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Anti-melanogenic properties of FBCC-EP850 derived from *Carex pumila* ThunbMirissa Hewage Dumindu Kavinda¹, Jinkuk Park¹, Nayeong Kim¹, Yung Hyun Choi², Gi-Young Kim^{1✉}¹Department of Marine Life Science, Jeju National University, Jeju 63243, Republic of Korea²Department of Biochemistry, College of Korean Medicine, Dong-Eui University, Busan 47227, Republic of Korea

ABSTRACT

Objective: To elucidate the anti-melanogenic potential of *Carex pumila* Thunb. extract (FBCC-EP850).

Methods: A collection of 180 plant extracts was tested for inhibition of mushroom tyrosinase activity using an *in vitro* assay. Among them, FBCC-EP850 exhibited the most promising inhibitory activity. Further analysis was conducted to investigate its mechanisms and therapeutic potential in reducing melanogenesis in B16F10 melanoma cells and zebrafish larvae.

Results: FBCC-EP850 inhibited mushroom tyrosinase activity in a dose-dependent manner, with a half-maximal inhibitory concentration of 45.83 µg/mL. FBCC-EP850 at concentrations up to 50 µg/mL demonstrated minimal cytotoxicity against B16F10 melanoma cells and no adverse effects on zebrafish larvae. Treatment with 50 µg/mL of FBCC-EP850 significantly reduced α-melanocyte stimulating hormone-induced melanin production and suppressed cellular tyrosinase activity in B16F10 melanoma cells. Additionally, FBCC-EP850 at 25 and 50 µg/mL effectively diminished hyperpigmentation in α-melanocyte stimulating hormone-stimulated zebrafish larvae. Its anti-melanogenic action could be attributed to modulation of the cAMP-CREB-MITF signaling pathway.

Conclusions: *Carex pumila* extract can inhibit melanogenesis by modulating the cAMP-CREB-MITF signaling pathway, which can be used as a promising candidate for treating hyperpigmentation disorders.

KEYWORDS: *Carex pumila* Thunb.; Melanogenesis; Tyrosinase; cAMP-CREB-MITF signaling

plays a critical role in protecting the skin from ultraviolet (UV) radiation-mediated damage by absorbing UV rays and neutralizing reactive oxygen species generated during exposure[3,4]. Melanin is synthesized within melanocytes, specifically in organelles called melanosomes, where it is produced and stored before being transferred to surrounding keratinocytes[5]. This well-coordinated process facilitates the even distribution of melanin across the skin, enhancing its ability to shield against UV-induced harm. Melanocytes produce two types of melanin: pheomelanin and eumelanin, with the relative proportions of these pigments influencing individual skin tone[6,7]. However, excessive melanin production can lead to hyperpigmentation disorders[8–10], which present significant challenges in dermatology and can negatively

Significance

Excessive melanin synthesis leads to hyperpigmentation disorders, and natural compounds have emerged as promising alternatives to synthetic treatments. *Carex pumila* Thunb. extract demonstrated significant potential as a natural inhibitor of melanogenesis, offering an effective alternative to conventional skin-whitening agents. This study deepens the understanding of the molecular mechanisms through which *Carex pumila* Thunb. extract exerts its anti-melanogenic properties, highlighting it as a promising candidate for the development of safe and effective treatments for hyperpigmentation conditions.

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1. Introduction

Skin pigmentation is primarily determined by melanin[1,2], which

impact quality of life.

Melanin synthesis, or melanogenesis, is primarily regulated by melanocortin 1 receptor (MC1R), a key G protein-coupled receptor located on melanocytes[1,11]. Exposure of UV radiation induces the secretion of α -melanocyte stimulating hormone (α -MSH) in both the skin and the pituitary gland[5,11]. When α -MSH binds to MC1R, it triggers the phosphorylation of cAMP response element-binding protein (CREB) and activates the cAMP/protein kinase A (PKA) pathway[1]. CREB, in turn, activates microphthalmia-associated transcription factor (MITF), a master regulator of melanocyte function, driving the expression of key melanogenic enzymes such as tyrosinase (TYR), which is crucial in melanin biosynthesis[5,11–14]. This intricate signaling network underscores the complexity of melanin regulation, highlighting the pivotal role of MC1R and its associated pathway in controlling melanocyte activity in response to environmental factors.

Despite the availability of various commercial skin-whitening agents[12–14], their long-term use has been associated with adverse effects[12,15]. As a result, there has been growing interest in natural alternatives for managing hyperpigmentation[16,17]. In this study, we screened 180 plant extracts and identified *Carex pumila* (*C. pumila*) Thunb. extract as the potent inhibitor of mushroom TYR activity *in vitro*. However, the anti-melanogenic potential of this perennial herb remains largely unexplored. Therefore, this research aims to investigate the effects of *C. pumila* Thunb. extracts on α -MSH-induced melanogenesis in B16F10 melanoma cells and zebrafish larvae.

2. Materials and methods

2.1. Plant extracts

A total of 180 plant extracts were sourced from the Freshwater Bioresources Culture Collection (FBCC) at the Nakdonggang National Institute of Biological Resources (Sanju, Gyeongsangbuk-do, Republic of Korea)[18]. These extracts, labeled FBCC-EP844 to FBCC-EP869, FBCC-EP871 to FBCC-EP873, FBCC-EP875 to FBCC-EP879, FBCC-EP881 to FBCC-EP910, FBCC-EP912 to FBCC-EP928, FBCC-EP1013 to FBCC-EP1036, FBCC-EP1038 to FBCC-EP1042, FBCC-EP1044 to FBCC-EP1057, FBCC-EP1059 to FBCC-EP1082, FBCC-EP1084 to FBCC-EP1093, and FBCC-EP1095 to FBCC-EP1116, were derived from various plant species using distilled water or 70% ethanol as solvents (Supplementary Table 1). For the ethanolic extracts, 100 g of plant material was macerated and extracted twice with 5 L of 70% ethanol at 25 °C over 24 h. The solution was then filtered, and the ethanol was removed using a rotary vacuum evaporator (Eyela, Tokyo, Japan) at 37 °C, yielding the dried

extract. For the aqueous extract, 100 g of plant material was similarly macerated and extracted twice with 5 L of distilled water at 100 °C for 2 h, followed by filtration. The aqueous solution was freeze-dried using a vacuum freeze dryer (Operon, Gimpo, Gyeonggi-do, Republic of Korea). Stock solution of the extracts was prepared by dissolving them in dimethyl sulfoxide at a concentration of 20 mg/mL.

2.2. Reagents and antibodies

Dulbecco's Modified Eagle's Medium, fetal bovine serum, and an antibiotic mixture were procured from Welgene (Gyeongsangbuk-do, Republic of Korea). The WST-8 assay kit was supplied by MediFab (Seoul, Republic of Korea). Phenylthiourea, ascorbic acid, mushroom TYR, α -MSH, and isobutyl methylxanthine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies for TYR (sc-20035), MITF (sc-71588), phospho-CREB (sc-81486), and β -actin (sc-69879) were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). All other reagents were acquired from Sigma-Aldrich.

2.3. *In vitro* mushroom TYR inhibition assay

The *in vitro* mushroom TYR inhibition assay was performed with slight modifications from previously established protocols[3]. In brief, 10 μ L of each plant extract (50 μ g/mL) from the 180 samples was mixed with 30 μ L of 1.5 mM *L*-tyrosine and 140 μ L of 100 mM phosphate buffer (pH 6.8) in a 96-well plate. The mixture was incubated at 37 °C for 10 min, and absorbance was measured at 490 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Afterward, 20 μ L of 210 U/mL mushroom TYR was added to each well and the reaction was incubated again at 37 °C for 10 min. The absorbance was measured at 490 nm. Ascorbic acid (0.5 mM) was used as a positive control. The percentage of mushroom TYR inhibition was calculated using the following formula:

$$\text{Inhibition rate (\%)} = [(A - B) - (C - D)] / (A - B) \times 100$$

where A represents the absorbance of the reaction with *L*-tyrosine and TYR, B is the absorbance of *L*-tyrosine alone, C is the absorbance of the reaction with *L*-tyrosine, TYR, and extracts, and D is the absorbance of *L*-tyrosine with extracts only. Among the tested extracts, FBCC-EP850 showed the highest inhibition rate in this assay (Supplementary Table 1). Further testing was conducted on FBCC-EP850 at concentrations ranging from 0 to 200 μ g/mL.

2.4. Cell culture and relative cell viability analysis

Mouse B16F10 melanoma cells (ATCC, Manassas, VA, USA) were cultured in a humidified incubator at 37 °C with 5% CO₂. The cells were seeded at a density of 1×10^4 cells/mL in 24-well plates and

treated with varying concentrations of FBCC-EP850 for 72 h. To evaluate cell viability, 10 μ L of WST-8 solution was added to the cell culture media at 24-hour intervals, and the cells were incubated for 1 h before measuring the absorbance at 450 nm. Cellular morphology was examined using a stereomicroscope (Macrotech, Goyang, Gyeonggi-do, Republic of Korea).

