Kunzea ericoides (kanuka) leaf extracts show moisturisation, anti-oxidant, and UVB-protection effects in HaCaT cells and anti-melanogenesis effect in B16F10 cells

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Research Article

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Abstract

The present study identified various compounds, such as chlorogenic acid, gallic acid, quercetin, and (E)-Ferulic acid in the kunzea ericoides (kanuka) leaf extract, showing its potential use in maintaining skin health. The influence of kanuka leaf extract upon epidermal cells concerning cytotoxicity and in vitro activities of moisturisation, anti-oxidation, UVB protection, and anti-melanogenesis effects were explored in the study. Kanuka leaf extract demonstrated significant promotion in the proliferation of HaCaT and B16F10 cells. After incubation with kanuka leaf extract, the content of ROS and DPPH in HaCaT was significantly decreased; at the same time, more SOD was produced. Furthermore, hyaluronidase-1 (HYAL-1) and HYAL-4 expressions were inhibited, while the aquaporin 3 (AQP-3) content was significantly increased. Kanuka leaf extract also inhibited the expressions of matrix metalloproteinases-1 (MMP-1) and MMP-14 in UVB radiated-HaCaT cells. In the B16F10 cell line, melanin and tyrosinase production were decreased under the presence of kanuka leaf extract, and the expressions of microphthalmia-associated transcription factor (MITF), tyrosinase-related protein-1 (TYRP-1) and TYRP-2 were also inhibited.

Introduction

Kunzea Ericoides, commonly known as kanuka (Maori) or white tea tree (English), is widely distributed in New Zealand [1]. As previously reported, kanuka products are well-known for their potent medicinal values in anti-oxidant, anti-inflammatory, anti-microbial, anti-tumour, and immunological applications [2]. Studies have identified multiple bioactive compounds in kanuka leaf extract, including chlorogenic acid, quercetin, ferulic acid, hydroxycinnamic acid, catechin, gallic acid, etc. [3]. Quercetin, gallic acid and chlorogenic acid have been proven effective in promoting cellular proliferation and alleviating inflammation and oxidative stress in human keratinocytes and fibroblasts [4-7]. Ferulic acid and catechin have also been applied to scavenge ROS and provide UV protection in epidermal cells [8, 9]. Our previous study about kanuka leaf extract indicates it can promote the proliferation, migration, and collagen synthesis ability of human dermal fibroblasts, showing its potential use in maintaining skin health [10].

The epidermis protects deeper structures by providing a physical barrier to resist damage from external factors such as UV radiation. Optimal skincare ingredients often exhibit capabilities to restore the full functionality of keratinocytes and melanocytes [11]. Therefore, the present study aims to verify the influence of kanuka leaf extract upon in vitro biological activities of epidermal cells involving HaCaT cells (immortalised human keratinocytes) and B16F10 cells (murine melanoma cells).

Methods and Materials

1. Materials and Chemicals

The chromatographic analysis was performed with a high-performance liquid chromatography (HPLC, Thermo Fisher Scientific, Orbitrap Exploris 480) system. Fluorescence images were acquired with a Pathway 435 bioimager (BD Biosciences). UVB radiation was performed using an Analytic Jena UVP

Crosslinker (GER). The absorbance was measured with Multiskan SkyHigh (Thermo Fisher Scientific, A51119500C). HaCaT and B16F10 cells were obtained from Servicebio (STCC11801G-1 and STCC20013G-1).

Reagent information: Kanuka leaf extract was produced by Technoble Co., Ltd. (JAPAN). Dulbecco's Modified Eagle Medium (DMEM, Gibco, C11995500BT), fetal bovine serum (FBS, Sigma-Aldrich, F8318), penicillin/streptomycin antibiotics (Gibco, 15140122)), Cell Counting Kit-8 (CCK-8) assay (Abcam, AB228554), Calcein/PI Cell Viability/Cytotoxicity Assay Kit (Beyotime, C2015), DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay (BOXBIO®, AKAO20M), SOD assay kit (BOXBIO®, AKAO001M), DCFDA/H2DCFDA cellular ROS Assay (Abcam, AB113851), L-Ascorbic acid (MCE, HY-B0166), α-melanocyte stimulating hormone (α-MSH, MCE, HY-P0252), arbutin (MCE, HY-N0192), Hyaluronic acid (HA, MCE, 9004-61-9), Tyrosinase Activity Assay Kit (Solarbio, BC4055), BCA Protein Assay Kit (Pierce™, Thermo Fisher Scientific).

Antibodies information: Aquaporin (AQP) 3 (Thermo Fisher Scientific, PA5-78811), anti-rabbit IgG Alexa Fluor 555 (Invitrogen), matrix metalloproteinase (MMP)-1 (Proteintech, 10371-2-AP), MMP-14 (Proteintech, 29111-1-AP), Hyaluronidase (HYAL)-1 (Proteintech, 66560-1-LG), HYAL-4 (Proteintech, 18139-1-AP), microphthalmia-associated transcription factor (MITF) (Abcam, Ab303530), tyrosinase-related protein (TYRP)-1 (Abcam, Ab235447) and TYRP-2 (Abcam, Ab74073), monoclonal Glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Abcam, Ab8245).

2. Preparation of Kanuka Leaf Extract

Components of kanuka leaf extract were determined by HPLC using a C18 column. The detection wavelength of CPL was set at 254 nm. The working samples of kanuka leaf extract were prepared in 50% methanol at 10 mg/mL, and the flow rate was 1 mL/min. Standard solutions were prepared by diluting the stock solution with 50% methanol to candidate concentrations.

3. Cell Culture

HaCaT and B16F10 cells were cultured at 37 ℃ in a 5% carbon dioxide humidified incubator with the complete culture medium (DMEM supplemented with 10% FBS and 1% penicillin/streptomycin antibiotics).

