

Network pharmacological study of ethyl acetate fraction of *Ophioglossum vulgatum* Linn. in alopecia treatment

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Research Article

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Abstract

Background

Ophioglossum vulgatum Linn. (*O. vulgatum*) is a herbal medicine of southwest provinces in China, which has been used for treatment of varieties diseases including wound or alopecia since ancient time. However, few of its pharmacological and pharmacochemical knowledge was known.

Methods

The ethyl acetate fraction of *O. vulgatum* (OpvE) was prepared with the reflex extraction and fractional extraction method. And the components in this fraction were identified with UPLC-Q/TOF-MS system and SCIEXOS database. The potential functions and regulatory pathways of these components were screened and predicted with some bioinformatic methods. MTT was engaged to determining the cell proliferation. Spectrophotometric method was carried according to the respond kits menus to identifying the ATP concentration and catalase activity. The transcription of the genes was semi-quantitatively studied by real-time PCR.

Results

The extract ratio of OpvE was $1.67 \pm 0.78\%$ (g/g). 24 Ingredients were identified, of which 12 compounds were found with positive and negative model of UPLC-Q/TOF-MS. 409 Candidate targets were obtained with bioinformatic analysis. And 14 pathways were selected with KEGG analysis ($P < 0.05$) as criterion. Finally, the cell proliferation promoting activity of OpvE (0.98–125 $\mu\text{g/mL}$) were determined with HaCaT cell assays *in vitro*. 250 $\mu\text{g/mL}$ OpvE showed cell toxicity. In addition, the ATP concentration and catalase (CAT) activity were increased with additional OpvE treatment (7.5–15 $\mu\text{g/mL}$ for ATP and 30–60 $\mu\text{g/mL}$ for CAT). Transcription of type I collagen pro- $\alpha 1$ chain (*COL 1A1*) fibronectin-1 (*FN1*), and tumor necrosis factor (*TNF- α*) were also increased in cell assays with 15, 30 and 60 $\mu\text{g/mL}$ OpvE respectively.

Conclusion

OpvE was identified as a hair growth promoting activity herbal, providing a new potential herbal medicine for the treatment of alopecia.

Background

Hair density decrease, hair thin and even hair loss appears to be increasing common in during age [1]. Many factors, such as the aging of hair follicles, reduced hormone secretion, moderated energy metabolism and the insufficient nutrient supplementation, are involved in this process [2]. Hence, the studies of hair follicles ageing process have been increasing in decades, the mechanisms are still less

known [3–5]. The previous researches indicated that the synthesis of type collagen in cell decreases during aging, resulting in incompact collagen network structure of skin, which prevents hair bulbs migrating down to the dermis layer deeply in the anagen phase of hair regeneration process [6–10]. The reported results showed the hair growth-promoting effect of collagen supplement [11].

Ophioglossum vulgatum Linn. (*O. vulgatum*) a genus of some inconspicuous ferns, belongs to Polypodiopsida [12]. It is an important folk's medicine and nutrients supplement in southwest China [13]. The detoxification, blood circulation promotion and fatigue amelioration activities of *O. vulgatum* have been reported, previously [14, 15]. In addition, the recent studies investigated dermatological, hemostatic, antiparasitic [16–18], antiviral [19] and wound healing [20, 21] properties of *O. vulgatum*. A patent mentioned that a *O. vulgatum* containing prescription can attenuate symptoms of respiratory infectious patients [22]. Mao Lingfei and her colleagues demonstrated that ethanol extract of *O. vulgatum* can significantly promote the epidermal growth factor (EGF) expression in the marginal tissue of acetic acid induced gastric ulcer in rats [23]. According to folktale, the oily extract of *O. vulgatum* can relieve skin aging and hair loss. But no scientific researches were engaged to verify this activity. Therefore, in this study we verified our hypothesis through experiments and prospected the biological mechanism.