2.5. Flow cytometry analysis

Flow cytometry was employed to assess both the total viable cell count and the proportion of dead cells. B16F10 melanoma cells were seeded at a density of 1×10^4 cells/mL in 6-well plates and treated with FBCC-EP850 at concentrations ranging from 0 to 200 μ g/mL for 72 h. Hydrogen peroxide (H_2O_2 , 100 μ M) was used as a positive control to induce cell death. After 72 hours of treatment, the cells were harvested, washed twice with phosphate-buffered saline (PBS), and incubated for 5 min with the Muse Cell Count and Viability Kit (Luminex, Austin, Texas, USA). Samples were analyzed using the Muse Cell Analyzer (Luminex).

2.6. Melanin content analysis

B16F10 cells were seeded at a density of 1×10^4 cells/mL in 6-well plates and treated with 500 ng/mL of α -MSH for 24 h, followed by treatment with FBCC-EP850 at concentrations of 0–50 μ g/mL for an additional 48 h. To assess extracellular melanin content, the culture media were collected and analyzed by measuring absorbance at 405 nm. A visible change in the color of the culture medium was also observed. For intracellular melanin quantification, cell pellets were washed with ice-cold PBS and then dissolved in 1 mol/L NaOH containing 10% dimethyl sulfoxide for 1 h at 100 °C. Absorbance was measured at 405 nm to evaluate intracellular melanin levels.

2.7. Intracellular TYR activity

Intracellular TYR activity was measured following a previously described protocol[19]. B16F10 cells were seeded at a density of 1×10^4 cells/mL and treated with 500 ng/mL of α -MSH for 24 h. Afterward, the cells were exposed to FBCC-EP850 at concentrations of 0–50 μ g/mL for an additional 48 h. The cells were then lysed in PBS containing 1% Triton X-100, followed by freezing at –20 °C for 2 h and subsequent thawing at 25 °C. Protein concentrations were determined using Bio-Rad Protein Assay Reagents, and 25 μ g of protein was incubated with 90 μ L of 5 mM *L*-dihydroxyphenylalanine at 37 °C for 30 min. The absorbance was measured at 405 nm to assess TYR activity.

2.8. Enzyme-linked immunosorbent assay (ELISA) assay

For the cAMP ELISA assay, B16F10 melanoma cells were seeded at a density of 1×10^5 cells/mL and incubated in serum-free Dulbecco's Modified Eagle's Medium for 16 h. The cells were pretreated with 1 mM isobutyl methylxanthine for 10 min, followed by exposure to 500 ng/mL α -MSH for 15 min. Afterward, cells were treated with varying concentrations of FBCC-EP850 (0–50 μ g/mL) and incubated for 30 min. A colorimetric ELISA kit (Cell Biolabs, San Diego, CA, USA) was used to quantify cAMP levels, with absorbance measured at 450 nm. The cAMP concentration was determined by generating a standard curve.

2.9. Reverse transcription–polymerase chain reaction (RT–PCR) analysis

B16F10 cells were seeded at a density of 1×10^4 cells/mL and treated with 500 ng/mL of α -MSH for 24 h. Following this, the cells were exposed to varying concentrations of FBCC-EP850 (0–50 μ g/mL) for 48 h. Total RNA extraction was extracted using the easy-BLUE Total RNA Extraction Kit (iNtRON Biotechnology, Seongnam, Gyeonggi-do, Republic of Korea). Reverse transcriptase was performed using MMLV reverse transcriptase (Bioneer, Daejeon, Republic of Korea), and PCR amplification was conducted using the EzWay Neo Taq PCR MasterMix (Koma Biotech, Seoul, Republic of Korea). Primer sequences for the target genes are provided in Table 1. Gene expression was normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the reference gene.

2.10. Western blotting analysis

B16F10 melanoma cells were seeded in 6-well plates at a density of 1×10^4 cells/mL. After treatment with 500 ng/mL α -MSH for 15 min (for pCREB) or 24 h (MITF and TYR), cells were further exposed to FBCC-EP850 (0–50 μ g/mL) for 30 min (pCREB) or 72 h (MITF and TYR). Proteins were extracted using RIPA lysis buffer (iNtRON Biotechnology) supplemented with protease and phosphatase inhibitors (Sigma-Aldrich). Protein concentrations were quantified using the Bio-Rad Protein Assay Kit (Bio-Rad). A total of 30 μ g of protein per sample was resolved on 10% sodium dodecyl sulfate-polyacrylamide gels, then transferred onto 0.45 μ m pore-sized PVDF membranes (Thermo Fisher Scientific). Membranes were incubated with primary antibodies (1:1000 dilution, 200 μ g/mL concentration) at 4 °C for 16 h, followed by washing and incubation with secondary antibodies (1:3000 dilution, 400 μ g/mL concentration). After washing, protein bands were detected using the SuperSignal West Pico PLUS Kit (Thermo Fisher Scientific) and visualized with the ImageQuant LAS 500 system (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Protein expression levels were normalized to β -actin as the control.

Table 1. Primer sequences used in this study.

Gene	Primer sequence (5'→3')	Size (bp)	Accession no.
<i>TYR</i>	F: GTCGTCACCTGAAAATCCTAACT R: CATCGCATAAAACCTGATGGC	111	NM 011661.6
<i>MITF</i>	F: CCCGTCTCTGGAAACTTGATCG R: CTGTACTCTGAGCAGCAGGTC	414	NM 001113198.2
<i>GAPDH</i>	F: ACCACAGTCCATGCCATCAC R: CACCACCCTGTTGCTGTAGC	450	NM 001289726.2

F: forward; R: reverse.

2.11. Zebrafish and ethical approval

Zebrafish were used in accordance with protocols approved by the Animal Care and Use Committee of Jeju National University (Jeju Special Self-governing Province, Republic of Korea; approval no: 2024-0016) and in compliance with established guidelines[20]. They were generated through natural mating and maintained in an embryo medium (prepared with 34.8 g NaCl, 1.6 g KCl, 5.8 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 9.78 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ per liter of double-distilled water, pH adjusted to 7.2), supplemented with 1% methylene blue, and incubated at 28 °C.

2.12. Evaluation of heart rates and melanogenesis in zebrafish larvae

At 2 days post-fertilization (dpf), zebrafish larvae ($n = 15$ per group) were treated with 200 μM phenylthiourea for 24 h, followed by a 24-hour exposure to 1 $\mu\text{g/mL}$ α -MSH. Subsequently, the larvae were immersed in different concentrations of FBCC-EP850 (0–50 $\mu\text{g/mL}$) for 72 h. Heart rates were measured by manually counting beats over a 1-minute interval and expressed as beats per minute. For melanin quantification, larvae were anesthetized with 0.02% tricaine methanesulfonate and mounted in 3% methylcellulose on a depression slide for imaging. Images were taken using a stereomicroscope (Olympus SZ2-ILST, Tokyo, Japan), and melanin pigmentation was analyzed using ImageJ software (National

Institutes of Health, Bethesda, MD, USA). Data are expressed as a percentage relative to the untreated control group.

2.13. Statistical analysis

Gene and protein expression levels, as well as melanin production in zebrafish larvae, were quantified using ImageJ software. Statistical evaluations were performed using SigmaPlot 12.5 (Systat Software, San Jose, CA, USA). Significant differences between experimental groups were assessed using one-way ANOVA with Bonferroni correction. Data are presented as mean $\pm$ standard error of the mean (SEM), and statistical significance was set at $P < 0.05$.

3. Results

3.1. FBCC-EP850 reduces *in vitro* mushroom TYR activity

FBCC-EP850 exhibited a dose-dependent reduction in mushroom TYR activity in *in vitro* assays (Figure 1A). Furthermore, the half-maximal inhibitory concentration (IC_{50}) of FBCC-EP850 for TYR activity was determined to be 45.83 $\mu\text{g/mL}$ (Figure 1B). These results suggest that FBCC-EP850 is a potent inhibitor of TYR activity *in vitro*.