4. Cytotoxicity Test and Optimal Concentration Screening of Kanuka Leaf Extract

The extract was dissolved in the complete culture medium to reach the candidate concentrations of 0.1%, 0.5%, 1%, 2.5%, and 5% (volume/volume, v/v)[12]. Cell viability of HaCaT and B16F10 cells was assessed using CCK-8 assay. After incubation with or without kanuka leaf extract for 48 h, ten μL of CCK-8 solution was added to each well. Then, the absorbance was measured on a microplate reader at a wavelength of 450 nm at 24, 48, and 72 h, respectively. Furthermore, the Calcein/PI Cell Viability/Cytotoxicity Assay Kit was applied following the manufacturer's protocol to confirm the optimal

concentration of kanuka leaf extract on HaCaT cells. The mean gray value PI staining (dead cell) was measured using Image J software and performed in triplicate.

5. In Vitro Wound Healing/Migration Assay

Kanuka leaf extract was added into the cell culture medium 48 hours before the scratch. A sterile 10 μL plastic pipette tip was used to create a wound in each well. The average wound area at different times (0, 24 or 48 h) was recorded and evaluated using Image J software. The cellular migration rate was decided by the following equation: migration rate = (1 – wound area / primary wound area) × 100%.

6. DPPH Assay

The scavenging effect of kanuka leaf extract was assessed using the DPPH assay. The extract was mixed with 250 mM of DPPH in a dose-dependent manner for 25 min at room temperature, and the absorbance was measured at 517 nm. The clearance rate was calculated following the manufacturer's protocol.

7. SOD Activity Analysis

The Superoxide Dismutase (SOD) activity was measured following the manufacturer's protocol. Extracting solution was added to the centrifuged cell sample at 1: (500 × cell count). After centrifuging for 10 min (4 ℃, 8000g), working solutions were mixed with the lysate solution (90 μL) by the order. The reaction mixture was incubated at 37 ℃ for 20 min, and the absorbance was then observed at 560 nm using a spectrophotometer.

8. Detection of ROS

Reactive Oxygen Species (ROS) was detected following the manufacturer's protocol. HaCaT cells were treated with or without kanuka leaf extract for 48 h, then were collected, washed, and incubated with 20 μM (DCFHA) at 37 °C in a dark condition for 45 min. The absorbance was measured immediately at 515 nm emission.

9. Immunofluorescence Cytochemistry (ICC)

Cells were fixed in 4% paraformaldehyde for 20 min and treated with Triton X in PBS for 30 min. Subsequently, cells were blocked and stained in the blocking buffer with primary antibodies at 4 ℃ for overnight. Then, they were washed and stained with secondary antibodies and DAPI in the blocking buffer for three hours at 4 ℃, protected from light. Primary AQP 3 polyclonal antibody was used at 0.5 mg/mL, and an anti-rabbit IgG Alexa Fluor 555 was used as the secondary antibody. Fluorescence results were observed and measured with Image J software.

10. UVB Protection Properties

The UVB protection experiment was performed following methods introduced by previous reports [13]. HaCaT cells were incubated in the presence or absence of kanuka leaf extract for 24 hours. Groups of blank control, negative control, positive control (L-Ascorbic acid, 100 μg/mL), and kanuka leaf extract were maintained. The cells from the last three groups were irradiated with UVB (30 mJ/ cm^2) and then incubated for another 24 hours. The viability of cells was measured using CCK-8 assay afterwards, and the absorbance was measured on a microplate reader at a wavelength of 450 nm.

11. Western Blotting Assay

Cultured cells were seeded in petri dishes and lysed in ice-cold RIPA buffer for 30 min on the ice after washing with PBS. Protein concentrations were determined by the BCA Protein Assay Kit. Proteins were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electroblotted in polyvinylidene difluoride (PVDF) membranes. The membranes were incubated overnight at 4 °C with primary antibodies. Primary antibodies: MMP-1, MMP-14, HYAL-1, HYAL-4 for HaCaT cells, and MITF, TYRP-1 and TYRP-2 for B16F10 cells. After washing with Tris Buffered Saline Tween (TBST), the membranes were incubated with the relevant HRP-conjugated secondary antibody for two hours at room temperature. Subsequently, the blots were developed using an enhanced chemiluminescence (ECL) kit. A monoclonal GAPDH antibody was used as an internal control. The experiment was performed in triplicate. Quantitative analyses were performed with Image J software, and the target protein results in each group were normalised with the corresponding internal control value.

12. Intracellular Melanin Production and Tyrosinase Activity Assay

B16F10 cells were incubated with or without kanuka leaf extract in the presence of 100 nM α-MSH for 24 h, and 100 μg/mL arbutin was applied as the positive control [14, 15]. The medium was incubated for 48 h, and the absorbance was measured on a microplate reader detected at 475 nm [16]. The cells were harvested in e-tubes and washed with cold PBS twice. After that, lysis buffer was used with 20 μL for each tube. After centrifuging at 12,000 rpm for 5 min, the pellet of each tube was collected and dissolved in 10% DMSO of 1 M NaOH solution at 50℃ for 10 min. The absorbance was checked at 405 nm using a microplate reader.

A Tyrosinase Activity Assay Kit was used to evaluate tyrosinase activity. B16F10 cells (5×10^7) were centrifuged and lysed in 1mL extract, and the supernatants were collected after further centrifugation (10 min at 12,000 g, 4℃). Subsequently, 20 μL cell lysate and 180 μL reagent I was added to the 96-well plates. The absorbance was immediately measured at 475 nm using a spectrophotometer and recorded as A1. Then, the plates were incubated at 37 ℃ for 3 min, and absorbance was measured again at the same condition and recorded as A2. The tyrosinase activity was estimated based on the manufacturer's protocol. One tyrosinase unit is defined as the amount of the enzyme for 1000 cells to catalyse and produce one nmol dopachrome in one minute.

13. Statistical Analyses

Data were expressed as the mean ± standard derivation (± s). All continuous variables have gone through the Kolmogorov-Smirnov Test. Comparisons between treatment groups were analysed using the independent sample t-test followed by Tukey's multiple range test using the Statistical Package for Social Sciences version 24 (SPSS Inc, Chicago, Illinois). Two-tailed P values < 0.05 were considered significant. Each experiment was performed in triplicate.

