplements.

Key words: *Coleus forskohlii*, CYP induction, fatty liver, herbal dietary supplement, forskolin

1 Introduction

There has been a recent increase in the use of herbal dietary supplements¹, as consumers perceive the term "natural" to indicate safety and herbal ingredients are believed to be effective due to their long use in complementary and alternative medicines. However, herbal ingredients are not subjected to routine safety examination, and recent studies have revealed that herbal products, especially those used for weight loss, can cause adverse events such as serious hepatic failure². In fact, hepatic failure induced by herbal dietary supplements reportedly accounts for 20% of hepatotoxicity cases in the United States³. With the increasing use of herbal dietary supplements, ensuring their safety is of top priority.

Herbs are composed of many unidentified compounds and the chemical composition of herbal ingredients varies depending on the production location, harvest time, and preparation by individual manufacturers⁴. Furthermore, herbal supplement products are generally produced as multi-ingredient formulations without standardization of herbal ingredients, which causes issues with research reproducibility and raises concerns about effectiveness as well as safety. Notably, the use of dietary supplements, including herbal supplements, by consumers is self-regulated, and concomitant use with orthodox medicines is known to occur without the physician’s knowledge⁵, raising the issue of drug-herb interactions⁶. Therefore, standardization is necessary for herbal supplement products, at least at an ingredient level, from a safety viewpoint.

*Coleus forskohlii* extract (CFE), a popular herbal ingredient, is used in weight-loss products. CFE has been used for centuries in Ayurvedic medicine to treat various diseases of the cardiovascular, respiratory, and central nervous systems.

Abbreviations: CFE, *Coleus forskohlii* extract ; CYP, cytochrome P450 ; Fr., fraction ; NMR, Nuclear Magnetic Resonance ; COSY, Correlation spectroscopy ; HSQC, Heteronuclear single-quantum correlation spectroscopy ; HMBC, Heteronuclear multiple-bond correlation spectroscopy
systems. CFE contains a diterpene compound, forskolin, which has been shown to activate adenylyl cyclase to enhance lipolysis and fat loss in cell culture and human studies. CFE generally contains 10% forskolin in dietary supplements; however, besides forskolin, there is no standardization of compounds in CFE as a dietary supplement.

In our previous studies, we observed that CFE induced hepatic cytochrome P450 (CYP) and fatty liver in mice, although these effects were not observed with pure forskolin. We also observed that CFE diminished the anticoagulant property of warfarin via induction of CYP2C in mice in vivo, and that CFE inhibited CYP2C from mouse and human liver microsomes in vitro, whereas pure forskolin did not. The compounds that induced fatty liver and hepatic CYP were soluble in ether and ethyl acetate supplements; however, besides forskolin, there is no standardization of compounds in CFE as a dietary supplement.

2 Experimental Procedures

2.1 Materials

Powdered CFE standardized with 10% (w/w) forskolin was obtained from Sabinsa Japan Co., Ltd. (Tokyo, Japan). This material contained 9.98% forskolin and 1.99% 1,9-dideoxyforskolin. Compounds commonly found in CFE (forskolin, 1,9-dideoxyforskolin, and 7-deacetyl-1,9-dideoxyforskolin) and crocetin dialdehyde were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Chemicals for the measurement of CYP enzyme activities were obtained from Promega Co. (Madison, WI, USA). All other reagents were purchased from FUJIFILM Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2 Preparation of fractionated samples