3.2. FBCC-EP850 exhibits cytotoxicity against B16F10 melanoma cells at concentrations above 100 $\mu\text{g/mL}$

To evaluate the safety profile of FBCC-EP850, its cytotoxic effect on B16F10 melanoma cells was investigated before assessing its impact on melanin production. After 72 hours of treatment, lower concentrations of FBCC-EP850 (0–50 $\mu\text{g/mL}$) did not cause noticeable changes in morphology, with cell populations remaining consistent. However, higher doses (100 and 200 $\mu\text{g/mL}$) led to a marked decrease in viable cells from 24 h to 72 h, with signs of

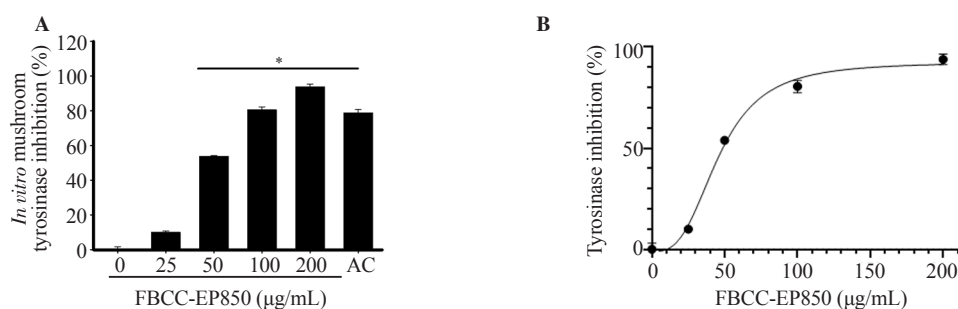


Figure 1. FBCC-EP850 inhibits *in vitro* mushroom tyrosinase activity. (A) Inhibition rate of *in vitro* mushroom tyrosinase activity was measured after treatment with various concentrations of FBCC-EP850 (0–200 $\mu\text{g/mL}$). Ascorbic acid (AC, 0.5 mM) served as a positive control. Data are represented as mean $\pm$ SEM of three independent experiments. * $P < 0.001$ vs. untreated control. (B) The FBCC-EP850 concentration required for 50% tyrosinase inhibition (IC_{50}) was calculated.

death, such as shrinkage, becoming evident at 200 $\mu\text{g/mL}$ after 48 h (Figure 2A). Additionally, low concentrations of FBCC-EP850 had no significant effect on cell viability after 72 h (Figure 2B). In contrast, higher concentrations of FBCC-EP850 progressively reduced cell viability at 24 h [(95.8 $\pm$ 0.5)% and (92.4 $\pm$ 0.3)% at

100 and 200 $\mu\text{g/mL}$, respectively]. This reduction became more pronounced at 48 h [(84.0 $\pm$ 0.3)% and (76.4 $\pm$ 0.6)% at 100 and 200 $\mu\text{g/mL}$, respectively] and 72 h [(78.8 $\pm$ 0.2)% and (71.2 $\pm$ 0.1)% at 100 and 200 $\mu\text{g/mL}$, respectively]. Flow cytometry analysis after 72 h (Figure 2C) showed a significant reduction in viable cell numbers

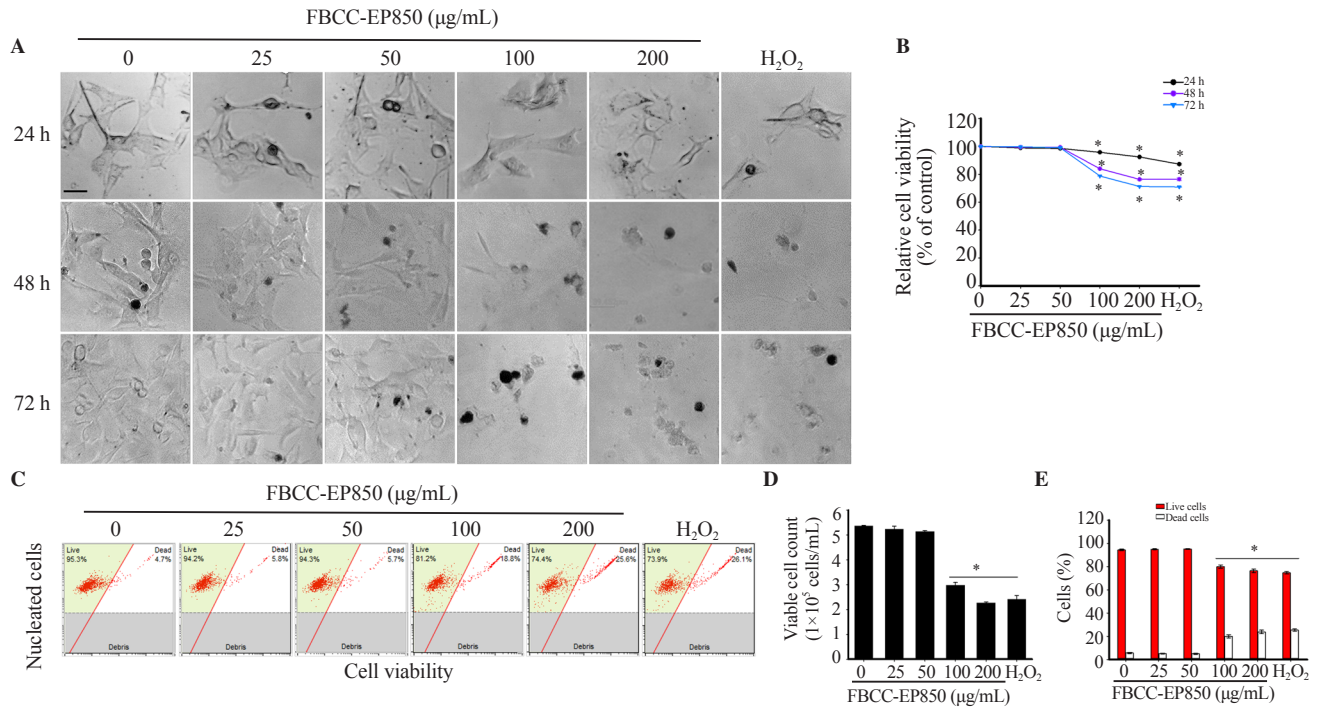


Figure 2. Low concentrations of FBCC-EP850 are not cytotoxic to B16F10 melanoma cells. B16F10 cells (1×10^4 cells/mL) were treated with the indicated concentrations of FBCC-EP850 (0–200 $\mu\text{g/mL}$) for 72 h. (A) Cell morphology was monitored every 24 h (scale bar = 40 μm). (B) Relative cell viability was determined using the WST-8 assay. (C) Cell populations were analyzed using flow cytometry. Hydrogen peroxide (H_2O_2 , 100 μM) was used as a positive control for cell death induction. (D) Viable cell count and (E) live and dead cell populations are displayed. The data are represented as mean $\pm$ SEM of three independent experiments. * $P < 0.001$ vs. untreated control.

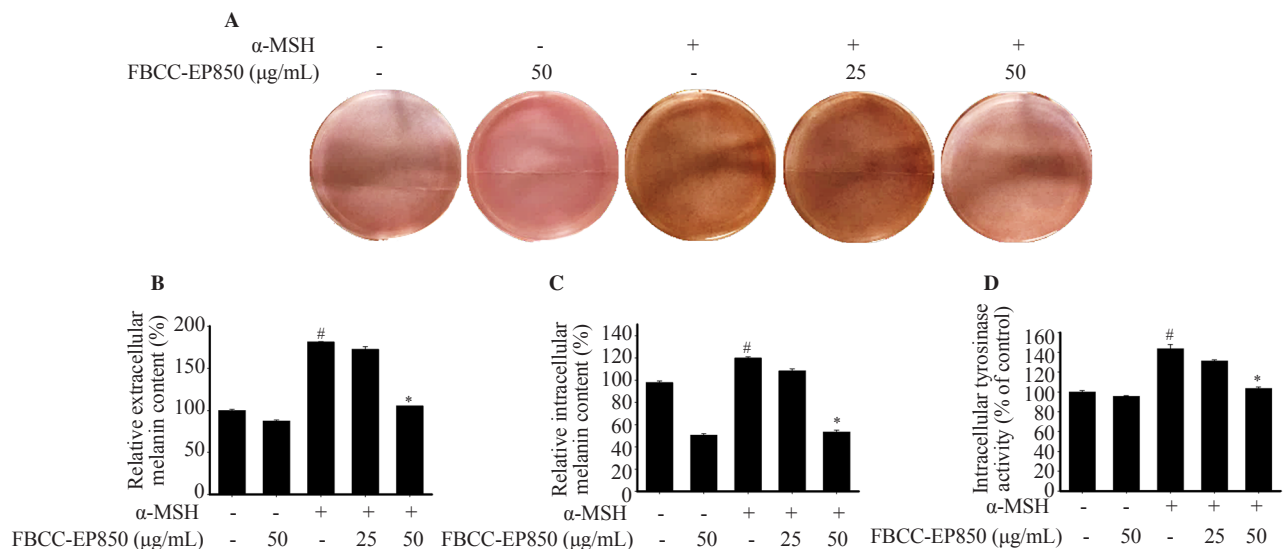


Figure 3. FBCC-EP850 inhibits α -melanocyte stimulating hormone (α -MSH)-induced melanogenesis. B16F10 cells (1×10^4 cells/mL) were stimulated with 500 ng/mL α -MSH for 24 h, followed by treatment with the indicated concentrations (0–50 $\mu\text{g/mL}$) of FBCC-EP850 for 48 h. (A) Color changes were observed in culture media. (B) Extracellular and (C) intracellular melanin contents, as well as (D) intracellular tyrosinase activity were measured. The data are represented as mean $\pm$ SEM of three independent experiments. # $P < 0.001$ vs. control cells; * $P < 0.001$ vs. α -MSH-treated cells.