Methods

Materials and chemicals

O. vulgatum was provided and identified by Ms. Cao Chun, an employee of Novartis Pharmaceuticals Corp. The collection of plant materials was performed in accordance with the relevant guidelines and regulations of *Specification on Good Agriculture and Collection Practices for Medicinal Plants* drafted by China Chamber of Commerce of Medicines & Health Products Importers & Exporters, China. HaCaT (human immortalized keratinocytes) cell line was purchased from the China National Experimental Cell Resource Sharing Platform (Beijing). DMSO (Dimethyl sulfoxide) and other organic reagents were purchased from Huadong Medicinal Technology Co., Ltd (Hangzhou, China). Fetal bovine serum and DMEM (Dulbecco's modified eagle medium) high glucose medium and antibiotics were obtained from Sigma-aldrich (St. Louis, MO, USA); ATP (adenosine triphosphate) quantitative kit and CAT (catalase) activity assay kit were purchased from Nanjing Jiancheng Chemical Industrial Co. Ltd (Nanjing, China); RNA preparation kit, cDNA synthesis kit, PCR reaction system and SYBR GREEN were obtained from TaKaRa Bio Inc (Dalian, China). The primers for *COL1A1*, *FN1*, *TNF- α* , *GAPDH* encoding gene polymerase chain reaction were supplied by Shanghai Shenggong Biotechnology Co. Ltd. (Shanghai, China).

Preparation of *O. vulgatum* extract

The forced air oven dried *O. vulgatum* was powdered and sifted into 40 mesh. 5 g of *O. vulgatum* powder was extracted twice with 150 mL slightly boiling 70% ethanol for 1.5 hours. The ethanol in crude extract was evaporated before the following extraction process. Firstly, the oleic pigment in crude extract could be removed by petroleum ether (1:1 v/v) extraction for several times. Then, the ethyl acetate fraction was

obtained by ethyl acetate (1:1 v/v) extraction for at least 3 times. The ethyl acetate fraction of *O. vulgatum* L. (OpvE) was rotary dried and stored in a refrigerator.

UPLC-Q/TOF-MS analysis

4 μ L 1 mg/mL OpvE methanol solution was loaded into a ACQUITY UPLC BEH C18 (150 $\times$ 2.1 mm, 1.7 μ m) column with an ultra-performance liquid chromatography (UPLC) (Waters, Milford, MA, America) system. The mobile phase was a solution A (acetonitrile containing 0.1% formic acid) mixed in solution B (0.1% formic acid in water) with a certain concentration gradient over time; 0–5 minutes 15%-20% A; 5–32 minutes 20–40% A; 32–37 minutes 40–95% A. The flow rate is 0.3 mL/min.

A time-of-flight mass spectrometry (TOF-MS) (AB Sciex Pte. Ltd., MA, USA) model was performed on mass spectrometry with an electrospray ionisation source (positive modes). The detection condition was: ion source gas 1 (Gas1): 55 psi, ion source gas 2 (Gas2): 55 psi, curtain gas (Cur): 35 psi, ion source temperature (TEM): 600°C, the electrospray voltage (ISVF): 4500/5500 eV; the mass spectrum scan range m/z : 50 ~ 1500 Da, MS scan accumulation time 0.25 s/spectrum, ion accumulation time 0.05 s/spectrum. The IDA-MS/MS with a high sensitivity mode condition was; positive and negative ion mode 60v.

The obtained molecular mass, as well as the secondary spectrum data, were comparatively screened with SCIEXOS, a ingredients database including more than 1500 Chinese herbal medicines. And the identity components were selected for the following analysis.

Target gene of OpvE prediction

The pharmaceutical components in OpvE were obtained by screening in the TCMSP (<http://lsp.nwu.edu.cn/tcmsp.php>) [24] with following characters; oral bioavailability (OB) $\geq$ 35%, half-life (HL) $\geq$ 4 h and drug likeness (DL) $\geq$ 0.13 h. The target genes of the selected components and identified ingredients were predicted by screening in Swiss (<http://www.swisstargetprediction.ch/index.php>) [25]. The annotation of selected genes was unified with UniProt (<https://www.uniprot.org/>) [26] for further analysis. TTD (<http://db.idrblab.net/ttd/>) [27], OMIM (<https://omim.org/>) [28], Genecard (<https://www.genecards.org/>) [29] and CTD (<https://ctdbase.org/>) [30] were screened with the key word; “hair growth”, “alopecia” to obtain disease related targets. The Cytoscape 3.4.1 software was engaged in preparation of ingredients-target and disease-target PPI (protein-protein interaction) network. The ingredients-target-disease CPPI (core PPI) were obtained by merge of the two PPI networks with the following topological parameters; degree centrality (DC) [31], betweenness centrality (BC) [32], closeness centrality (CC) [33], eigenvector centrality (EC) [34], neighborhood centrality (NC) [35] and local average connectivity (LAC) [36]. With this CPPI network, the candidate genes were selected. Finally, the KEGG (Kyoto Encyclopedia of Genes and Genomes) online analysis was employed to conducting functions and biological pathways of the candidate gene, with $P < 0.05$ as the criterion.