Previous studies revealed that the induction of hepatic CYP and fatty liver by CFE in vivo can be easily evaluated by an increase in the liver/body weight ratio. Therefore, the ability of target components in the materials to cause CYP and fatty liver was confirmed by dietary administration to mice and evaluation of increases in the liver/body weight ratio. Powdered CFE standardized with 10% (w/w) forskolin (original CFE) was washed with an acid and alkaline solution. After neutralization, the materials were extracted 4 times with methanol to isolate methanol soluble materials, and then washed with hexane to remove lipid soluble materials. The target compounds were contained in the methanol soluble fraction and were absent from the hexane soluble fraction. Thus, the methanol soluble material washed with hexane was applied to further fractionation by column chromatography. In brief, 74 g of the extracted material dissolved in methanol was applied to an open column (10.5 cm × 80 cm) packed with 3.5 kg silica (spherical, 110 µm diameter; Fuji Silysiachemical Ltd., Kasugai, Japan) and eluted with hexane/ethyl acetate (7:3 ratio). The eluate was fractionated into 18 portions. Aliquots of the eluate were applied to a TLC plate (HPTLC Silica gel 60 F254; Merck, Darmstadt, Germany) developed in hexane/ethyl acetate (7:3 ratio) and visualized by spraying with 50% methanol containing 10% sulfuric acid, followed by heating. According to the TLC analysis, the 18 eluate fractions were combined into 5 major eluate fractions (Fig. 1). The solvent in each eluate was removed under reduced pressure (60°C, 48 h) to obtain the final fractionated materials for use in further studies. The yield of each fraction (Fr.) was: 4.0 g for Fr. 1; 3.7 g for Fr. 2; 20.5 g for Fr. 3, 4.2 g for Fr. 4; and 9.1 g for Fr. 5. Aliquots of the fractions were dissolved at a concentration of 10 mg/mL, and analyzed by TLC, as described above.

2.3 Assay of CYP induction and fatty liver in mice

Fractionated materials and the original un fractionated sample were added separately to an AIN-93G semi-purified diet (Oriental Yeast Co., Ltd., Tokyo, Japan) at 0.5% (w/w) as test diets. Male C57BL/6 mice (5-week-old, obtained from Japan Clea, Tokyo, Japan) were fed either the AIN-93G semi-purified diet (control diet) or the test diets for one week. To examine the effect of a brown pigment compound (confirmed as crocetin dialdehyde) found in Fr. 2-4, crocetin dialdehyde was mixed with the AIN-93G diet (6 mg/3 g diet) and given to C57BL mice for one week at a dose of 300 mg/kg body weight. At the end of the feeding study, the mice were anesthetized with pentobarbital and exsanguinated from the inferior vena cava using heparin as an anticoagulant. Livers were immediately removed, weighed, and snap-frozen on dry ice. The plasma and liver samples were stored at −80°C until analysis. All animal procedures were conducted in accordance with the National Institute of Health and Nutrition guidelines for the Care and Use of Laboratory Animals, and were approved by the ethics committee of the National Institute of Health and Nutrition, Japan (approval number and date: No. 1409, April 1st, 2014).

Analysis of drug-metabolizing enzymes was performed as follows. The liver was rinsed with 0.9% (w/v) NaCl, homogenized in 50 mmol/L Tris-HCl buffer (pH 7.4) containing 0.25 mol/L sucrose, and separated by centrifugation at 10,000 g at 4°C for 30 min. The supernatant was centrifuged at 105,000 g at 4°C for 60 min and used as microsomes to determine the total CYP content and CYP activities. The total CYP content was determined as previously.
The activities of various CYP enzymes were determined using the P450-Glo™ CYP1A1 System (Luciferin-CEE) assay, CYP1A2 System (Luciferin-1A2) assay, CYP2C9 System (Luciferin-H) assay, CYP3A4 System (Luciferin PPXE) assay and the NADPH Regeneration System, according to the manufacturer’s instructions (Promega). Luminescence signals were measured by luminometry (GloMax-Multi Detection System; Promega) and activities were expressed as relative light units (RLU)/mg protein/min.

Hepatic lipids were extracted according to the Bligh and Dyer method\textsuperscript{17}. Concentrations of triglyceride, cholesterol and phospholipid were measured using a Triglyceride E Test Wako, a Cholesterol E Test Wako, and a Phospholipid C Test Wako (Wako Pure Chemical Industries, Ltd.), respectively.

Protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

\subsection*{2.4 Determination of compounds}

To identify compounds in Fr. 2 that induced fatty liver in the results of the feeding assay, the components in the fraction were further fractionated. In brief, 457 mg of Fr. 2 was subjected to silica gel column chromatography using hexane/diethyl ether (3:2 ratio) to give 6 fractions and their corresponding yields: Fr. 2-1 (4 mg), Fr. 2-2 (75 mg), Fr. 2-3 (199 mg), Fr. 2-4 (43 mg), Fr. 2-5 (104 mg), and Fr. 2-6 (44 mg). A large amount of material was observed in Fr. 2-3, the second largest amount was present in Fr. 2-5, and a brown pigment compound was present in Fr. 2-4. Fractions 2-3 (199 mg) and 2-4 (43 mg) were further purified by silica gel chromatography and developed with hexane/dichloromethane (1:9 ratio), and the following two purified compounds were isolated: 26 mg of 14-deoxycoleon U (1) from Fr. 2-3 and 4 mg of crocetin dialdehyde (2) from Fr. 2-4 (Fig. 2). The chemical structures of the isolated compounds were determined by \textsuperscript{1}H- and \textsuperscript{13}C-NMR and 2D NMR (COSY, HSQC, HMBC).

Since no commercially available known standard substance exists as a reference compound for Fr. 5 (Fig. 1), we attempted to identify its major compounds. Specifically, the material in Fr. 5 was dissolved in dichloromethane, and acetylated using acetic anhydride and pyridine. After stirring for 12 h, the reaction mixture was washed with 5% Na\textsubscript{2}CO\textsubscript{3} and then with 5% HCl to give a crude extract. This extract was then purified using silica gel column chromatography with hexane/diethyl ether (Fig. 2). The isolated compound was analyzed and identified by \textsuperscript{1}H- and \textsuperscript{13}C-NMR.
and 2D-NMR (COSY, HSQC, HMBC).

The 1H-NMR attribution data of 14-deoxycoleon U(1) was as follows. 1H-NMR (800 MHz, CDCl3) δ 7.73 (1H, s, H-14), 7.12 (1H, s, 6-OH), 3.06 (1H, sep, J = 6.4 Hz, H-15), 2.95 (1H, ddd, J = 13.6, 9.6, 7.2 Hz, H-1β), 2.03 (1H, ddt, J = 12.8, 4.8, 0.8 Hz, H-3α), 1.88 (1H, m, H-2β), 1.75 (1H, ddd, 14.4, 9.6, 5.6 Hz, H-1α), 1.68 (3H, s, H-20), 1.64-1.69 (1H, m, H-2α), 1.47 (3H, s, H-18 or -19), 1.46 (3H, s, H-18 or -19), 1.45-1.48 (1H, m, H-3β), 1.32 (3H, d, J = 6.4Hz, H-16 or -17), 1.30 (3H, d, J = 6.4 Hz, H-16 or -17). 13C-NMR (200 MHz, CDCl3) 179.8 (C, C-7), 145.2 (C, C-12), 143.1 (C, C-5), 142.9 (C, C-6 or -11), 140.7 (C, C-6 or 11), 138.1 (C, C-9), 132.5 (C, C-13), 121.0 (C, C-8), 116.5 (CH, C-14), 40.6 (C, C-10), 36.4 (CH3, C-4). 36.4 (C, C-3), 30.4 (CH3, C-1), 27.9 (CH3, C-20), 27.9 (CH3, C-18 or -19), 27.4 (CH, C-15), 27.1 (CH3, C-18 or -19), 22.5 (CH3, C-16 or -17), 22.3 (CH3, C-16 or -17), 17.9 (CH3, C-2).

The 1H-NMR attribution data of crocein dialdehyde (2) was as follows. 1H-NMR (800 MHz, CDCl3) δ 9.47 (1H, s, CHO), 6.95 (1H, m, H-3), 6.77 (1H, dd, J = 8.0, 2.4 Hz, H-7), 6.74-6.73 (2H, m, H-4 and -8), 6.46 (1H, dd, J = 8.0, 2.4 Hz, H-5), 2.04 (3H, brs, H-18), 1.91 (3H, d, J = 0.8 Hz, H-17). 13C-NMR (200 MHz, CDCl3) 194.5 (CH, C-1), 148.8 (CH, C-3), 145.4 (CH, C-4), 137.4 (C, C-2), 137.1 (C, C-5), 136.7 (CH, C-6), 132.0 (CH, C-7), 123.7 (CH, C-8), 12.8 (CH3, C-18), 9.7 (CH3, C-17).