Results

1. HPLC Analysis

The HPLC analysis of the kanuka leaf extract revealed multiple peaks, which matched standards of chlorogenic acid, gallic acid, quercetin, and (E)-Ferulic acid (Fig.1a). The contents of each compound were not further investigated in the study.

2. Cytotoxicity Test and Optimal Concentration Screening

In HaCaT cells, the concentration of 0.5% (v/v) demonstrated the highest cell viability at each time point (24 h, 48 h or 72 h). Notably, the concentration of 0.1% did not show this proliferation promotion effect; at the same time, higher concentration (> 2.5%) groups demonstrated significant cytotoxicity (Fig.1b). Similar results were observed in the calcein/PI cell viability/cytotoxicity assay after applying kanuka leaf extract. The fluorescence intensity of live cells showed that the HaCaT cell proliferation was significantly inhibited in the 2.5% and 5% groups, implying dose-dependent cytotoxicity of kanuka leaf extract (Fig.1c). As for B16F10, the concentration of 0.5% demonstrated the highest cell viability at each time point (24 h, 48 h or 72 h) (Fig.1d). The concentration of 0.5% was proved to be superior in maintaining cell viabilities in both HaCaT and B16F10 cell lines.

3. Migration Promotion

To further confirm the optimal concentration, comparisons were made between different groups about the migration influence of kanuka leaf extract. As presented in Fig.2a, kanuka leaf extract demonstrated noticeable cell proliferation and migration promotion. Specifically, at both 24 h and 48 h, the 0.5% group showed the highest cell migration rates compared to other groups. The difference was statistically significant compared to the blank control (BC) group ($p < 0.01$, Fig.2b). Therefore, the optimal concentration was determined as 0.5% for subsequent assays to obtain the maximal effect of kanuka leaf extract.

4. Anti-oxidative Effects

The epidermal and dermal cells are continuously exposed to physiological levels of ROS originating from cellular metabolic activities. UV radiation also generates ROS that can contribute to structural damage to the skin. Accumulation of oxidative damage is one of the critical mechanisms of ageing [17, 18]. The odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from anti-oxidants to the corresponding hydrazine. Compared to the positive control group (L-ascorbic acid, 100 μg/mL), kanuka

leaf extract showed a higher level of DPPH scavenging ability ($p < 0.05$, Fig.2c). SODs form the front line of defence against reactive oxygen species (ROS)-mediated injury [19]. In the SOD activity analysis, kanuka leaf extract showed a higher level of SOD activity compared to L-ascorbic acid ($p < 0.05$), both of which were statistically higher than BC ($p < 0.001$, Fig.2d). In the cellular ROS analysis, kanuka leaf extract exhibited more substantial ROS inhibitory effects than L-ascorbic acid ($p < 0.01$, Fig.2e), further validating the strong anti-oxidative efficacy of kanuka leaf extract.

5. Moisturisation Effects

HA is an essential component of the extracellular matrix (ECM) in the skin, which helps to inhibit water loss during skin ageing. HA (100 μg/mL) was set as the positive control in the moisturising capability analysis [20]. AQP3, a significant membrane protein in keratinocytes, was compared among groups to validate moisturisation effects. ICC results showed that the kanuka leaf extract group exhibited a more intense fluorescence signal compared to BC ($p < 0.01$, Fig.3a). The mean gray values of the HA group were statistically lower than the kanuka leaf extract group ($p < 0.05$, Fig.3b).

HYAL plays a crucial role in HA degradation, and high HYAL expression often leads to HA insufficiency in the skin [21]. In western blotting assays, the internal control GAPDH was comparable among the three groups (Fig.3c). Based on the blots value, HYAL-1 and HYAL-4 expressions in HaCaT cells were significantly prohibited under the existence of kanuka leaf extract compared to BC ($p < 0.01$, Fig.3d and 3e). At the same time, expressions of HYAL-1 and HYAL-4 in the HA group were lower than kanuka leaf extract ($p < 0.01$, Fig.3d and 3e).

6. UVB Protection Effects

In the preliminary experiment, while evident growth inhibition was observed in all groups after UVB exposure, the cell viability of HaCaT cells significantly increased under the presence of kanuka leaf extract (Fig.4a). Ascorbic acid is a potent antioxidant that neutralises oxidative stress in electron donation/transfer. It demonstrates an excellent ability for UVB-induced ROS scavenging when applied topically [22]. An additional comparison between L-ascorbic acid and kanuka leaf extract was presented in Fig.4b. Kanuka leaf extract effectively alleviated damages from UVB exposure and exhibited superior UVB-protection efficacy than L-ascorbic acid ($p < 0.05$, Fig.4b).

In the present study, the production of MMP-1 and MMP-14 significantly increased after UVB radiation ($p < 0.001$, Fig.4c-4e). However, the effect was significantly attenuated by kanuka leaf extract ($p < 0.01$, Fig.4c-4e).

7. Inhibitory Effect of Melanogenesis

Arbutin, a melanin synthesis inhibitor, showed a profound anti-melanogenesis effect in B16F10[21]. In the present study, the melanin production in B16F10 was significantly increased after α-MSH stimulation ($p < 0.001$, Fig.5a). Arbutin and kanuka leaf extract inhibited the production of melanin contents in B16F10 cells ($p < 0.01$, Fig.5a). Tyrosinase is a rate-limiting enzyme in the melanin biosynthetic pathway.

The expression of tyrosinase in B16F10 was significantly increased after α-MSH stimulation ($p < 0.001$, Fig.5b). Kanuka leaf extract inhibited tyrosinase expression activated by α-MSH ($p < 0.01$, Fig.5b). It showed a superior inhibitory effect than arbutin ($p < 0.05$, Fig.5b).