Cell proliferation identification

HaCaT cell, 1.0×10^4 cells/well, was grown with DMEM high glucose medium containing 10% fetal bovine serum in a 96-well plate and incubated in a 37°C, 5% CO₂ incubator (Thermo Fisher Scientific, Waltham, MA, USA) over night. The freshly prepared same medium containing OpvE with a successful gradient concentration from 0.98 to 250 µg/mL, was added to growing the cell 14–16 hours, separately. 6 Replicates were prepared for each concentration. The cell concentration was detected with MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) kit according to the instruction.

ATP concentration and CAT enzyme activity measurement

HaCaT cell, 5.0×10^5 cells/mL, was grown in a 24-well plate with the described condition. On the following day, the freshly prepared medium containing 7.5, 15, 30, 60 and 120 µg/mL OpvE was pipetted into the wells for cells treatment respectively, for 14–16 hours. The cells were collected for intracellular ATP content and CAT activity determination, according to the specific kit provided methods. For ATP measurement, the cell precipitant and ATP standard solution was treated with sample solution and specific reagents in ATP measurement kit. The assays were incubated at 37°C for 30 min to catalyzing synthesis of phosphocreatine. After mixed with molybdenum blue reagent, the absorption of each reactive assays was determined with a photometer (Agilent Technologies, Inc., Milford, MA, USA) at 636 nm. The ATP concentration was calculated with respect to the standard solution and the negative control assay. CAT activity could also be measured with a spectrophotometric method. H₂O₂ mixed with ammonium molybdate produces a yellow complex, which could be spectrophotometrically quantified at 405 nm. CAT catalyzes the decomposition of H₂O₂. There for the absorbance change at 405 can be used to determining the activity of CAT.

RNA preparation and real-time PCR

HaCaT cell was grown in 24-well plate and handled with the described methods. And the concentration of OpvE in medium was 15, 30, 60 and 120 µg/mL, respectively. The collected cells were washed with PBS solution. And total RNA was prepared by Trizol-chloroform method [37], The concentration of RNA was identified with a nano-drop spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Waltham, MA, USA) at 260/280 nm. The mRNA was reverse transcribed and used as template for target genes amplification. A 12.5 µL real-time PCR assay contained reverse transcribed template, amplification mixture and specific primer pairs (Table. 1), respectively. The amplifying condition was, activating at 95°C for 3 min, followed by 45 cycles of 2 sec at 95°C, 15 sec at 60°C, and 15 sec at 72°C.

Table 1
Specific primers sequence

Primers	Gene	Sequence
GAPDH-F	Glyceraldehyde 3-phosphate dehydrogenase (<i>GAPDH</i>)	GCA CCG TCA AGG CTG AGA AC
GAPDH-R		TGG TGA AGA CGC CAG TGG A
COL1A1-F	Alpha-1 type I collagen (<i>COL1A1</i>)	TAG GGT CTA GAC ATG TTC AGC TTT G
COL1A1-R		CGT TCT GTA CGC AGG TGA TTG
FN1-F	Fibronectin 1 (<i>FN1</i>)	ACC CAG TCC CGA AGG CAC TA
FN1-R		CCT GTA CAT CTA AAG GCA TGA AGC A
TNF- α -F	Tumour Necrosis Factor alpha (<i>TNF-α</i>)	CTC TTC TGC CTG CTG CAC TTT G
TNF- α -R		ATG GGC TAC AGG CTT GTC ACT C

Statistical analysis

All data in this research are expressed as means $\pm$ standard deviation (SD). And the difference between two groups was statistically analyzed by unpaired t-test using GraphPad Prism 8.3.0 software (GraphPad Software Inc., La Jolla, CA, USA). A *P*-value < 0.05 was considered significantly different.