The NMR attribution data of forskolin A (3) was as follows. 1H-NMR (800 MHz, CDCl3) δ 5.92 (1H, dd, J = 16.8, 10.4 Hz, H-14), 5.81 (1H, dd, J = 4.8, 2.4 Hz, H-6a), 5.57 (1H, t, J = 2.4 Hz, H-1β), 5.55 (1H, d, J = 4.8 Hz, H-7a), 5.21 (1H, dd, J = 16.8, 0.8 Hz, H-15β), 4.95 (1H, dd, J = 10.4, 0.8 Hz, H-15A), 4.74 (1H, s, 9α-OH), 3.15 (1H, d, J = 16.0 Hz, H-12α), 2.44 (1H, d, J = 16.0 Hz, H-12β), 2.42 (1H, d, J = 3.2 Hz, H-5α), 2.10 (3H, s, 1α-OAc), 2.03 (3H, s, 6β-OAc), 2.02 (3H, s, 7β-OAc), 1.66 (3H, s, Me-17), 1.64 (1H, m, H-2α), 1.58 (1H, m, H-3α), 1.53 (3H, s, Me-20), 1.34 (3H, s, Me-16), 1.16 (1H, dt, J = 13.6, 2.4 Hz, H-3β), 1.05 (3H, s, Me-18), 1.00 (3H, s, Me-19). 13C-NMR (200 MHz, CDCl3) 205.2 (C, C-11), 169.8 (C, 6β-COCH3), 169.8 (C, 7β-COCH3), 168.4 (C, 1α-COCH3), 145.99 (CH, C-14), 110.4 (CH3, C-15), 138.1 (C, C-9), 81.7 (C, C-8), 81.5 (C, C-9), 76.6 (CH, C-7), 74.0 (CH, C-1), 69.4 (CH, C-6), 48.7 (CH3, C-12), 43.5 (C, C-10), 43.0 (CH, C-5), 37.0 (CH3, C-3), 33.8 (C, C-4), 32.7 (CH3, C-18), 31.0 (CH3, C-16), 23.4 (CH3, C-19), 23.2 (CH3, C-17), 23.1 (CH3, C-2), 21.7 (CH3, 1α-COCH3), 21.4 (CH3, 6β-COCH3), 20.8 (CH3, 7β-COCH3), 19.6 (CH3, C-20).

2.5 Statistical analysis

Data are presented as the mean ± standard error (SE) for individual groups and were statistically analyzed using one-way ANOVA with Tukey’s multiple comparison test. Differences at p < 0.05 were considered significant. All statistical analyses were performed using Prism 5.0 (Graphpad Software Inc., La Jolla, CA, USA).

3 Results

3.1 Characterization of fractionated samples

The ingredients of CFE were crudely divided using solvent extraction, and the effects of the crude extract on

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Fig. 3  TLC profile of 5 fractionated and unfractonated samples in CFE.

RI-value of each spot is as follows: 7-deacetyl-1,9-dideoxyforskolin, 4.0; forskolin, 5.2; 1,9-dideoxyforskolin, 7.1, and main spot of Fr. 2 containing 14-deoxycoleon U(1) and brown pigment (crocein dialdehyde (2)), 5.7; and main spot of Fr. 5 containing forskolin A (3), 3.0.
the induction of CYP and fatty liver were confirmed by observing increases in the liver/body weight ratio using a feeding assay in mice. The target compounds were methanol-soluble, but not hexane-soluble. The crude extract was further separated into 5 fractions by column chromatography (Fig. 1). The TLC profile of the 5 fractions is shown in Fig. 3. The following commercially-available components were found: 1.9-dideoxyforskolin in Fr. 1, forskolin in Fr. 3, and 7-deacetyl-1,9-dideoxyforskolin in Fr. 4. There were no reference standards available for Fr. 2 and Fr. 5; however, a brown pigment was observed in Fr. 2.