One of the significant melanogenesis regulators is MITF, which activates tyrosinase expression [15]. Continuously, as presented in the western blotting results, the expression of MITF, TYRP-1 and TYRP-2 in B16F10 cells was increased after incubation with α-MSH (Fig.5c-5f). However, the expressions of these genes were inhibited in varying degrees in the presence of kanuka leaf extract (Fig.5c-5f). The quantitative results of these blots showed that kanuka leaf extract significantly inhibited the expression of MITF, TYRP-1 and TYRP-2 ($p < 0.01$, Fig.5d-5f). In addition, further comparison showed that kanuka leaf extract was comparable to arbutin in inhibiting MITF, TYRP-1 and TYRP-2 expression, offering a promising effect in preventing the melanogenesis process (Fig.5d-5f) [15].

Discussion

As illustrated before, skin ageing is a complex process resulting from intrinsic and extrinsic factors [23]. Specifically, aged skin is often characterised by loss of elasticity, coarse wrinkling, rough texture, irregular pigmentation, and dryness [24]. Keratinocytes, the primary cell type in the epidermis (approximately 90% of all), contribute to resisting dryness by retaining sufficient moisturisation [25]. Melanocytes, the melanin-producing cells, are another critical cell type in the epidermis, which determines the colour of the skin and provides crucial protection against UV radiation. The epidermal-melanin unit is composed of a single melanocyte and neighbouring keratinocytes. Keratinocytes also modulate melanocyte function and melanogenesis. Affected by UV radiation, oxidative stress, and dehydration in the skin are among the most visible signs of ageing. Hence, restoring the full functionalities of keratinocytes and melanocytes in daily skincare is essential.

Kanuka products, especially essential oil and leaf extract, have been confirmed effective in anti-microbial, anti-oxidative, anti-inflammatory, and sedative activities [26-29]. Developed technologies have been applied to clarify components in kanuka leaf extracts and thereby illustrate underlying mechanisms for its current application [30]. In accordance with previous research, HPLC assay results in the present study demonstrated that chlorogenic acid, gallic acid, quercetin, and (E)-ferulic acid were the most abundant bioactive compounds in kanuka leaf extract [3, 12]. These compounds were known for their extraordinary anti-oxidative, anti-cancer, anti-inflammatory, and anti-diabetic effects. For instance, chlorogenic acid has been proven to promote wound healing through remarkable anti-inflammatory effects in vitro and in vivo [5]. Quercetin has been applied to prevent UV-induced photoaging in humans targeting JAK2 and PKCdelta pathways, and it also shows a promising effect in promoting hair regeneration [4, 7]. Gallic acid and (E)-ferulic acid have also been used as antioxidants to prevent photoaging in human skin [6, 8, 31, 32]. Our previous study about kanuka leaf extract indicates it can promote the proliferation, migration, and collagen synthesis ability of human dermal fibroblasts. It might promote wound healing in rats by enhancing the function of dermal fibroblasts [10]. These previous findings have provided the potential to apply kanuka leaf extract as a skincare ingredient.

AQP3 is a vital transmembrane protein expressed in keratinocytes, which can transport water and glycerol into cells [33]. The present study proved that kanuka leaf extracts might improve dehydration in the skin by increasing the expression of AQP3 and water content of the epidermal layer. Skin HA is inclined to decrease with age, and this reduction might contribute to increased wrinkles, reduced elasticity, and declined moisture content in aged skin. As reported, HA is synthesised by hyaluronic acid synthase (HAS) and degraded by HYAL. In the present study, kanuka leaf extract significantly suppressed the expressions of HYAL-1 and HYAL-4, further inhibiting HA degradation. Though the underlying mechanisms were not further investigated in the study, the HAS genes, which can accumulate intermediate-sized HA in keratinocytes, are believed to be involved in the process [34].

ROS is produced in the normal process of cellular oxidative metabolism and is considered the leading cause of intrinsic ageing [35]. Similarly, photoageing is also accountable for ROS production and accumulation, leading to extrinsic ageing [18, 36]. As the most critical component of the ECM, collagen is essential for preventing skin damage from UV radiation and maintaining the moisture balance to prevent wrinkles and preserve the elasticity of the skin [37]. However, excessive ROS accumulation may lead to abnormal collagen production and synthesis. ROS significantly activates the synthesis of several collagen-degrading enzymes, such as matrix metalloproteinases (MMPs), which ultimately promote skin ageing [35, 38]. In the present study, kanuka leaf extract exhibited a significant ROS-inhibiting effect and DPPH scavenging ability [3]. Continuously, the increased expression of MMP-1 or MMP-14 in HaCaT cells after UVB exposure was attenuated under the existence of kanuka leaf extract. Previous studies have also confirmed that increased MMP-1 expression often facilitates the decomposition of type I collagen, the dominant collagen type in ECM. Based on this, it is conceivable that kanuka leaf extract may attenuate skin ageing by maintaining balanced collagen synthesis and decomposition.

Hyperpigmentation is another significant manifestation of skin ageing, often resulting from the UV-induced accumulation of melanin pigments in the skin [39]. UV radiation stimulates keratinocytes to release excessive α-MSH, and the latter upregulates the expression of MITF in melanocytes [13, 40, 41]. As one of the most crucial melanogenesis regulators, MITF activates melanogenesis-related genes of tyrosinase and tyrosinase-related proteins (TYRPs) [42]. As a result, more melanin was produced after UV exposure, leading to abnormal pigmentation in the skin. The reduced expression of MITF, TYRP1 and TYRP2 in the study demonstrated that kanuka leaf extract could inhibit melanogenesis-related genes, therefore reducing melanin production during the process of skin ageing. The study did not investigate underlying mechanisms, but the results confirmed kanuka leaf extract as an effective agent in inhibiting melanogenesis.

Conclusions

In the present study, bioactive compounds like chlorogenic acid, gallic acid, quercetin, and (E)-Ferulic acid were identified as the main components in the kanuka leaf extract, and these compounds are known as effective anti-oxidants which can attenuate oxidative stress in the process of skin ageing. The cytotoxicity, moisturisation, anti-oxidation, UVB-protection, and anti-melanogenesis effects of kanuka

leaf extract were investigated by observing the in vitro activities of HaCaT and B16F10 cells. The results confirmed that kanuka leaf extract can potentially delay skin ageing. Further research, both in vitro and in vivo, is needed to illustrate the underlying mechanisms of these effects.