Results

OpvE preparation and ingredients identification

84 $\pm$ 39 mg OpvE was extracted from 5 grams of *O. vulgatum* powder. The extractive efficiency is 1.67 $\pm$ 0.78% (g/g). The chemical components in OpvE were identified with a UPLC-Q/TOF-MS system. The signal of positive and negative ion mode was shown in Fig. 1A and B respectively. 14 and 22 compounds in the positive and negative ion mode were identified by comparing with the standard primary accurate mass, isotope distribution ratio and MS/MS of the compound TCM MS/MS Library in the TCM secondary database of the SCIEXOS. In which, 12 compounds were found in both positive and negative ion mode, namely: kaempferol 3-O-(6-O-caffeoyl)- β -D-glucosyl-(1 $\rightarrow$ 2)- α -L-rhamnose-7-O- α -L-rhamnoside, 3-O-methylquercetin-7-O- β -D-glucosyl-4'-O- β -D-glucoside, 3-O-methyl quercetin 4'-O- β -D-glucoside, ophioglonin, luteolin, ophioglonol, ophioglonol prenyl ether, methyl linolenate, ortho-dibutyl phthalate, linoleic acid monoglyceride, linoleic acid and palmitic acid. Quercetin and quercetin 3-O-(6-O-caffeoyl)- β -D-glucosyl-(1 $\rightarrow$ 2)- α -L-rhamnose-7-O- α -L-rhamnoside only appeared in the positive ion mode. Additional 10 Compounds, 3,4-dihydroxybenzoic acid, ophioglonin-7-O- β -D-glucopyranoside, ophioglonol-4'-O- β -D-glucoside, 7,10,13-hexadecenoic acid methyl ester, methyl stearate, 11,13-eicosadienoic acid methyl ester,

linoleic acid isopropyl ester, methyl palmitate, methyl oleate and (Z)-hexadecenoic acid methyl ester were only found in negative model.

Bioinformatic analysis and pharmacological activity prediction

Some internet based pharmacological tools and database were engaged to predicting the activity of OpvE. Firstly, the target genes were screened and selected in the DrugBank database and UniProt database. Over 1490 targets were selected for ingredients-target network (Fig. 2A, and Supplementary Material 1 for more details).

The target genes regulated functional cytokine or proteins were merged with the ingredients-target network, which resulted another 5373 nodes and 145617 edges network (Fig. 2B). After analyzed with sequence-based method, the topology-based method with $DC \geq 66$ was engaged for candidate genes selection. In addition, more topology parameters, $DC \geq 107$, $BC \geq 545.910$, $CC \geq 0.503$, $EC \geq 0.016$, $NC \geq 17.648$ and $LAC \geq 15.930$, were designed, with which 409 candidate targets was selected (Fig. 2B).

KEGG analysis was performed on these candidate genes, and 14 pathways were selected with $P < 0.05$ as criterion (Fig. 2C, and Supplementary Material 2–4 showed more geneset enrichment analysis results). The result indicated the most correlative function of OpvE is cell cycle and cell proliferation regulation. This prediction was demonstrated with HaCaT cell assays *in vitro*.

OpvE promoting cell proliferation

The MTT assay is generally used for evaluation of cell survival and (or) proliferation. HaCaT cells were treated with OpvE of concentration from 0.98 to 250 $\mu\text{g/mL}$, respectively, in this study. In comparison with that of Ctrl (control assays), high concentration OpvE (250 $\mu\text{g/mL}$) showed significant cell toxicity activities. On the contrary, OpvE present marked promotional activity of cell proliferation at low concentration $> 125 \mu\text{g/mL}$ (Fig. 3).

OpvE stimulating cell energy metabolism

The principal source of cellular ATP is oxidative phosphorylation. The intracellular of ATP per μg protein respects to cell vitality. It was very interested that 7.5 $\mu\text{g/mL}$ OpvE showed significant highest promoting activity. And the increasing of concentration resulted decrease in activity (Fig. 4A). CAT is one of enzymes involved in reduction of H_2O_2 , which is produced by oxidative phosphorylation. Obviously, high concentration OpvE, 30 $\mu\text{g/mL}$, was required on stimulating activity of CAT (Fig. 4B).

OpvE improving the transcription of COL1A1, FN1 and TNF- α

Type collagen pro- $\alpha 1$ chain (COL1A1) and fibronectin-1 (FN1) are two of cell structural proteins. And tumor necrosis factor (TNF- α) is known as a cell growth regulator. OpvE showed capability on promoting

the transcription of *COL1A1*, *FN1* and *TNF- α* ($P < 0.01$) (Fig. 5), at the concentration range from 15 to 60 $\mu\text{g/mL}$. But it exhibited the contrary activities when the concentration increased to 120 $\mu\text{g/mL}$.