3.2 Effects of fractionated samples on mouse liver in vivo
Each fraction was administered as a diet to mice, and the effects on the liver/body weight ratio and induction of CYP and fatty liver in vivo were verified. Dietary administration of the fractionated samples increased the liver weight and liver/body weight ratio, and a greater effect was observed in Fr. 2 and 5 (Table 1). Induction of CYP was observed for all fractions, although some differences were observed in the type of CYP for each fraction (Fig. 4). On the other hand, Fr. 2 produced a remarkable increase in liver triglyceride (Fig. 5). There was no effect on the contents of phospholipid and cholesterol following the administration of each fraction.

3.3 Identification of active compounds
To determine the active compounds in Fr. 2 (Fig. 1), column chromatography was conducted and compounds 1 and 2 were isolated. Based on the evaluation of spectroscopic data, Compounds 1 and 2 were shown to be structurally identical to 14-deoxycoleon U and crocetin dialdehyde, respectively \cite{18,19}. 14-Deoxycoleon U (compound 1) was identified as the main component of Fr. 2, while crocele-

<table>
<thead>
<tr>
<th>CFE</th>
<th>Control</th>
<th>Fraction 1</th>
<th>Fraction 2</th>
<th>Fraction 3</th>
<th>Fraction 4</th>
<th>Fraction 5</th>
<th>Unfractionated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (g)</td>
<td>19.6 ± 0.2</td>
<td>18.6 ± 0.6</td>
<td>18.3 ± 0.3</td>
<td>19.6 ± 0.1</td>
<td>19.5 ± 0.1</td>
<td>19.7 ± 0.4</td>
<td>20.1 ± 0.2</td>
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<tr>
<td>Liver weight (g)</td>
<td>1.05 ± 0.03</td>
<td>1.59 ± 0.04*</td>
<td>1.85 ± 0.06*</td>
<td>1.52 ± 0.03*</td>
<td>1.55 ± 0.04*</td>
<td>1.88 ± 0.11*</td>
<td>1.86 ± 0.04*</td>
</tr>
<tr>
<td>Liver weight (%/body weight)</td>
<td>5.35 ± 0.16</td>
<td>8.57 ± 0.19*</td>
<td>10.13 ± 0.33*</td>
<td>7.79 ± 0.18*</td>
<td>7.95 ± 0.20*</td>
<td>9.51 ± 0.37*</td>
<td>9.26 ± 0.16*</td>
</tr>
<tr>
<td>Average daily food intake (g)</td>
<td>3.0 ± 0.02</td>
<td>3.0 ± 0.04</td>
<td>2.9 ± 0.02</td>
<td>3.0 ± 0.07</td>
<td>3.0 ± 0.02</td>
<td>3.0 ± 0.04</td>
<td>3.0 ± 0.03</td>
</tr>
<tr>
<td>Calculated CFE dose (mg/kg body weight)</td>
<td>0</td>
<td>869 ± 20</td>
<td>876 ± 5</td>
<td>806 ± 23</td>
<td>819 ± 13</td>
<td>804 ± 16</td>
<td>790 ± 13</td>
</tr>
</tbody>
</table>

Male C57BL/6 mice were fed diets containing 0.5% (w/w) of fractionated or unfractonated CFE for one week. Values are expressed as means ± SE for 4-5 mice. * Significantly different versus control, \( p < 0.05 \).
tin dialdehyde (compound 2) was identified as the brown pigment (Fig. 3), which had an Rf value of 5.7.

As crocetin aldehyde was available in an appropriate amount for the in vivo mouse study, mice were administered a dose of 300 mg/kg body weight (2 mg/g diet) for one week. However, the expected effects on liver weight were not observed, suggesting that crocetin aldehyde is not involved in the induction of fatty liver. Thus, 14-deoxycoleon U, the major component in Fr. 2, was suggested to be related to the induction of fatty liver.