Statements & Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

Xuefeng He, Peishan Li and Weijian Tang conceived and designed the study; Xuefeng He and Shixin Zhao performed the experiment and drafted the manuscript; Peishan Li and Hengdeng Liu analysed the data; Julin Xie and Jinming Tang revised the manuscript. All authors read and approved the final manuscript.

Ethics approval

Not applicable.

Data availability statement

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

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Figures

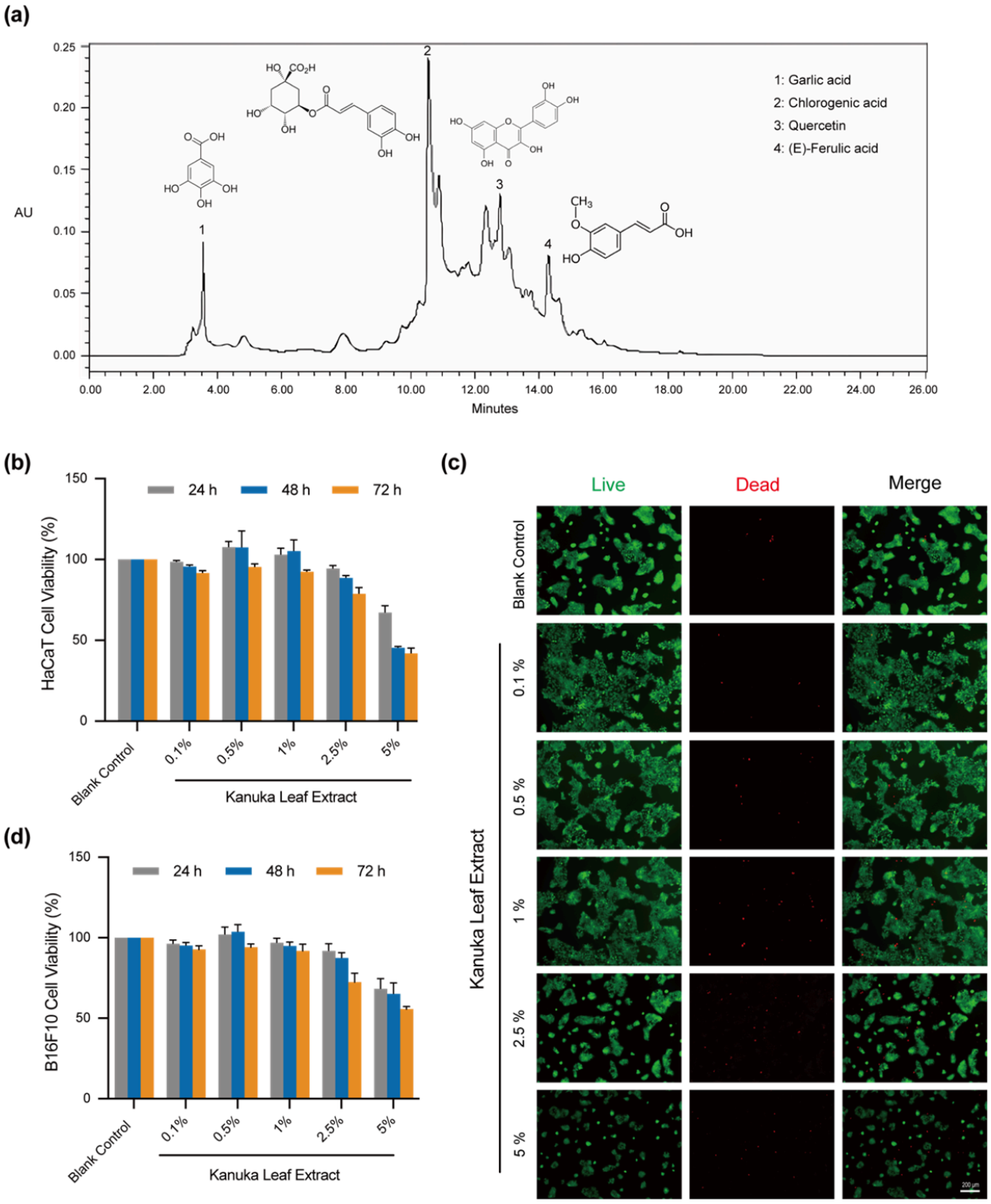

Figure 1

HPLC assay of kanuka leaf extract and optimal concentration screening of kanuka leaf extract in HaCaT and B16F10 cells (a) Kanuka leaf extract and its active components were determined by HPLC assay. (b) CCK-8 assay results showed HaCaT cell viability after incubation with different concentrations of Kanuka leaf extract. (c) The optimal concentration of kanuka leaf extract was further confirmed with a Calcein/PI Cell Viability/Cytotoxicity Assay Kit. The live cells were stained with green fluorescence, and the dead

cells with red fluorescence. Scale bar = 200 μm. (d): CCK-8 assay results showed B16F10 cell viability after incubation with different concentrations of kanuka leaf extract. HPLC:high-performanceliquid chromatography.