from 5.4×10^5 cells/mL to 3×10^5 and 2.3×10^5 cells/mL at 100 and 200 $\mu\text{g/mL}$, respectively (Figure 2D), along with an increase in the proportion of dead cells from $(5.2 \pm 0.7)\%$ to $(20.0 \pm 1.4)\%$ and $(23.7 \pm 1.6)\%$ at 100 and 200 $\mu\text{g/mL}$, respectively (Figure 2E). However, FBCC-EP850 at concentrations ≤ 50 $\mu\text{g/mL}$ did not exhibit cytotoxic effects against B16F10 melanoma cells after 72 h, indicating that FBCC-EP850 is non-cytotoxic to B16F10 melanoma cells at concentrations up to 50 $\mu\text{g/mL}$.

3.3. FBCC-EP850 suppresses α -MSH-induced melanin production and intracellular TYR activity

α -MSH stimulation resulted in a significant enhancement of

melanin production in B16F10 melanoma cells, as evidenced by the increased dark brown pigmentation (Figure 3A). However, treatment with 50 $\mu\text{g/mL}$ of FBCC-EP850 substantially lightened this pigmentation. In addition, treatment with 50 $\mu\text{g/mL}$ of FBCC-EP850 significantly diminished both extracellular (Figure 3B) and intracellular (Figure 3C) melanin levels elevated by α -MSH. Furthermore, 50 $\mu\text{g/mL}$ of FBCC-EP850 decreased α -MSH-induced intracellular TYR activity from $(143.9 \pm 4.2)\%$ to $(103.7 \pm 1.4)\%$ in B16F10 melanoma cells (Figure 3D). These results demonstrate that FBCC-EP850 effectively inhibits α -MSH-induced melanin production by downregulating intracellular TYR activity in B16F10 melanoma cells.

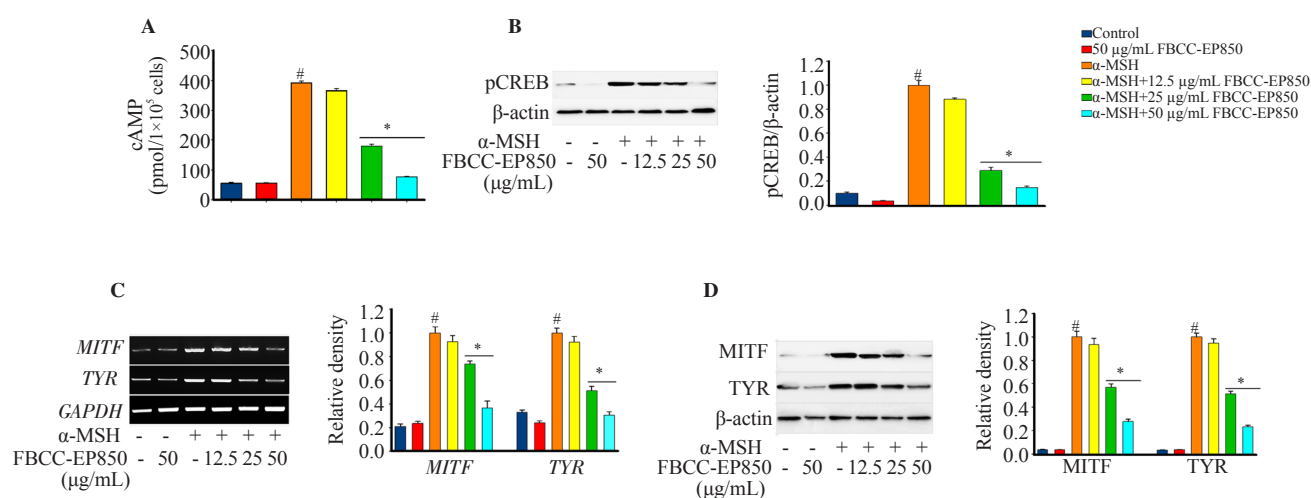


Figure 4. FBCC-EP850 attenuates α -MSH-induced melanogenesis. (A) B16F10 melanoma cells were pretreated with isobutyl methylxanthine (1 mM) for 10 min, followed by treatment with 500 ng/mL of α -MSH for 15 min and FBCC-EP850 (0–50 $\mu\text{g/mL}$) for 30 min. Intracellular cyclic adenosine monophosphate (cAMP) levels were measured using ELISA. (B) B16F10 melanoma cells were pretreated with 500 ng/mL of α -MSH for 15 min and FBCC-EP850 (0–50 $\mu\text{g/mL}$) for 30 min. Western blotting was performed to evaluate pCREB protein expression. (C–D) B16F10 melanoma cells were pretreated with 500 ng/mL of α -MSH for 24 h, followed by treatment with FBCC-EP850 (0–50 $\mu\text{g/mL}$) for 48 h. RT-PCR and Western blot analyses were performed to evaluate MITF and TYR gene and protein expression. The data are represented as mean $\pm$ SEM of three independent experiments. [#] $P < 0.001$ vs. control cells; ^{*} $P < 0.001$ vs. α -MSH-treated cells.

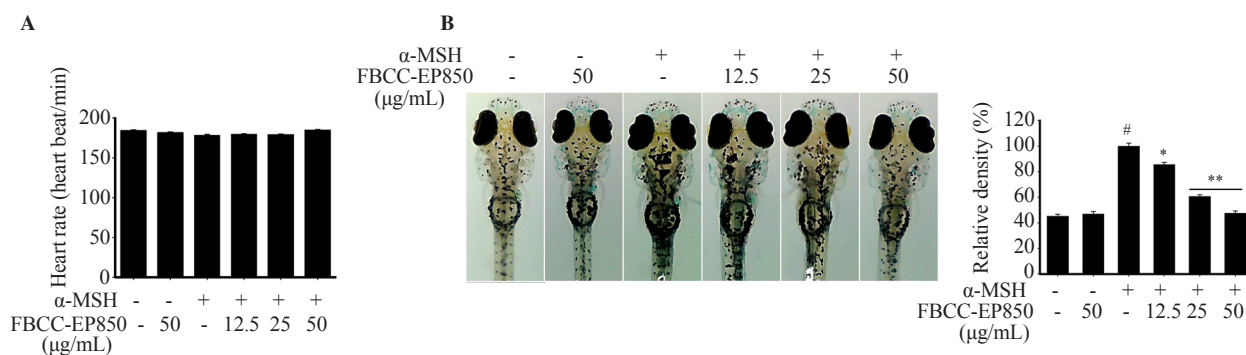


Figure 5. FBCC-EP850 inhibits melanogenesis in zebrafish larvae. Zebrafish larvae at 2 days post-fertilization (dpf) were treated with phenylthiourea (200 μM) for 24 h, followed by stimulation with α -MSH (1 $\mu\text{g/mL}$) for another 24 h. The larvae were then exposed to the indicated concentrations of FBCC-EP850 for 72 h. (A) Average heart rate of zebrafish larvae ($n = 15$) was manually measured at a 1-minute interval. (B) Microscopic evaluation of zebrafish larvae pigmentation was performed and its relative density was measured. The data are represented as mean $\pm$ SEM of three independent experiments. [#] $P < 0.001$ vs. control zebrafish larvae; ^{*} $P < 0.05$ and ^{**} $P < 0.001$ vs. α -MSH-stimulated zebrafish larvae.

3.4. FBCC-EP850 inhibits α -MSH-induced melanogenic signaling pathways

The effects of FBCC-EP850 on the melanogenesis signaling pathways in B16F10 melanoma cells were evaluated in this study. Treatment with FBCC-EP850 significantly decreased α -MSH-induced increase in the levels of cAMP in a dose-dependent manner (Figure 4A), with no significant change observed at 12.5 μ g/mL. Moreover, FBCC-EP850 treatment diminished the phosphorylation of CREB induced by α -MSH, with a marked decrease evident at 25 and 50 μ g/mL (Figure 4B). Additionally, FBCC-EP850 significantly suppressed the expression of MITF and TYR, at both transcriptional (Figure 4C) and translational levels (Figure 4D). These results indicate that FBCC-EP850 effectively inhibits α -MSH-induced melanogenesis by inhibiting the cAMP-CREB-MITF-TYR pathway in B16F10 melanoma cells.