Discussion

Hair is a unique feature of mammals, covering almost the entire body surface, which undertakes a variety of physiological functions, including avoiding or reducing the body from various damages (such as ultraviolet rays, trauma) and regulating body temperature [38]. It has not only a cosmetic effect, but also a sign of body health [38]. Although modern medical methods can effectively improve hair loss in a short period of time, the treatment options available so far are extremely limited [39]. The Chinese nationality has a long history, and has many knowledges for preventing and curing hair loss, which have been passed on through generations. Based on these knowledges, many safe and effective ingredients were discovered and developed with scientific theories and methods, which are benefit for hair growth. *O. vulgatum*, is a kind of fern, which has been used as food and dietary supplement in Yunnan, Guizhou and Sichuan Provinces in China since ancient times [40]. However, there are few studies on the chemical consists and biological activity of *O. vulgatum* in decades. Hence, we did some research to demonstrate and evaluate cell growth promoting activity of it.

In this study, we used reflux extraction and fractional extraction to prepare the extract of *O. vulgatum* L. to obtain ethyl acetate fraction (OpvE). UPLC-Q/TOF-MS was engaged in identifying components in OpvE. 3-O-methyl-quercetin-7-O-diglucose-4'-O-glucoside, linoleic acid, quercetin, *Ophioglossum vulgatum* alcohol, etc. 24 ingredients were identity to TCM database of the SCIEXOS. Based on the prediction of these chemical components by network pharmacology, we speculate that OpvE has the activity of promoting cell proliferation and energy metabolism. We verified the analytic results with some studies, *in vitro*. In HaCaT cell assays, OpvE (0.98 to 250 $\mu\text{g/mL}$) showed promoting cell proliferation activity. And the results indicated that OpvE could increase the intracellular ATP content and CAT activity at a relative low concentration, which were 7.5 and 30 $\mu\text{g/mL}$. At the same time, the transcription of the skeletal proteins COL1A1 and FN1, and the cell signaling factor TNF- α are also stimulated with OpvE treatment (15–60 $\mu\text{g/mL}$).

ATP (adenosine triphosphate) is one of mainly energy-carrying molecule in all living creatures, signaling molecular, as well as basic material of DNA synthesis [41]. Therefore, increased ATP concentration in cell is one of the key signal for cell multiplication. Mitochondria are the organelle where oxidative phosphorylation and ATP synthesis takes place, a process of oxygen free radicals generation [42]. It has been known that free radicals can directly impair function and structure of micro mitochondria. This is a key factor for inducing and accelerating diseases and aging [43]. In particular, increasing the anti-oxidation capability of cell is specially important for fast growing cell [44]. CAT in mitochondria is an essential antioxidant enzyme [45]. According to the results of the current research, OpvE promoted cell growth through improve ATP levels and enhance the activity of CAT enzymes in mitochondria. This can effectively prevent fast growing induced cell damage.

The hair follicle regeneration process is still less known. The previous studies reported that increasing the content of type I collagen in skin cells can not only alleviate skin aging, but also benefit the regeneration of hair follicle stem cells [46]. Collagen type I alpha, COL1A1, is a component of extracellular matrix and is closely related to cell growth, proliferation and differentiation [47]. Fibronectin 1 (FN1) is a kind of fibronectin, which is mainly distributed in connective tissue and the basement membrane [48]. It has the physiological function of promoting the connection between cells and fibrous matrix, and participates in cell adhesion, differentiation, wound healing, blood coagulation and body defense [48]. Our research results show that the extract of *O. vulgatum* promotes the transcription of structural proteins COL1A1 and FN1 and cell proliferation regulator (TNF- α) in cells. The results indicated the potential hair follicle promotional function of OpvE.