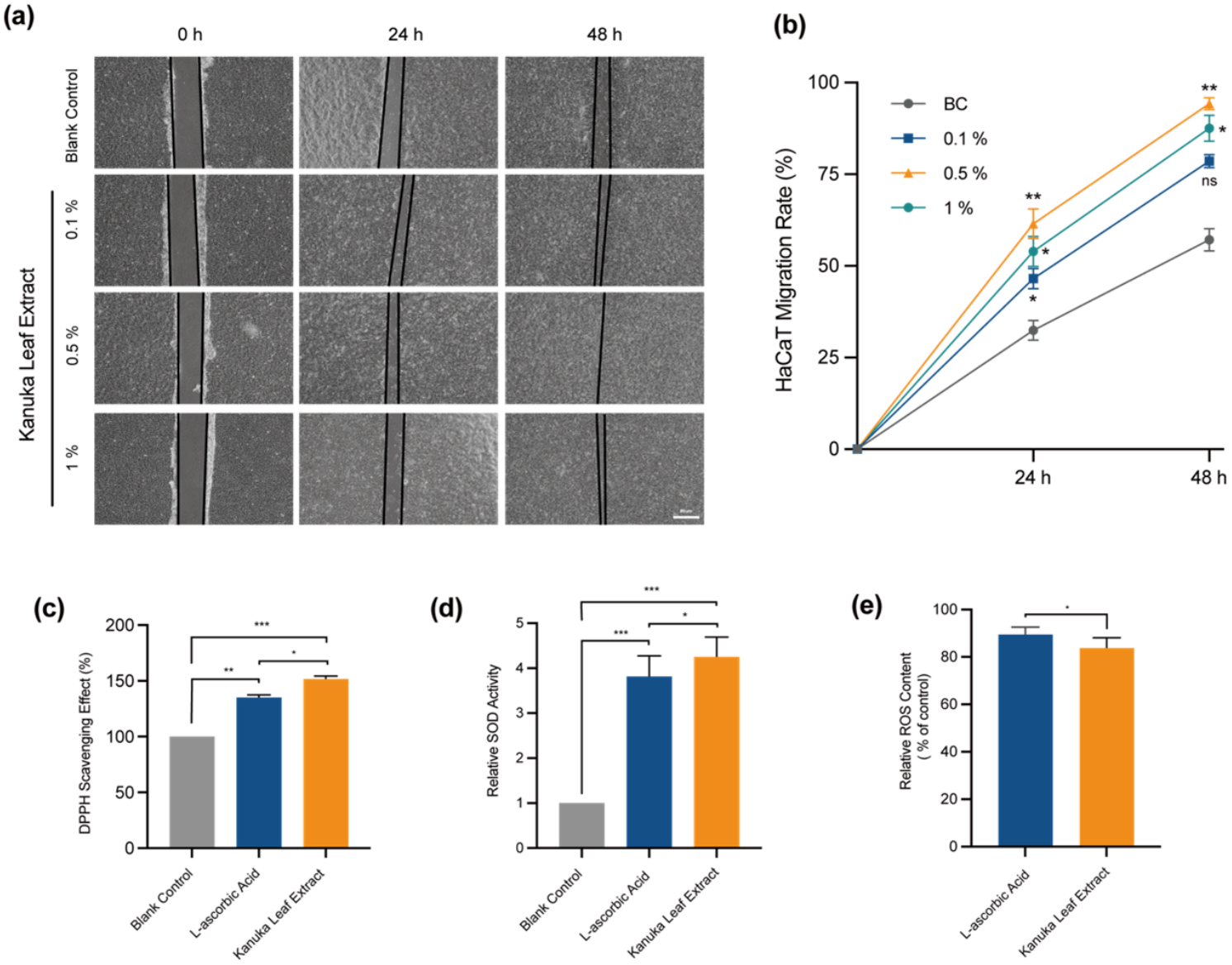

Figure 2

Migration promotion and anti-oxidative effects of kanuka leaf extract in HaCaT cells (a) Kanuka leaf extract promoted migration activities of HaCaT cells. Scale bar = 80 μm. (b) Cell migration rates were presented in percentage compared to the primary wound area. The migration rate in each group at each time point was separately compared with the blank control. L-ascorbic acid (100 μg/mL) was determined as the positive control in the anti-oxidative analysis. (c): The results of the DPPH scavenging effect were presented in relative percentages compared to the blank control group. (d) The SOD inhibition rate was presented in relative rate compared to blank control. (e) ROS inhibition ability was measured based on ROS OD values, and the results were normalised with the value in the blank control group. DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase. Data represent means ± standard deviation. ***: P < .001, **: P < .01, *: P < .05, ns: not significant.