3.5. FBCC-EP850 decreases α -MSH-induced melanin pigmentation in zebrafish larvae without inducing toxicity

To evaluate the efficacy of FBCC-EP850 *in vivo*, concentrations that did not affect heart rates or cause morphological abnormalities in zebrafish larvae were identified. Concentrations of 12.5, 25, and 50 μ g/mL were selected for subsequent experiments (Figure 5A). Treatment with FBCC-EP850 resulted in a dose-dependent reduction in melanin pigmentation compared to the α -MSH-stimulated group (Figure 5B). These results underscore the potential of FBCC-EP850 as an effective inhibitor of melanin pigmentation in zebrafish larvae, suggesting its promise as a therapeutic candidate for conditions related to abnormal melanogenesis.

4. Discussion

The growing popularity of skin-whitening products, especially among communities of color, has led to increasing dermatologic public health concerns worldwide[15,21,22]. Consequently, identifying compounds that can safely inhibit melanogenesis without causing adverse effects is essential. Natural compounds have garnered significant attention in this context due to their perceived benefits, including reduced cytotoxicity and enhanced effectiveness in inhibiting melanin biosynthesis compared to synthetic alternatives[13,23,24]. In this study, FBCC-EP850 demonstrated potent anti-melanogenic effects while maintaining a low toxicity profile, making it a promising candidate for further development into skincare products targeting hyperpigmentation disorders.

Melanin biosynthesis is initiated when α -MSH binds to the MC1R, triggering the activation of the cAMP-CREB-MITF-TYR signaling

pathway[25]. Upon binding of α -MSH, intracellular cAMP levels rise, leading to the phosphorylation of CREB through PKA activation. This cascade ultimately derives the transcription of *MITF* and *TYR*, key regulators of melanin synthesis[26,27]. MITF, in particular, is a master transcription factor crucial for melanocyte development and proliferation, and melanin production[28,29]. Mutation in *MITF* is associated with conditions such as albinism and melanoma due to disruptions in its ability to regulate TYR expression, the rate-limiting enzyme in melanin synthesis[3,30–32]. Our results demonstrate that FBCC-EP850 significantly inhibited *in vitro* mushroom TYR activity, indicating its potential as a direct TYR inhibitor. Furthermore, FBCC-EP850 effectively reduced α -MSH-induced increases in cAMP, CREB phosphorylation, and the transcription and translation of MITF and TYR. These findings suggest that FBCC-EP850 targets multiple stages of the cAMP-CREB-MITF-TYR pathway, offering a multifaceted approach to inhibit melanogenesis at the cellular level. Despite these promising outcomes, further investigation is needed to identify the specific active compounds of FBCC-EP850 that contribute to its anti-melanogenic effects. Previous research by Masuda *et al.* revealed that a methanol extract of *C. pumila* leaves exhibited approximately 58% TYR inhibition at a concentration of 500 μ g/mL, though the major components were not identified[33]. Kurihara *et al.* successfully isolated oligostilbenes, such as (-)- ϵ -viniferin and from *C. pumila*[34], while Kawabata *et al.* identified kobophenol B, a tetra-stilbene from the same plant[35]. More recent studies have demonstrated that stilbenes inhibit melanin production by interfering with the cAMP/PKA/CREB pathway, thereby reducing melanosome transport[36]. Given this, it is plausible that FBCC-850 contains high levels of stilbenes, which may account for its potent anti-melanogenic activity. Nevertheless, further studies should focus on isolating and characterizing the specific compounds responsible for FBCC-EP850 in different experimental models and evaluating its clinical applicability will provide valuable insights into its therapeutic potential. A more comprehensive understanding of the effects of FBCC-EP850 across various conditions and cell types will further aid in optimizing its clinical use.

In conclusion, FBCC-EP850 derived from *C. pumila* Thunb. is a promising agent for inhibiting melanogenesis. It exhibits potent inhibitory effects both *in vitro* and *in vivo*, underscoring its potential as a valuable therapeutic candidate for addressing hyperpigmentation disorders. However, further research is essential to fully elucidate molecular mechanisms underlying its anti-melanogenic properties. In addition, long-term safety, stability, and efficacy studies are necessary to ensure its viability as a therapeutic agent. Investigating potential synergistic effects with other anti-melanogenic compounds could further enhance the clinical potential of FBCC-EP850. Continued exploration of FBCC-EP850 could ultimately lead to the development of innovative treatments for hyperpigmentation,

providing safer and more effective solutions for individuals seeking skin-whitening options.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

MHDK, JP, and GYK designed the experiment. MHDK, JP, and NK operated the experiment and processed the experimental data. YHC and GYK visualized and ensured the authenticity of the experimental data. MHDK wrote the manuscript. YHC and GYK contributed to the revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Anti-melanogenic properties of FBCC-EP850 derived from *Carex pumila* Thunb.

Running title: Anti-melanogenic properties of FBCC-EP850

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Supplementary Table 1. FBCC code and plant sources used in this study and *in vitro* mushroom tyrosinase inhibition activity.

FBCC code	Species name	Extracted parts	Collection date	Extraction solvents	Drying methods	<i>In vitro</i> mushroom tyrosinase inhibition activity (%)
FBCC-EP844	<i>Carex pumila</i> Thunb.	under ground	01/04/2019	water	freeze drying	-24.51 ±1.88
FBCC-EP845	<i>Carex pumila</i> Thunb.	above ground	01/04/2019	water	freeze drying	-39.69 ±4.96
FBCC-EP846	<i>Lemna perpusilla</i> Torr.	whole	01/04/2019	water	freeze drying	-41.63 ±1.78
FBCC-EP847	<i>Lycopus lucidus</i> var. hirtus	under ground	01/04/2019	water	freeze drying	-26.46 ±3.21
FBCC-EP848	<i>Lycopus lucidus</i> var. hirtus	above ground	01/04/2019	water	freeze drying	-7.00 ±0.89
FBCC-EP849	Linderniaceae Borsch, K. Müll. & Eb. Fisch., 2005	whole	01/04/2019	70% ethanol	reduced pressure concentration	-43.97 ±2.94
FBCC-EP850	<i>Carex pumila</i> Thunb.	under ground	01/04/2019	70% ethanol	reduced pressure concentration	55.64 ±0.58
FBCC-EP851	<i>Carex pumila</i> Thunb.	above ground	01/04/2019	70% ethanol	reduced pressure concentration	-26.46 ±1.78
FBCC-EP852	<i>Lemna perpusilla</i> Torr.	whole	01/04/2019	70% ethanol	reduced pressure concentration	-43.58 ±3.55
FBCC-EP853	<i>Lycopus lucidus</i> var. hirtus	under ground	01/04/2019	70% ethanol	reduced pressure concentration	-39.30 ±0.67
FBCC-EP854	<i>Lycopus lucidus</i> var. hirtus	above ground	01/04/2019	70% ethanol	reduced pressure concentration	-7.00 ±4.42
FBCC-EP855	<i>Vallisneria spinulosa</i> S. Z. Yan	under	01/08/2019	water	freeze drying	-44.75 ±0.58

		ground				
FBCC-EP856	<i>Trapa incisa</i> Siebold & Zucc.	above ground	01/08/2019	water	freeze drying	-70.82 ±1.21
FBCC-EP857	<i>Najas minor</i> All.	whole	01/08/2019	water	freeze drying	-47.86 ±3.00
FBCC-EP858	<i>Ammannia multiflora</i> (W. A. Nicholson) Rehder	above ground	01/08/2019	water	freeze drying	-46.30 ±4.75
FBCC-EP859	<i>Vallisneria spinulosa</i> S. Z. Yan	whole	01/08/2019	water	freeze drying	-33.85 ±4.96
FBCC-EP860	<i>Marsilea quadrifolia</i> L.	whole	01/08/2019	water	freeze drying	-49.42 ±5.92
FBCC-EP861	<i>Vallisneria spinulosa</i> S. Z. Yan	under ground	01/08/2019	70% ethanol	reduced pressure concentration	-50.19 ±1.78
FBCC-EP862	<i>Trapa incisa</i> Siebold & Zucc.	above ground	01/08/2019	70% ethanol	reduced pressure concentration	-35.41 ±1.17
FBCC-EP863	<i>Najas minor</i> All.	whole	01/08/2019	70% ethanol	reduced pressure concentration	-58.37 ±2.76
FBCC-EP864	<i>Ammannia multiflora</i> (W. A. Nicholson) Rehder	above ground	01/08/2019	70% ethanol	reduced pressure concentration	-17.08 ±0.31
FBCC-EP865	<i>Vallisneria spinulosa</i> S. Z. Yan	whole	01/08/2019	70% ethanol	reduced pressure concentration	-35.94 ±4.01
FBCC-EP866	<i>Marsilea quadrifolia</i> L.	whole	01/08/2019	70% ethanol	reduced pressure concentration	-44.13 ±4.74
FBCC-EP867	<i>Trapa bispinosa</i> Roxb.	whole	01/15/2019	water	freeze drying	-37.72 ±2.82
FBCC-EP868	<i>Moliniopsis japonica</i> (Hack.) Hayata	under ground	01/15/2019	water	freeze drying	-21.71 ±5.77
FBCC-EP869	<i>Moliniopsis japonica</i> (Hack.) Hayata	above ground	01/15/2019	water	freeze drying	-25.62 ±1.11
FBCC-EP871	<i>Scirpus wichurai</i> var. asiaticus (Beetle) T. Koyama ex Ohwi	above ground	01/15/2019	water	freeze drying	-2.85 ±0.62
FBCC-EP872	<i>Scirpus mitsukurianus</i> Makino	under	01/15/2019	water	freeze drying	-1.07 ±1.87