Ferns are an important evolutionary marker for land plants on the earth, which occupy an important position in the composition of the flora. There are a lot of medicinal value ferns used by Chinese since ancient time. Such as *Lycopodium annotinum* L., *Selaginella tamariscina*, *Equisetum hyemale* L., *Lygodium japonicum* (Thunb.) Sw., *Davallia mariesii* Moore ex Bak., *Scutellaria barbata* D. Don, *Pyrrosia lingua* (Thunb.) Farwell, *Dryopteris crassirhizoma* Nakai (Rhizoma Dryopteris Crassirhizomae) are listed in the Pharmacopoeia of the People's Republic of China [49]. In comparison of aforementioned ones, *O. vulgatum* is not a popular used herb. It was reported high content of unsaturated fatty acids [50, 51], as well as galactoglycerolipids [52], flavonoids [53, 54] and polysaccharides [55] in *O. vulgatum*. Even though, the pharmacochemical characterization was still rarely known. This limited the bioactivity studies.

Conclusion

In summary, our research demonstrated that the ethyl acetate fraction of *O. vulgatum* has a significant promotional activities of epidermal cell proliferation and energy metabolism. And it can stimulate the expression of *COL1A1* and *FN1*, which indicated the hair growth-promoting effects. We hope our study will give an insight into the research of *O. vulgatum*.

Abbreviations

O. vulgatum

Ophioglossum vulgatum Linn

OpvE

Ethyl acetate fraction of *O. vulgatum*

UPLC

Ultra-performance liquid chromatography

TOF-MS

Time-of-flight mass spectrometry

EGF

Epidermal growth factor

HaCaT
Human immortalized keratinocytes
DMSO
Dimethyl sulfoxide
DMEM
Dulbecco's modified eagle medium
MTT
3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
ATP
Adenosine triphosphate
CAT
Catalase
GAPDH
Glyceraldehyde 3-phosphate dehydrogenase
COL1A1
Alpha-1 type I collagen
FN1
Fibronectin 1
TNF- α
Tumour Necrosis Factor alpha
Cur
Curtain gas
TEM
Ion source temperature
ISVF
Electrospray voltage
OB
Oral bioavailability
HL
Half-life
DL
Drug likeness
DC
Degree centrality
BC
Betweenness centrality
CC
Closeness centrality
EC
Eigenvector centrality

NC
Neighborhood centrality
LAC
Local average connectivity
PPI
protein-protein interaction
CPPI
core PPI
KEGG
Kyoto Encyclopedia of Genes and Genome
SD
Standard deviation.

Declarations

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None.

Authors' contributions

LZ: Conceptualisation, Methodology, Data curation, Writing-Original draft preparation, Visualisation, Investigation, Validation, Formal analysis. YL: Supervision, Methodology, Data curation, Validation, Writing-Reviewing and Editing. HD: Formal analysis, Software, Data curation, Writing-Review and Editing. HL: Investigation, Writing-Reviewing and Editing. BD: Resources, Project administration, Validation, Writing-Reviewing and Editing. The author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

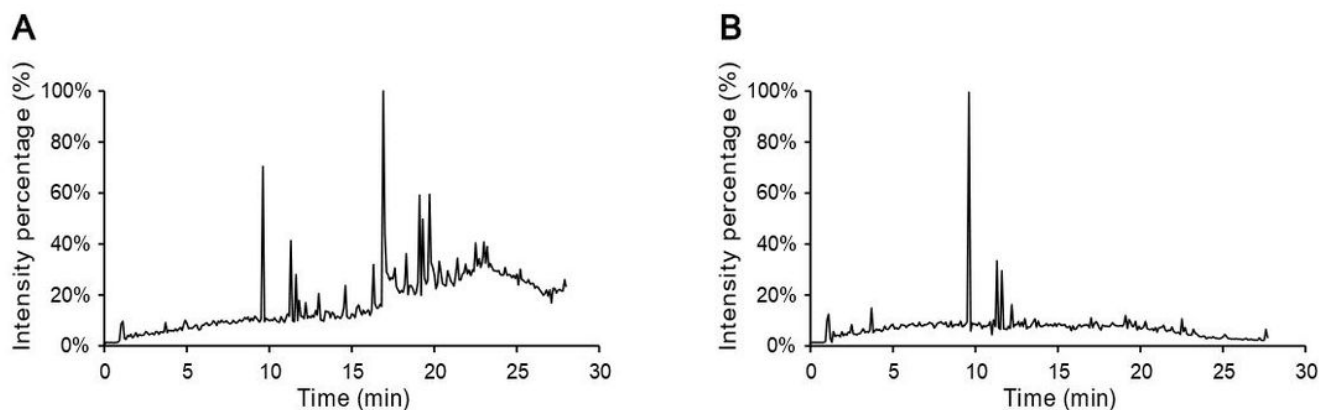


Figure 1

(B) The ingredients-target-disease networks of OpvE. The target genes were marked in yellow. (C) The enriched metabolic pathways with KEGG analysis.