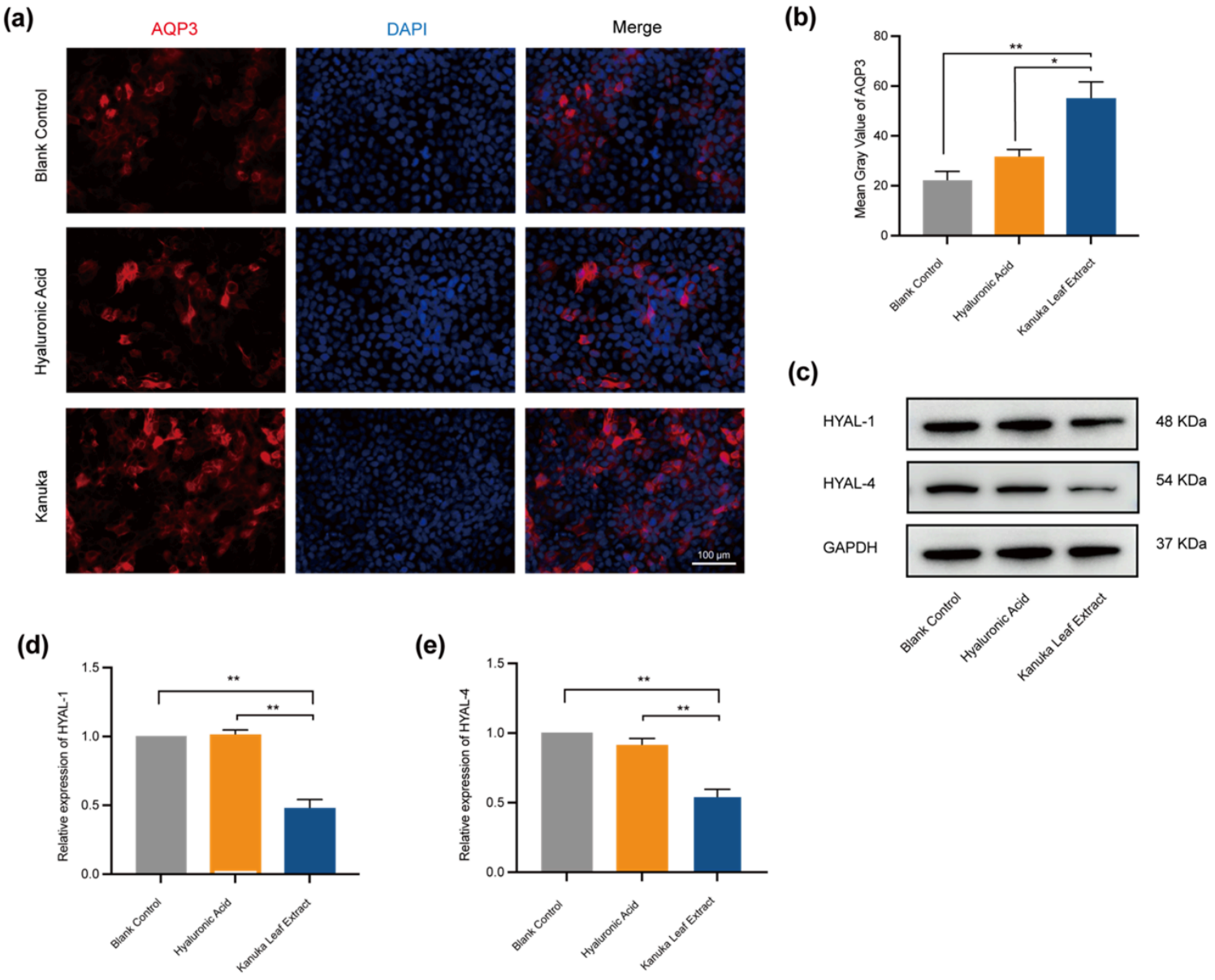

Figure 3

Moisturisation Effects of kanuka leaf extract in HaCaT cells. Hyaluronic acid (100 μg/mL) was determined as the positive control in the moisturising capability analysis. (a) The AQP3 contents were demonstrated by fluorescence intensity in ICC analysis. Scale bar = 100 μm. (b) Mean gray values of AQP3 were measured and compared among groups. (c) The expression of HYAL-1 and HYAL-4 in HaCaT cells was measured after treatment, and GAPDH was determined as the internal control. (d-e): The blots of HYAL-1 and HYAL-4 were measured in Image J software and normalised to GAPDH expression. Then, all results were presented in relative value compared to the blank control. HA: hyaluronic acid; AQP3: aquaporin 3; ICC: immunofluorescence cytochemistry; HYAL-1: hyaluronidase-1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase. Data represent means ± standard deviation. **: P < .01, *: P < .05.

