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Anti-aging of phenolic-rich *Acanthus ebracteatus* Vahl. extracts

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Abstract

Background Sea holly has long been used in traditional Thai medicine for longevity, skin inflammation, skin dullness and as a treatment for itchy skin. The white flower cultivar *Acanthus ebracteatus* Vahl. has been documented to perform better remedies than the purple flower *A. ilicifolius*. Nonetheless, preparation of the phenolic-rich extract of *A. ebracteatus*, including safety and cellular assessments relevant for inflammation and aging treatment is sparingly presented. In this study, the extracts of *A. ebracteatus* were prepared and comparatively quantified on total phenolic contents. In vitro activities by means of ABTS, DPPH, FRAP and mushroom tyrosinase inhibitory assays were undertaken. The extracts were UPLC analyzed and examined on cellular safety and activities.

Results The phenolic-rich extracts of *A. ebracteatus* were prepared and standardized in verbascoside. The extracts were noted to have in vitro antioxidant and anti-tyrosinase activities. Cellular activities, co-cultures and ex vivo human skin appointed for cutaneous aging treatment, i.e., photoaging, are promising including antioxidant, anti-melanogenesis, anti-inflammatory (IL-6 and IL-8), anti-MMP and collagen production stimulating as well as anti-senescence activities.

Conclusions Sea holly is highlighted as a potential source for beneficial cutaneous phenolics, especially verbascoside. The extracts were proven to be safe and efficient for cutaneous aging treatment indicated by their antioxidant, anti-melanogenesis, collagen stimulating, anti-inflame and anti-senescence activities. The plant extracts are therefore appointed for use as innovative agents for anti-aging purposes.

Keywords Medicinal plant, Cellular biological activity, Specialty material, *Acanthus ebracteatus* Vahl., Inflammatory, Anti-aging agents
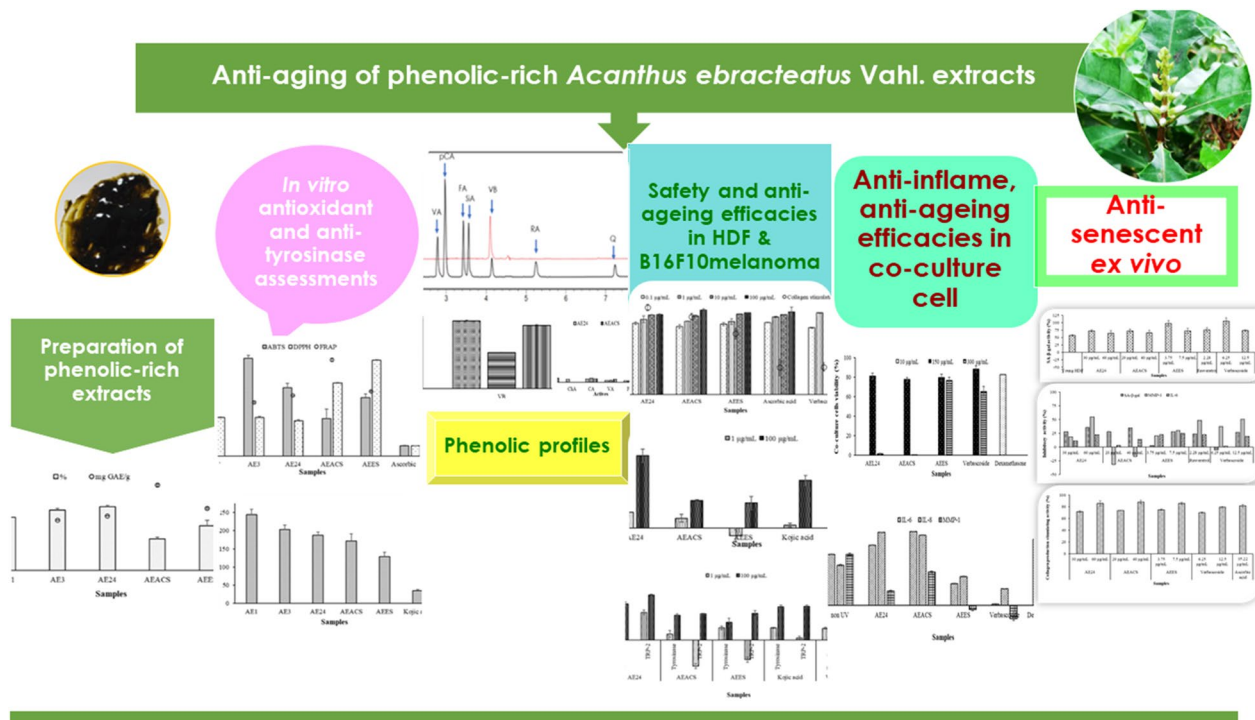
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Graphical Abstract