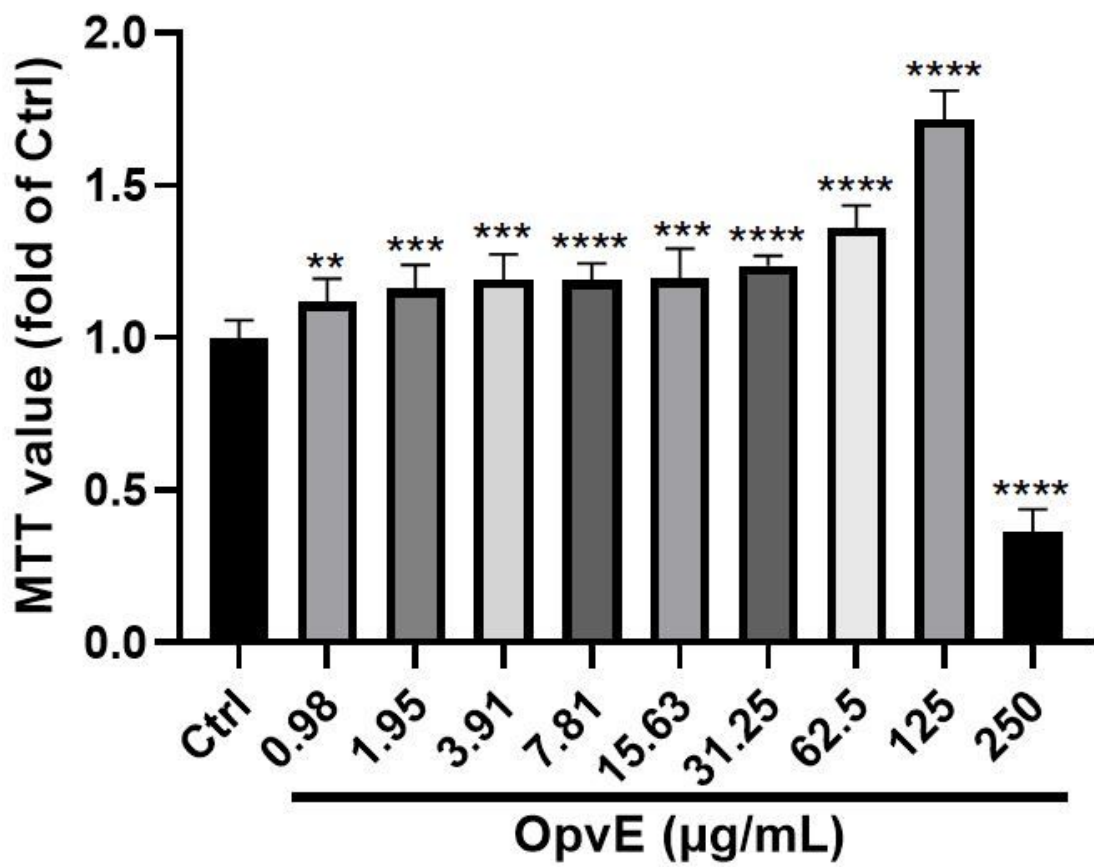


Figure 3

The cell promotional activity of OpvE on HaCaT cells. All the values are denoted as means $\pm$ SD from 8 biological repeats. ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ vs the Ctrl.