		ground				
FBCC-EP873	<i>Trapa bispinosa</i> Roxb.	whole	01/15/2019	70% ethanol	reduced pressure concentration	-20.64±1.92
FBCC-EP875	<i>Moliniopsis japonica</i> (Hack.) Hayata	above ground	01/15/2019	70% ethanol	reduced pressure concentration	-21.00±2.22
FBCC-EP876	<i>Scirpus wichurai</i> var. asiaticus (Beetle) T. Koyama ex Ohwi	under ground	01/15/2019	70% ethanol	reduced pressure concentration	-21.71±1.41
FBCC-EP877	<i>Scirpus wichurai</i> var. asiaticus (Beetle) T. Koyama ex Ohwi	above ground	01/15/2019	70% ethanol	reduced pressure concentration	-21.71±2.33
FBCC-EP878	<i>Scirpus mitsukurianus</i> Makino	under ground	01/15/2019	70% ethanol	reduced pressure concentration	34.16±1.72
FBCC-EP879	<i>Scirpus mitsukurianus</i> Makino	above ground	01/29/2019	water	freeze drying	-18.15±2.52
FBCC-EP881	<i>Scirpus juncooides</i> Roxb.	above ground	01/29/2019	water	freeze drying	-25.98±2.33
FBCC-EP882	<i>Juncus papillosus</i> Franch. & Sav.	under ground	01/29/2019	water	freeze drying	-11.03±1.85
FBCC-EP883	<i>Juncus papillosus</i> Franch. & Sav.	above ground	01/29/2019	water	freeze drying	-5.34±4.28
FBCC-EP884	<i>Arthraxon hispidus</i> (Thunb.) Makino	whole	01/29/2019	water	freeze drying	-5.69±2.14
FBCC-EP885	<i>Scirpus mitsukurianus</i> Makino	above ground	01/29/2019	70% ethanol	reduced pressure concentration	-17.79±0.31
FBCC-EP886	<i>Scirpus juncooides</i> Roxb.	under ground	01/29/2019	70% ethanol	reduced pressure concentration	21.00±1.07
FBCC-EP887	<i>Scirpus juncooides</i> Roxb.	above ground	01/29/2019	70% ethanol	reduced pressure concentration	-122.99±5.54
FBCC-EP888	<i>Juncus papillosus</i> Franch. & Sav.	under ground	01/29/2019	70% ethanol	reduced pressure concentration	-48.85±12.11

FBCC-EP889	<i>Juncus papillosus</i> Franch. & Sav.	above ground	01/29/2019	70% ethanol	reduced pressure concentration	-100.00 ±2.59
FBCC-EP890	<i>Arthraxon hispidus</i> (Thunb.) Makino	whole	01/29/2019	70% ethanol	reduced pressure concentration	-131.61 ±5.47
FBCC-EP891	<i>Penthorum chinense</i> Pursh	under ground	02/12/2019	water	freeze drying	-105.17 ±0.86
FBCC-EP892	<i>Penthorum chinense</i> Pursh	above ground	02/12/2019	water	freeze drying	-78.16 ±3.59
FBCC-EP893	<i>Paspalum distichum</i> var. indutum Shinnery	whole	02/12/2019	water	freeze drying	-101.15 ±6.97
FBCC-EP894	<i>Cyperus glomeratus</i> L.	under ground	02/12/2019	water	freeze drying	-93.1 ±2.99
FBCC-EP895	<i>Cyperus glomeratus</i> L.	above ground	02/12/2019	water	freeze drying	-102.87 ±3.98
FBCC-EP896	<i>Hemarthria compressa</i> var. japonica (Hack.) Y. N. Lee	under ground	02/12/2019	water	freeze drying	-104.02 ±4.98
FBCC-EP897	<i>Penthorum chinense</i> Pursh	under ground	02/12/2019	70% ethanol	reduced pressure concentration	25.29 ±5.54
FBCC-EP898	<i>Penthorum chinense</i> Pursh	above ground	02/12/2019	70% ethanol	reduced pressure concentration	-36.78 ±0.5
FBCC-EP899	<i>Paspalum distichum</i> var. indutum Shinnery	whole	02/12/2019	70% ethanol	reduced pressure concentration	-130.46 ±2.63
FBCC-EP900	<i>Cyperus glomeratus</i> L.	under ground	02/12/2019	70% ethanol	reduced pressure concentration	-70.69 ±3.11
FBCC-EP901	<i>Cyperus glomeratus</i> L.	above ground	02/12/2019	70% ethanol	reduced pressure concentration	-50.57 ±8.19
FBCC-EP902	<i>Hemarthria compressa</i> var. japonica (Hack.) Y. N. Lee	under ground	02/12/2019	70% ethanol	reduced pressure concentration	-133.91 ±3.26
FBCC-EP903	<i>Hemarthria compressa</i> var. japonica (Hack.) Y. N.	above	03/06/2019	water	freeze drying	-100.00 ±3.76

	Lee	ground				
FBCC-EP904	<i>Arthraxon hispidus</i> (Thunb.) Makino	whole	03/06/2019	water	freeze drying	-83.33 ±4.07
FBCC-EP905	<i>Zoysia sinica</i> Hance	under ground	03/06/2019	water	freeze drying	-122.99 ±5.61
FBCC-EP906	<i>Zoysia sinica</i> Hance	above ground	03/06/2019	water	freeze drying	-108.05 ±4.9
FBCC-EP907	<i>Juncus krameri</i> Franch. & Sav.	under ground	03/06/2019	water	freeze drying	-32.66 ±2.72
FBCC-EP908	<i>Juncus krameri</i> Franch. & Sav.	above ground	03/06/2019	water	freeze drying	-70.35 ±4.94
FBCC-EP909	<i>Hemarthria compressa</i> var. japonica (Hack.) Y. N. Lee	above ground	03/06/2019	70% ethanol	reduced pressure concentration	-83.92 ±0.75
FBCC-EP910	<i>Arthraxon hispidus</i> (Thunb.) Makino	whole	03/06/2019	70% ethanol	reduced pressure concentration	-71.86 ±2.72
FBCC-EP912	<i>Zoysia sinica</i> Hance	above ground	03/06/2019	70% ethanol	reduced pressure concentration	-88.44 ±7.98
FBCC-EP913	<i>Juncus krameri</i> Franch. & Sav.	under ground	03/06/2019	70% ethanol	reduced pressure concentration	-30.65 ±1.15
FBCC-EP914	<i>Juncus krameri</i> Franch. & Sav.	above ground	03/06/2019	70% ethanol	reduced pressure concentration	-53.77 ±0.75
FBCC-EP915	<i>Arthraxon hispidus</i> (Thunb.) Makino	whole	03/27/2019	water	freeze drying	-68.34 ±2.18
FBCC-EP916	<i>Polygonum muricatum</i> Meisn.	whole	03/27/2019	water	freeze drying	-61.31 ±3.77
FBCC-EP917	<i>Penthorum chinense</i> Pursh	under ground	03/27/2019	water	freeze drying	-70.35 ±3.45
FBCC-EP918	<i>Penthorum chinense</i> Pursh	above ground	03/27/2019	water	freeze drying	-64.82 ±3.05
FBCC-EP919	<i>Lobelia chinensis</i> Lour.	whole	03/27/2019	water	freeze drying	-76.38 ±3.77
FBCC-EP920	<i>Ammannia coccinea</i> Rottb.	whole	03/27/2019	water	freeze drying	-104.52 ±1.15