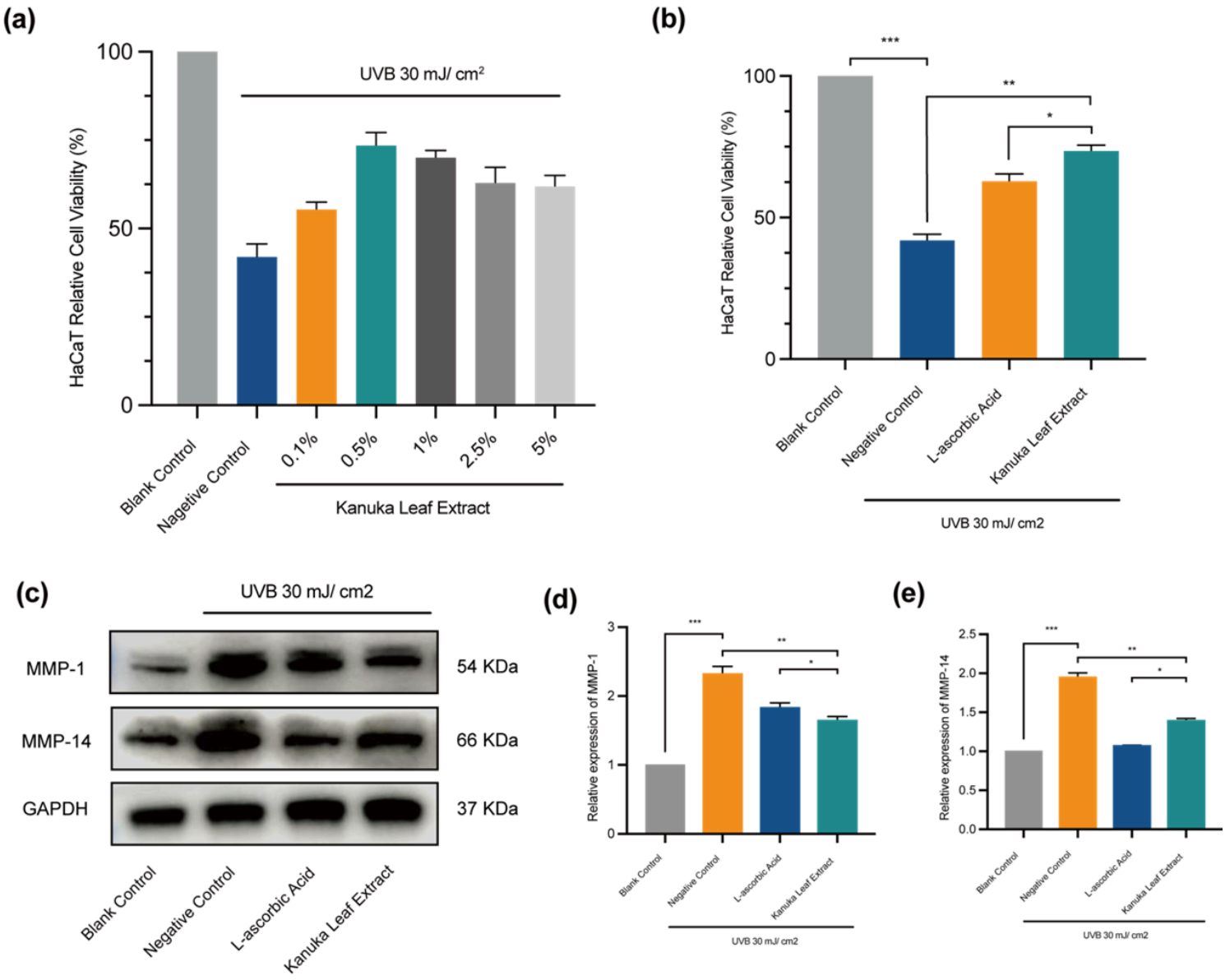

Figure 4

UVB protection effects of kanuka leaf extract in HaCaT cells (a) CCK-8 assay results showed that cell viability of HaCaT significantly increased under the presence of kanuka leaf extract after UVB radiation (30 mJ/ cm^2). (b) Groups of negative control, positive control (L-Ascorbic acid, 100 μg/mL) and kanuka leaf extract (0.5%) were irradiated with UVB (30 mJ/ cm^2) and incubated for another 24 h. CCK-8 assay was conducted to detect cell viability. (c) The expression of MMP-1 and MMP-14 in HaCaT cells was demonstrated with immunoblot after treatment, and GAPDH was determined as the internal control. (d-e): The blots of MMP-1 and MMP-14 were measured with ImageJ software and normalised to GAPDH expression. Then, all the results were presented in relative value compared to the blank control. MMP-1: matrix metalloproteinases-1. Data represent means ± standard deviation. ***: P < .001, **: P < .01, *: P < .05.

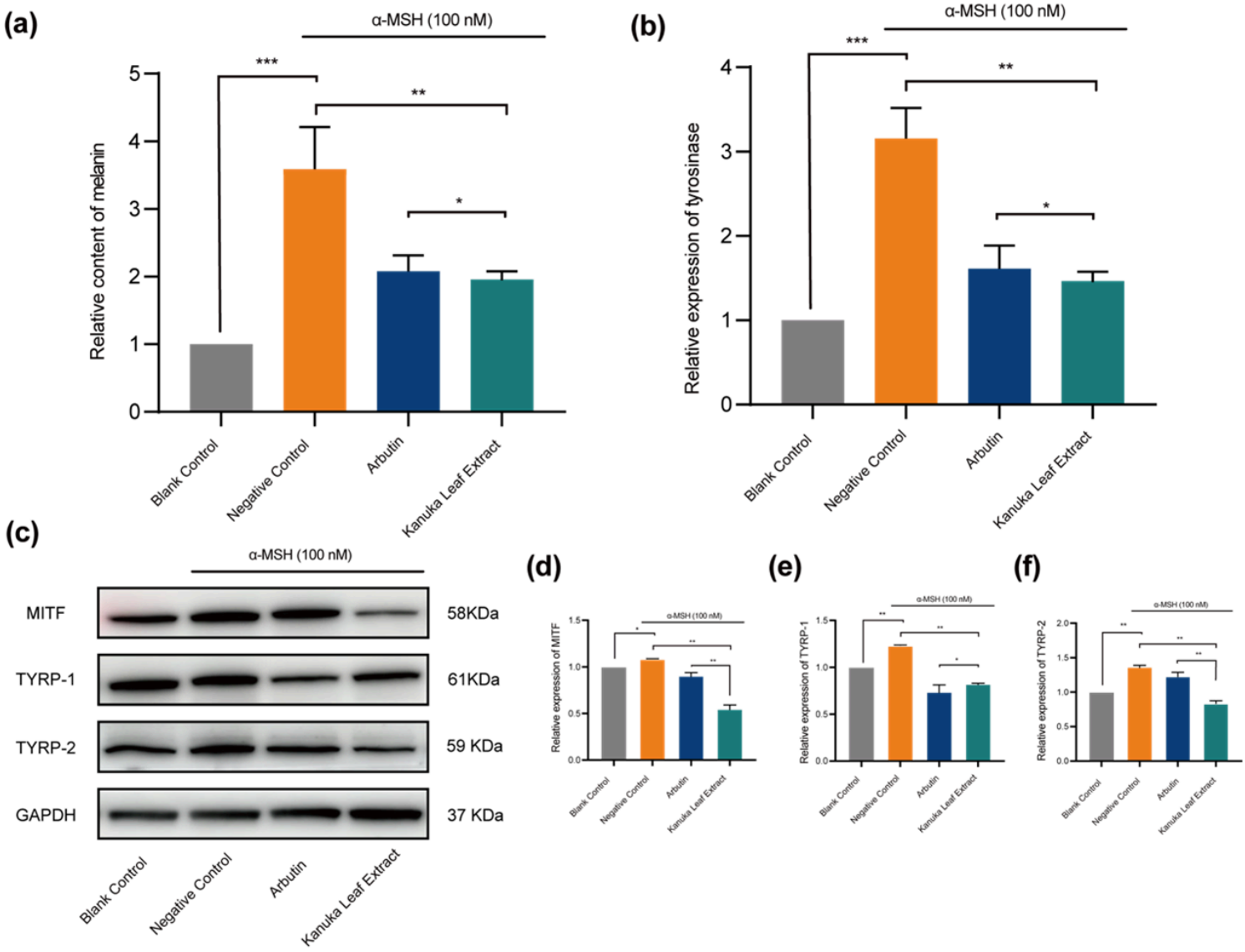

Figure 5

Anti-melanogenesis effect of kanuka leaf extract in B16F10 cells. α-MSH (100 nM) was applied to stimulate melanin production in B16F10 cells, and arbutin was maintained as the positive control. (a) Melanin content was presented in relative value compared to the blank control. (b) The tyrosinase activity was presented in relative value compared to the blank control. (c): MITF, TYRP-1 and TYRP-2 expressions were measured and compared, and GAPDH was determined as the internal control. (d-f): The MITF, TYRP-1, and TYRP-2 blots were measured with ImageJ software and normalised to GAPDH expression. Then, all the results were presented in relative value compared to the blank control. MITF: microphthalmia-associated transcription factor, TYRP-1: tyrosinase-related protein-1. Data represent means ± standard deviation. ***: P < .001, **: P < .01, *: P < .05.

Supplementary Files

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