Since a known reference standard was not commercially available, we purified the substances in Fr. 5 (Fig. 1). The major chemical component was identified as forskolin A (compound 3, analyzed as an acetylated derivative of forskolin A) by comparing the data to previously established ¹H- and ¹³C-NMR data.¹⁰

**4 Discussion**

CFE is a popular herbal ingredient in weight-loss supplements, and the weight-loss effect is attributed to the action of forskolin, which activates adenylate cyclase and enhances lipolysis and fat loss. We previously reported that CFE induced hepatic CYP and fatty liver in mice, while pure forskolin did not.¹² Thus, the elimination or decrease in the concentration of compounds in CFE associated with CYP-induction and fatty liver would assist in producing a standardized, safe ingredient for dietary supplements and phytopharmaceuticals. Hepatic failure is a major concern with respect to herbal dietary supplements and phytopharmaceuticals. Hepatic failure is not observed, suggesting that crocetin aldehyde is not involved in the induction of fatty liver.

For CYP induction in an in vitro study in mice, we observed that pure forskolin had only a slight effect on CYP2C activity in mouse and human liver microsomes. In the present study, Fr. 3, which is mainly composed of forskolin, showed a clear induction of CYP, suggesting that compounds other than forskolin were responsible for inducing CYP. Dig and Staudinger reported that 10 to 100 µM of forskolin concentrations induced CYP3A gene expression through the pregnane X receptor in cultured rodent hepatocytes. In contrast, Hebbani et al. reported that forskolin, 1-deoxyforskolin, and 1,9-dideoxyforskolin did not induce any significant mRNA expression of CYP3A, CYP2B, and CYP2C in rat hepatocytes. In the present study, CYP-inducing activity was assessed in vivo in mice because in vitro assay systems are incapable of evaluating the effects of absorption, metabolism, physio-

Fig. 5 Effect of fractionated samples on hepatic lipids in mice. Male C57BL/6 mice were fed diets containing 0.5% (w/w) of fractionated (Fr.1 to 5) or un fractionated (W) CFE for one week. * Significantly different versus control, p < 0.05.
CFE Compounds Inducing Fatty Liver

The effects of ingested materials on the human body depend on the amount consumed. In mouse studies, CYP induction and fatty liver were observed with administration of 0.05% CFE and 0.5% CFE diets, which correspond to doses of 60 mg and 600 mg CFE/kg body weight, respectively. Equivalent CFE doses in humans were extrapolated as 4.9 mg/kg body weight for CYP induction and 49 mg/kg body weight for fatty liver. Accordingly, the dose of CFE required to induce fatty liver in humans is estimated to be higher than 3,000 mg/day. Currently, the intake of CFE from dietary supplements is reported to be up to 1,000 mg/day. In humans, diarrhea was reported as a negative side effect following the ingestion of a CFE-containing dietary supplements, and was classified as a minor adverse reaction. However, we did not observe diarrhea in the study conducted on mouse models, even when mice were administered the higher 5% CFE diet. This suggests that there is a species difference in adverse reactions with CFE, and liver dysfunction with CFE intake in mice is unlikely to occur in humans at the typical amount consumed from dietary supplements. There are also individual differences in the effects of ingested materials; thus, inducing fatty liver by CFE ingestion in humans remains a possibility, and careful clinical observation is necessary.

Natural products, including CFE, contain multiple compounds that may differ due to production location, harvest time, and ingredient preparation. Standardization of CFE ingredients is important to ensure the effectiveness and safety of CFE as a dietary supplement ingredient. In the case of CFE, it is critical to identify the compounds that induce fatty liver, and eliminate them from forskolin, which is considered the main compound responsible for the weight loss effect exerted by CFE and its other known pharmacological effects. The present data will be useful for preparing standardized ingredients for CFE that do not cause hepatic dysfunction.

5 Conclusion

We assayed the compounds present in CFE that induce CYP and fatty liver, and identified the presence of various components as CYP-inducers and 14-deoxycoleon U (compound 1) as a possible inducer of fatty liver. The fatty liver inducer in CFE was present in the same fraction as crocetin dialdehyde (compound 2), which could be used as a marker pigment component, as it can be easily separated from forskolin, the active compound is responsible for the weight loss effect exerted by CFE and its other known pharmacological effects. The present data will be useful for preparing standardized ingredients for CFE that do not cause hepatic dysfunction.

Acknowledgment

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Conflicts of Interest

The authors indicated no potential conflicts of interest.

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