FBCC-EP921	<i>Arthraxon hispidus</i> (Thunb.) Makino	whole	03/27/2019	70% ethanol	reduced pressure concentration	-77.89 ±1.51
FBCC-EP922	<i>Polygonum muricatum</i> Meisn.	whole	03/27/2019	70% ethanol	reduced pressure concentration	-80.40 ±4.15
FBCC-EP923	<i>Penthorum chinense</i> Pursh	under ground	03/27/2019	70% ethanol	reduced pressure concentration	-9.05 ±1.74
FBCC-EP924	<i>Penthorum chinense</i> Pursh	above ground	03/27/2019	70% ethanol	reduced pressure concentration	-21.11 ±1.15
FBCC-EP925	<i>Lobelia chinensis</i> Lour.	whole	03/27/2019	70% ethanol	reduced pressure concentration	-78.89 ±1.57
FBCC-EP926	<i>Ammannia coccinea</i> Rottb.	whole	03/27/2019	70% ethanol	reduced pressure concentration	-58.79 ±3.87
FBCC-EP927	<i>Ottelia alismoides</i> (L.) Pers.	whole	04/09/2019	water	freeze drying	-87.44 ±0.87
FBCC-EP928	<i>Ottelia alismoides</i> (L.) Pers.	whole	04/09/2019	70% ethanol	reduced pressure concentration	-59.60 ±4.87
FBCC-EP1013	<i>Salix koreensis</i> Andersson	leaves	08/27/2019	water	freeze drying	-98.48 ±3.94
FBCC-EP1014	<i>Salix gracilistyla</i> Miq.	leaves	08/27/2019	water	freeze drying	-82.83 ±0.87
FBCC-EP1015	<i>Salix koriyanagi</i> Kimura ex Goerz	leaves	08/27/2019	water	freeze drying	-123.74 ±1.91
FBCC-EP1016	<i>Salix subfragilis</i> Andersson	branches	08/27/2019	water	freeze drying	-106.06 ±2.00
FBCC-EP1017	<i>Salix subfragilis</i> Andersson	flowers	08/27/2019	water	freeze drying	-72.22 ±11.04
FBCC-EP1018	<i>Salix subfragilis</i> Andersson	leaves	08/27/2019	water	freeze drying	-102.53 ±2.66
FBCC-EP1019	<i>Salix koreensis</i> Andersson	branches	08/27/2019	70% ethanol	reduced pressure concentration	-106.06 ±1.31
FBCC-EP1020	<i>Salix gracilistyla</i> Miq.	leaves	08/27/2019	70% ethanol	reduced pressure concentration	-94.44 ±2.66
FBCC-EP1021	<i>Salix koriyanagi</i> Kimura ex Goerz	leaves	08/27/2019	70% ethanol	reduced pressure concentration	-95.45 ±1.52
FBCC-EP1022	<i>Salix subfragilis</i> Andersson	branches	08/27/2019	70%	reduced pressure	-93.43 ±6.87

FBCC-EP1023	<i>Salix subfragilis</i> Andersson	flowers	08/27/2019	ethanol 70%	concentration reduced pressure	-104.55 ±4.61
FBCC-EP1024	<i>Salix subfragilis</i> Andersson	leaves	08/27/2019	ethanol 70%	concentration reduced pressure	-125.25 ±3.58
FBCC-EP1025	<i>Salix pseudolasiogyne</i> H. Lév.	branches	09/03/2019	water	freeze drying	-61.11 ±0.44
FBCC-EP1026	<i>Salix pseudolasiogyne</i> H. Lév.	flowers	09/03/2019	water	freeze drying	-107.58 ±3.30
FBCC-EP1027	<i>Salix pseudolasiogyne</i> H. Lév.	leaves	09/03/2019	water	freeze drying	-78.79 ±9.67
FBCC-EP1028	<i>Salix chaenomeloides</i> Kimura	branches	09/03/2019	water	freeze drying	-72.22 ±1.16
FBCC-EP1029	<i>Salix chaenomeloides</i> Kimura	leaves	09/03/2019	water	freeze drying	-95.45 ±2.73
FBCC-EP1030	<i>Juncus effusus</i> var. <i>decipiens</i> Buchenau	above ground	09/03/2019	water	freeze drying	-101.01 ±0.87
FBCC-EP1031	<i>Salix pseudolasiogyne</i> H. Lév.	branches	09/03/2019	ethanol 70%	concentration reduced pressure	-97.47 ±1.58
FBCC-EP1032	<i>Salix pseudolasiogyne</i> H. Lév.	flowers	09/03/2019	ethanol 70%	concentration reduced pressure	-83.01 ±7.58
FBCC-EP1033	<i>Salix pseudolasiogyne</i> H. Lév.	leaves	09/03/2019	ethanol 70%	concentration reduced pressure	-86.41 ±3.34
FBCC-EP1034	<i>Salix chaenomeloides</i> Kimura	branches	09/03/2019	ethanol 70%	concentration reduced pressure	-74.76 ±2.18
FBCC-EP1035	<i>Salix chaenomeloides</i> Kimura	leaves	09/03/2019	ethanol 70%	concentration reduced pressure	-87.38 ±2.94
FBCC-EP1036	<i>Juncus effusus</i> var. <i>decipiens</i> Buchenau	above ground	09/03/2019	ethanol 70%	concentration reduced pressure	-80.10 ±3.59
FBCC-EP1038	<i>Ranunculus sceleratus</i> L.	whole	09/06/2019	water	freeze drying	-75.73 ±2.22
FBCC-EP1039	<i>Veronica peregrina</i> L.	whole	09/06/2019	water	freeze drying	-66.02 ±7.17
FBCC-EP1040	<i>Carex doniana</i> Speng.	whole	09/06/2019	water	freeze drying	-79.61 ±4.27
FBCC-EP1041	<i>Iris pseudacorus</i> L.	whole	09/06/2019	water	freeze drying	-84.47 ±5.47

FBCC-EP1042	<i>Ixeris debilis</i> (Thunb.) A. Gray	whole	09/06/2019	water	freeze drying	-110.68 ±7.65
FBCC-EP1044	<i>Ranunculus sceleratus</i> L.	whole	09/06/2019	70% ethanol	reduced pressure concentration	-90.29 ±4.96
FBCC-EP1045	<i>Veronica peregrina</i> L.	whole	09/06/2019	70% ethanol	reduced pressure concentration	-93.20 ±5.27
FBCC-EP1046	<i>Carex doniana</i> Speng.	whole	09/06/2019	70% ethanol	reduced pressure concentration	-94.66 ±3.03
FBCC-EP1047	<i>Iris pseudacorus</i> L.	whole	09/06/2019	70% ethanol	reduced pressure concentration	-86.41 ±3.34
FBCC-EP1048	<i>Ixeris debilis</i> (Thunb.) A. Gray	whole	09/06/2019	70% ethanol	reduced pressure concentration	-103.40 ±5.27
FBCC-EP1049	<i>Scirpus radicans</i> Schkuhr	whole	09/16/2019	water	freeze drying	-71.84 ±4.05
FBCC-EP1050	<i>Carex maackii</i> Maxim.	whole	09/16/2019	water	freeze drying	-85.44 ±1.83
FBCC-EP1051	<i>Carex pumila</i> Thunb.	above ground	09/16/2019	water	freeze drying	-75.24 ±6.77
FBCC-EP1052	<i>Carex pumila</i> Thunb.	under ground	09/16/2019	water	freeze drying	-60.68 ±4.14
FBCC-EP1053	<i>Iris setosa</i> Pall. ex Link	whole	09/16/2019	water	freeze drying	-81.07 ±7.00
FBCC-EP1054	<i>Veronica anagallis-aquatica</i> L.	whole	09/16/2019	water	freeze drying	-83.74 ±4.07
FBCC-EP1055	<i>Scirpus radicans</i> Schkuhr	whole	09/16/2019	70% ethanol	reduced pressure concentration	-98.52 ±2.59
FBCC-EP1056	<i>Carex maackii</i> Maxim.	whole	09/16/2019	70% ethanol	reduced pressure concentration	-104.43 ±3.49
FBCC-EP1057	<i>Carex pumila</i> Thunb.	above ground	09/16/2019	70% ethanol	reduced pressure concentration	-85.71 ±1.54
FBCC-EP1059	<i>Iris setosa</i> Pall. ex Link	whole	09/16/2019	70% ethanol	reduced pressure concentration	-88.18 ±5.59
FBCC-EP1060	<i>Veronica anagallis-aquatica</i> L.	whole	09/16/2019	70%	reduced pressure	-79.80 ±8.24