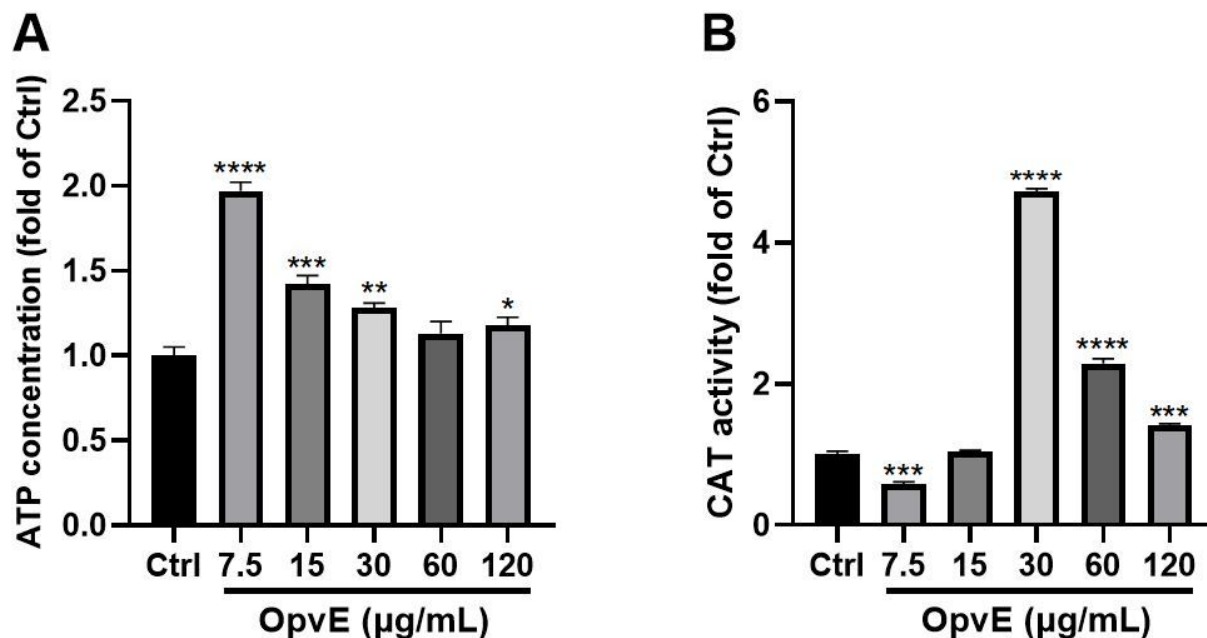


Figure 4

OpvE promoting (A) ATP synthesis and (B) CAT activity in HaCaT cell. All the values are denoted as means $\pm$ SD from 3 biological repeats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ vs the Ctrl.

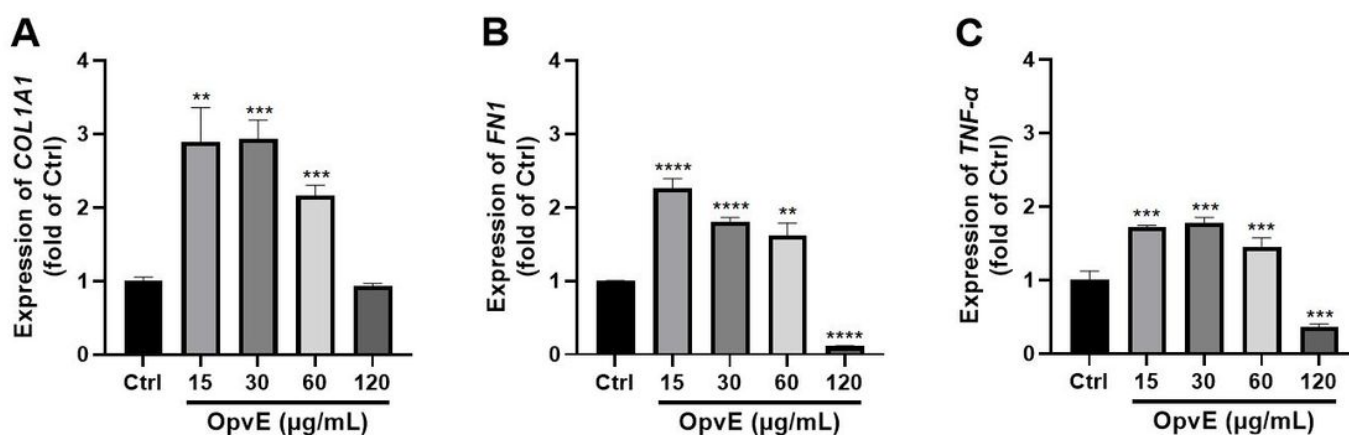


Figure 5

OpvE improving the transcription of (A) *COL1A1*, (B) *FN1* and (C) *TNF-α*. All the values are denoted as means $\pm$ SD from 3 biological repeats. ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ vs the Ctrl.

Supplementary Files

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