Background

Plants that are mainly used in traditional medicine are gaining great interest for use in innovative applications in new industries, especially pharmaceutic and cosmetic products [1–3]. Of these, phytochemical and pharmacological profiles will warrant application. Phenolics, the secondary metabolites with a phenylpropanoid skeleton, are the important health-beneficial phyto-active compounds [4]. Phenolics are therefore regarded as having actives useful for a variety of therapeutics and pharmaceutics [5, 6] and being applicable for anti-aging products [7].

Sea holly leaves have long been applied in traditional Thai medicine for treatment of inflamed skin [8]. The crop is therefore widely cultivated in Thailand to support its traditional uses that applied in the format of tincture (soaking in 95%EtOH). The white flower cultivar, i.e., *Acanthus ebracteatus* Vahl. has been recognized to have better performance than the purple cultivar *A. ilicifolius*. The herb is used in a longevity recipe and is also claimed to be a useful treatment for dull and itching skin [9]. In addition, the antimicrobial activity of *A. ebracteatus* water extract against *Staphylococcus aureus*

and *S. epidermidis*, skin disorder-related microbes, was reported [10].

A. ebracteatus has been reported to contain biologically active phenolics [7] in addition to verbascoside [9]. Of these, verbascoside has been increasingly used for the treatment of human diseases and disorders [11]. Plants are highlighted as the promising and reliable sources for biologically active phenolics, especially the agricultural crops [1–3]. Nonetheless, assessments of *A. ebracteatus* against inflammatory mediators, i.e., cytokines related to skin inflammation and skin aging [12, 13], for the treatment of photoaging are sparingly presented [1, 4]. The phytochemical and pharmacological profiles of the crop appointed for innovative topical product development are inadequate.

The present study is therefore designed to prepare phenolic-rich extracts of *A. ebracteatus* leaves (AEL) with a concise and feasible upscaling method. The leaves of *A. ebracteatus* were macerated in 95% EtOH in accordance with its traditional preparation with this solvent and challenged for different lengths of an extraction time. The extraction was further modified to give additional extracts in order to achieve the extracts' quality in terms of chemical and biological profiles. The extracts' actives

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were examined in parallel with in vitro activities related to skin aging. Thereafter, the candidate extracts were tested for their safety and biological activities in cell cultures, co-cultures and ex vivo human skin. Accordingly, the safety and efficacy of the extract were confirmed. This phytomedicine is therefore appointed as a promising source of innovative extracts for antioxidant, anti-inflame and anti-aging purposes.

Methods

Materials

AEL were harvested from the farm located at Nan Province, Thailand during the late of November 2020. The voucher specimen (MKAE11NAN20) was deposited at our laboratory herbarium. All chemicals used were analytical grade unless otherwise specified. The solvents for extractions were purchased from Merck (Darmstadt, Germany). ABTS, DPPH and ferric FRAP reagents for antioxidant activity assessments were supplied by Fluka (MO, USA), as were Folin–Ciocalteu reagent, the standards and $FeSO_4$ including those of phenolic content analysis. Reference compounds for UPLC analysis were brought from Fluka. The UPLC standards were diluted at various concentrations in AcCN (Labscan, Gliwice, Ireland). De-ionized water was prepared using a Milli-Q water purification system (Millipore, MA, USA). Chemicals and reagents for enzyme inhibitory activities were from Sigma-Aldrich (MO, USA). Mediums for cellular assessments were from Gibco (NY, USA), including the supplements.

Preparation of AEL extracts

The dried powder AEL was macerated in 95% EtOH for 1, 3 and 24 h. The whole was filtrated and concentrated to dryness under *vacuo* giving AE1, AE3 and AE24 extracts. The extractive yield was calculated. In addition, AEL was extracted in EtOAc for 24 h, and worked up as usual to afford AEACS extract. The marc residue was additionally extracted with 95%EtOH for 24 h, filtered and concentrated to dryness resulted in AEES extract. Each extraction was undertaken for more two times.