				ethanol	concentration	
FBCC-EP1061	<i>Lamium album</i> var. <i>barbatum</i> (Siebold & Zucc.) Franch. & Sav.	whole	09/30/2019	water	freeze drying	-111.82±10.03
FBCC-EP1062	<i>Ixeris polycephala</i> Cass.	whole	09/30/2019	water	freeze drying	-90.15±4.45
FBCC-EP1063	<i>Potentilla supina</i> L.	whole	09/30/2019	water	freeze drying	-109.85±0.74
FBCC-EP1064	<i>Bidens radiata</i> var. <i>pinnatifida</i> (Turcz. ex DC.) Kitam.	whole	09/30/2019	water	freeze drying	-97.04±3.41
FBCC-EP1065	<i>Equisetum arvense</i> L.	whole	09/30/2019	water	freeze drying	-91.13±2.13
FBCC-EP1066	<i>Carex miyabei</i> Franch.	whole	09/30/2019	water	freeze drying	-81.28±1.71
FBCC-EP1067	<i>Lamium album</i> var. <i>barbatum</i> (Siebold & Zucc.) Franch. & Sav.	whole	09/30/2019	70% ethanol	reduced pressure concentration	-121.67±2.22
FBCC-EP1068	<i>Ixeris polycephala</i> Cass.	whole	09/30/2019	70% ethanol	reduced pressure concentration	-107.39±1.54
FBCC-EP1069	<i>Potentilla supina</i> L.	whole	09/30/2019	70% ethanol	reduced pressure concentration	-117.73±4.20
FBCC-EP1070	<i>Bidens radiata</i> var. <i>pinnatifida</i> (Turcz. ex DC.) Kitam.	whole	09/30/2019	70% ethanol	reduced pressure concentration	-70.94±3.49
FBCC-EP1071	<i>Equisetum arvense</i> L.	whole	09/30/2019	70% ethanol	reduced pressure concentration	-105.91±9.30
FBCC-EP1072	<i>Carex miyabei</i> Franch.	whole	09/30/2019	70% ethanol	reduced pressure concentration	-84.73±3.84
FBCC-EP1073	<i>Eleocharis mamillata</i> var. <i>cyclocarpa</i> Kitag.	whole	10/14/2019	water	freeze drying	-94.09±2.26
FBCC-EP1074	<i>Ailanthus altissima</i> f. <i>erythrocarpa</i> (Carrière) Rehder	branches	10/14/2019	water	freeze drying	-90.64±0.00
FBCC-EP1075	<i>Ailanthus altissima</i> f. <i>erythrocarpa</i> (Carrière) Rehder	leaves	10/14/2019	water	freeze drying	-106.15±3.35
FBCC-EP1076	<i>Carex neurocarpa</i> Maxim.	above ground	10/14/2019	water	freeze drying	-64.10±4.44
FBCC-EP1077	<i>Carex neurocarpa</i> Maxim.	under	10/14/2019	water	freeze drying	-64.62±2.77

		ground				
FBCC-EP1078	<i>Iris sanguinea</i> Donn ex Hornem.	whole	10/14/2019	water	freeze drying	-44.10 ±12.07
FBCC-EP1079	<i>Eleocharis mamillata</i> var. <i>cyclocarpa</i> Kitag.	whole	10/14/2019	70% ethanol	reduced pressure concentration	-83.59 ±12.65
FBCC-EP1080	<i>Ailanthus altissima</i> f. <i>erythrocarpa</i> (Carrière) Rehder	branches	10/14/2019	70% ethanol	reduced pressure concentration	-62.05 ±3.47
FBCC-EP1081	<i>Ailanthus altissima</i> f. <i>erythrocarpa</i> (Carrière) Rehder	leaves	10/14/2019	70% ethanol	reduced pressure concentration	-111.28 ±1.18
FBCC-EP1082	<i>Carex neurocarpa</i> Maxim.	above ground	10/14/2019	70% ethanol	reduced pressure concentration	-71.79 ±8.47
FBCC-EP1084	<i>Iris sanguinea</i> Donn ex Hornem.	whole	10/14/2019	70% ethanol	reduced pressure concentration	-65.64 ±3.64
FBCC-EP1085	<i>Rosa multiflora</i> Thunb.	branches	05/28/2019	water	freeze drying	-66.67 ±2.22
FBCC-EP1086	<i>Rosa multiflora</i> Thunb.	leaves	05/28/2019	water	freeze drying	-93.33 ±3.64
FBCC-EP1087	<i>Hydrocotyle maritima</i> Honda	whole	07/08/2019	water	freeze drying	-78.46 ±1.54
FBCC-EP1088	<i>Typha orientalis</i> C. Presl.	whole	07/09/2019	water	freeze drying	-70.77 ±2.66
FBCC-EP1089	<i>Stachys japonica</i> Miq.	whole	07/09/2019	water	freeze drying	-80.00 ±5.55
FBCC-EP1090	<i>Salvia plebeia</i> R. Br.	whole	07/09/2019	water	freeze drying	-18.46 ±0.77
FBCC-EP1091	<i>Rosa multiflora</i> Thunb.	branches	05/28/2019	70% ethanol	reduced pressure concentration	-61.54 ±2.04
FBCC-EP1092	<i>Rosa multiflora</i> Thunb.	leaves	05/28/2019	70% ethanol	reduced pressure concentration	-73.85 ±6.15
FBCC-EP1093	<i>Hydrocotyle maritima</i> Honda	whole	07/08/2019	70% ethanol	reduced pressure concentration	-91.28 ±4.89
FBCC-EP1095	<i>Stachys japonica</i> Miq.	whole	07/09/2019	70% ethanol	reduced pressure concentration	-80.51 ±5.24
FBCC-EP1096	<i>Salvia plebeia</i> R. Br.	whole	07/09/2019	70% ethanol	reduced pressure concentration	11.28 ±2.91

FBCC-EP1097	<i>Cyperus difformis</i> L.	whole	03/13/2020	70% ethanol	reduced pressure concentration	-45.92 ±0.74
FBCC-EP1098	<i>Eleocharis mamillata</i> var. <i>cyclocarpa</i> Kitag.	whole	03/13/2020	70% ethanol	reduced pressure concentration	-9.44 ±2.23
FBCC-EP1099	<i>Lindernia dubia</i> (L.) Pennell	whole	03/13/2020	70% ethanol	reduced pressure concentration	-45.92 ±1.97
FBCC-EP1100	<i>Sparganium erectum</i> L.	above ground	03/13/2020	70% ethanol	reduced pressure concentration	-45.06 ±1.97
FBCC-EP1101	<i>Sparganium erectum</i> L.	under ground	03/13/2020	70% ethanol	reduced pressure concentration	-35.19 ±5.72
FBCC-EP1102	<i>Sparganium erectum</i> L.	flowers	03/13/2020	70% ethanol	reduced pressure concentration	-33.91 ±6.53
FBCC-EP1103	<i>Alnus japonica</i> (Thunb.) Steud.	branches	03/20/2020	70% ethanol	reduced pressure concentration	-21.89 ±2.90
FBCC-EP1104	<i>Alnus japonica</i> (Thunb.) Steud.	leaves	03/20/2020	70% ethanol	reduced pressure concentration	-34.33 ±1.49
FBCC-EP1105	<i>Alnus japonica</i> (Thunb.) Steud.	barks	03/20/2020	70% ethanol	reduced pressure concentration	-24.46 ±2.44
FBCC-EP1106	<i>Hydrilla verticillata</i> (L. f.) Royle	whole	03/20/2020	70% ethanol	reduced pressure concentration	-54.08 ±0.98
FBCC-EP1107	<i>Polygonum sagittatum</i> L.	whole	03/20/2020	70% ethanol	reduced pressure concentration	-37.77 ±1.93
FBCC-EP1108	<i>Murdannia keisak</i> (Hassk.) Hand-Mazz.	whole	03/20/2020	70% ethanol	reduced pressure concentration	-45.92 ±0.74
FBCC-EP1109	<i>Commelina communis</i> L.	whole	03/27/2020	70% ethanol	reduced pressure concentration	-45.92 ±2.60
FBCC-EP1110	<i>Najas marina</i> L.	whole	03/27/2020	70% ethanol	reduced pressure concentration	-49.79 ±1.34

FBCC-EP1111	<i>Najas minor</i> All.	whole	03/27/2020	70% ethanol	reduced pressure concentration	-38.20±0.37
FBCC-EP1112	<i>Myriophyllum spicatum</i> L.	whole	03/27/2020	70% ethanol	reduced pressure concentration	-44.21±1.12
FBCC-EP1113	<i>Typha laxmannii</i> Lepech.	whole	03/27/2020	70% ethanol	reduced pressure concentration	-3.86±1.34
FBCC-EP1114	<i>Alisma canaliculatum</i> A. Braun & C. D. Bouché	whole	03/27/2020	70% ethanol	reduced pressure concentration	-42.06±1.62
FBCC-EP1115	<i>Cyperus difformis</i> L.	whole	04/03/2020	water	reduced pressure concentration	-48.93±4.29
FBCC-EP1116	<i>Eleocharis mamillata</i> var. <i>cyclocarpa</i> Kitag.	whole	04/03/2020	water	reduced pressure concentration	-37.77±3.58