Total phenolic content (TPC) of AEL extracts

TPC of each extract was first examined with Folin–Ciocalteu reagent by the literature method [15] and expressed as g of gallic acid equivalents per g of extract (g GAE/g). Briefly, a serial dilution of standard gallic acid was prepared to generate a calibration curve. The sample was mixed with water, the Folin–Ciocalteu reagent, and 2% Na_2CO_3, then incubated prior to absorbencies recorded. All measurements were performed in triplicate.

In vitro antioxidant assessments of AEL extracts

In vitro antioxidant activity was comparatively assessed by 3 different assays. AEL extracts' capabilities to scavenge $ABTS^{\bullet+}$ and DPPH radicals were assessed in a comparison with the standard ascorbic acid. The inhibitory concentration at 50% (IC_{50}) was calculated. In addition, the reducing power of 1 mg of the AEL extracts was tested in FRAP reagent and expressed as an equivalent concentration (EC) of 1 mg $FeSO_4$ [15]. The assessments were performed in triplicate.

In vitro tyrosinase inhibitory effect

AEL extracts' capabilities on mushroom tyrosinase activity were determined using the dopachrome method using *L*-Dopa as the substrate. The assessment was conducted in a comparison with the standard kojic acid [15] in triplicate. In short, a sample that was a mixture of a phosphate buffer and mushroom tyrosinase was incubated, *L*-Dopa was then added to the mixture, incubated, and absorbencies measured. The enzyme deactivation efficacy of each extract was monitored, and IC_{50} was calculated.

Phenolic profiles of AEL extracts

UPLC analysis was carried out on an ACQUITY H-Class system equipped with an ACQUITY UPLC PDA eλ detector. Gallic (GA), protocatechuic (PA), chlorogenic (ChA), caffeic (CA), vanillic (VA), *p*-coumaric (*p*CA), ferulic (FA), sinapic (SiA), and rosmarinic (RA) acids, verbascoside (VB), and quercetin (Q) at various concentrations in AcCN were used to prepare calibration curves. Phenolics of AEL extracts were successively separated by a gradient mobile phase consisting of AcCN (A) and 3% aq. AcOH (B). The eluent was programmed as follows: 0 min 100%B, 1.5 min 95%B, 3 min 85% B, 5 min 80%B and 8 min 70%B at a flow rate of 0.6 mL/min [15]. The analysis was conducted in three cycles.

Safety and biological activity assessment in human dermal fibroblasts (HDF)

HDF (ATCC, USA) were used in this study. Cellular safety assessment was preliminary examined with sulforhodamine B (SRB) assay as previously described [2].

Antioxidant activity assessment

Cellular antioxidant activity of AEL extracts and the standard were tested by the literature method [2]. In short, the sample-treated cells were treated with the fresh medium containing 150 μM H_2O_2, incubated, fixed, washed, dyed with SRB, and solubilized. Thereafter, cell viability was examined and calculated in a comparison with the solvents-treated group.

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Cellular collagen production activity

HDF were treated with the samples, incubated, lysed, dyed with Sirius Red, centrifuged, and washed with 0.01 M HCl. The cell pellets were dissolved in 0.01 M NaOH. The collagen content was examined in addition to protein content (Bradford assay). The relative ratio of collagen production was calculated in a comparison with that of the control. Thereafter, collagen stimulation activity (%) was presented [2].

Safety and biological activity assessment in B16F10 melanoma

B16F10 melanoma cells (ATCC, USA) were used in this study. Cellular safety was monitored with the SRB assay [3].

Cellular melanin production activity

Cells were treated with the samples. Melanin content was measured in parallel with total protein content analysis. The anti-melanogenesis (%) was calculated [3].

Inhibitory effect against tyrosinase

Cells were treated with the samples, washed, lysed, incubated, centrifuged, treated with *L*-DOPA and incubated. Tyrosinase activity was determined by means of DOPAchrome formation. The enzyme activity was compared with the standard mushroom tyrosinase, and the inhibitory activity (%) was calculated [3].

Inhibitory effect against tyrosinase related proteins-2 (TRP-2)

The supernatant of the lysis treated cells was mixed with phenylthiourea, EDTA and sodium phosphate buffer. Thereafter, the mixture was added with DOPAchrome containing *L*-DOPA and $NaIO_4$, and incubated. The reduction of DOPAchrome was measured. The reaction mixture with bovine serum albumin instead of the cell supernatant was used as a negative control. The inhibitory activity (%) against TRP-2 was presented [3].

Safety and biological activity assessment in HaCaT and HDF co-culture

Safety study in co-culture model was conducted by the literature method as per biological activity [16]. Briefly, HDF were seeded in 48-well plate for 3 days. Thereafter, human keratinocytes (HaCaT) were additionally seeded onto the HDF for further 24 h. Thereafter, the co-cultures were treated with the extracts or the standards, incubated for 24 h, exposed with UVA (1 J/cm^2) and UVB (30 mJ/cm^2), incubated for 24 h. Cell viability (%) was monitored with CellTiter-Glo luminance.

Cellular anti-inflammatory activity of the AEL extracts was examined in terms of the mediator contents. Cytokines, i.e., IL-6, IL-8, and MMP-1 were examined in
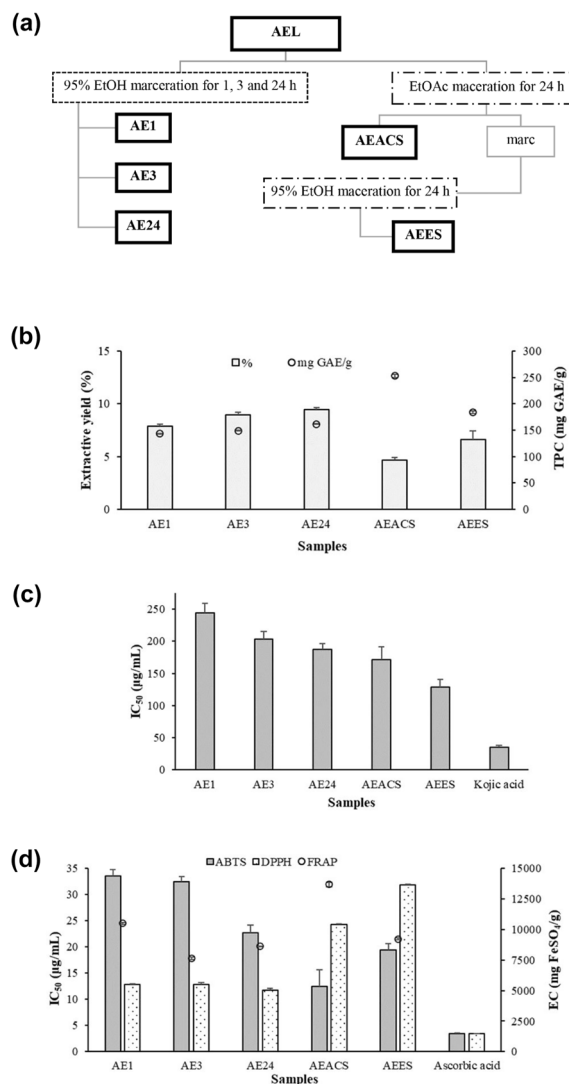
Fig. 1 Preparation scheme of AEL extracts (**a**), AEL extracts with extractive yields, TPC (**b**), in vitro antioxidant (**c**) activities and tyrosinase inhibition (**d**) of AEL extracts

a comparison with the control groups, i.e., vehicle control and non-UV exposure.

Safety and biological activity assessments in human ex vivo skin model

Ex vivo skin tissues (8-mm diameter, NativeSkin®) from the abdominal region of a healthy 58-year-old female donor were obtained. According to the manufacturer, full ethical approval for the study protocol had been obtained from the French ethical research committee, and authorization had been provided by the French Ministry of Research. Under French Law (L.1245 CSP), this skin model was considered a product and element of the body

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collected during a surgical procedure and used for scientific research. The collection procedures complied with the authorized protocol as per the harvest and treatment methods during the course of the tissue preparation for the study. Safety of the samples in term of cell viability was investigated with CellTiter-Flour™ Cell Viability Assay in triplicate during two independent experiments [17].

HDF were senescence-induced, treated with the extracts, and assessed onto senescence-associated β-galactosidase (SA-*β*-gal) activity in a comparison with vehicle control and the standards. In addition, cytokines, i.e., IL-6 and MMP-1 that are senescence-associated secretory phenotype (SASP) in HDF were examined following the extracts treatments in a comparison with the vehicle control and the standards as previously described [17, 18].

Statistical analysis
Data were presented as the means $\pm$ SD and a one-way ANOVA test was used to evaluate the difference between groups using the program SPSS version 16.0. The level of significance was at $p < 0.05$.

Results and discussion
Preparation of the phenolics-rich AEL extracts
The powdered, dried leaves of AEL were first macerated in 95% EtOH, which is the solvent used in the traditional recipe yielding the anti-inflammatory extract [4], for 1, 3 and 24 h as illustrated in Fig. 1a. The extractive yields and the principal actives content, i.e., total phenolics (TPC) were compared. An extraction at a longer time significantly ($p < 0.05$) increased in the extractive yield and TPC (Fig. 1b), which were highest at 24 h (AE24). It should be noted that the extractive yields of all the AEL extracts in the present study were better than those achieved with percolation (5.25%) for 5 days [4]. Furthermore, the extract preparation modification successively yields higher TPC values than a previous report (114.30 ± 0.11 mg GAE/g) [7]. Notably, an extraction for 24 h was determined to be the optimal time for AEL extract preparation. This extraction time was selected for further extracting modification to achieve the AEL extracts' quality in terms of chemical and biological profiles.

To enhance the phenolic-rich extract, the leaves were additionally macerated in EtOAc for 24 h, which was the extraction time with the highest yield and TPC, as noted, to give the AEACS extract. The marc residue was further extracted with 95% EtOH for 24 h to yield the AEES extract. These latter extracts demonstrated obvious ($p < 0.01$) TPC achievement, although the yields were

suppressed. The selected active principles were isolated. Of these, the TPC value can be used for quality control purposes in the application of the natural extract in topical products [19]. Accordingly, AEL extracts with greater TPC values should exhibit superior activity. Thus, in vitro assessments of the extracts' activity related to skin aging [20] were thereafter performed.

In vitro antioxidant and anti-tyrosinase activities
Oxidation events initiate and exacerbate several skin disorders, including skin aging [14]. Oxidative stresses escalate inflammation that accumulates during aging [13]. Accordingly, an assessment of antioxidant activity is appointed for screening the pharmacological profiles of AEL extracts.

The in vitro antioxidant activities of the extracts were assessed by ABTS, DPPH and FRAP assays. All of the AEL extracts exhibited antioxidant activity, although they were less potent than the standard ascorbic acid (Fig. 1c). AEACS was the most potent ($p < 0.01$) anti-ABTS radical among the AEL extracts, with the greatest ability to reduce Fe^{3+} to Fe^{2+}. However, AE24 was the best DPPH radical scavenger. Thus, the present AEL extracts can function as primary and secondary antioxidants in accordance with these in vitro results [21]. In addition, with an IC_{50} of 59.05 ± 0.23 μg/mL, the AEL extracts of the present study had better anti-DPPH radical activities than those of a previous report [7]. That was governed by the greater TPC values of the AEL extracts in this study. Accordingly, TPC should be considered a marker of the antioxidant activity of AEL extracts.

To broaden the application of AEL extracts for skin aging treatment, the potency of the extracts against photoaging [1] was examined in vitro by means of anti-tyrosinase activity. AEL extracts were shown to suppress the melanogenesis enzyme tyrosinase (Fig. 1d). Of these, AEES was the best extract, followed by AEACS, AE24, AE3 and AE1. However, the AEL extracts were weaker than the standard kojic acid inhibiting mushroom tyrosinase.

Thus, the AEL extracts with high TPC values and prominent in vitro biological activities, i.e., AEACS, AEES and AE24 were included for further assessments of their phenolic profiles and their safety and biological activities in cell cultures, i.e., cell lines, co-cultures and ex vivo human skin.

Phenolic profiles of AEL extracts
Phenolics with core structures are secondary metabolites with several beneficial health activities and are commonly regarded as antioxidants [4]. Antioxidative phenolics are applicable in health promotion products [5] that claim to have anti-aging and skin dullness treatment
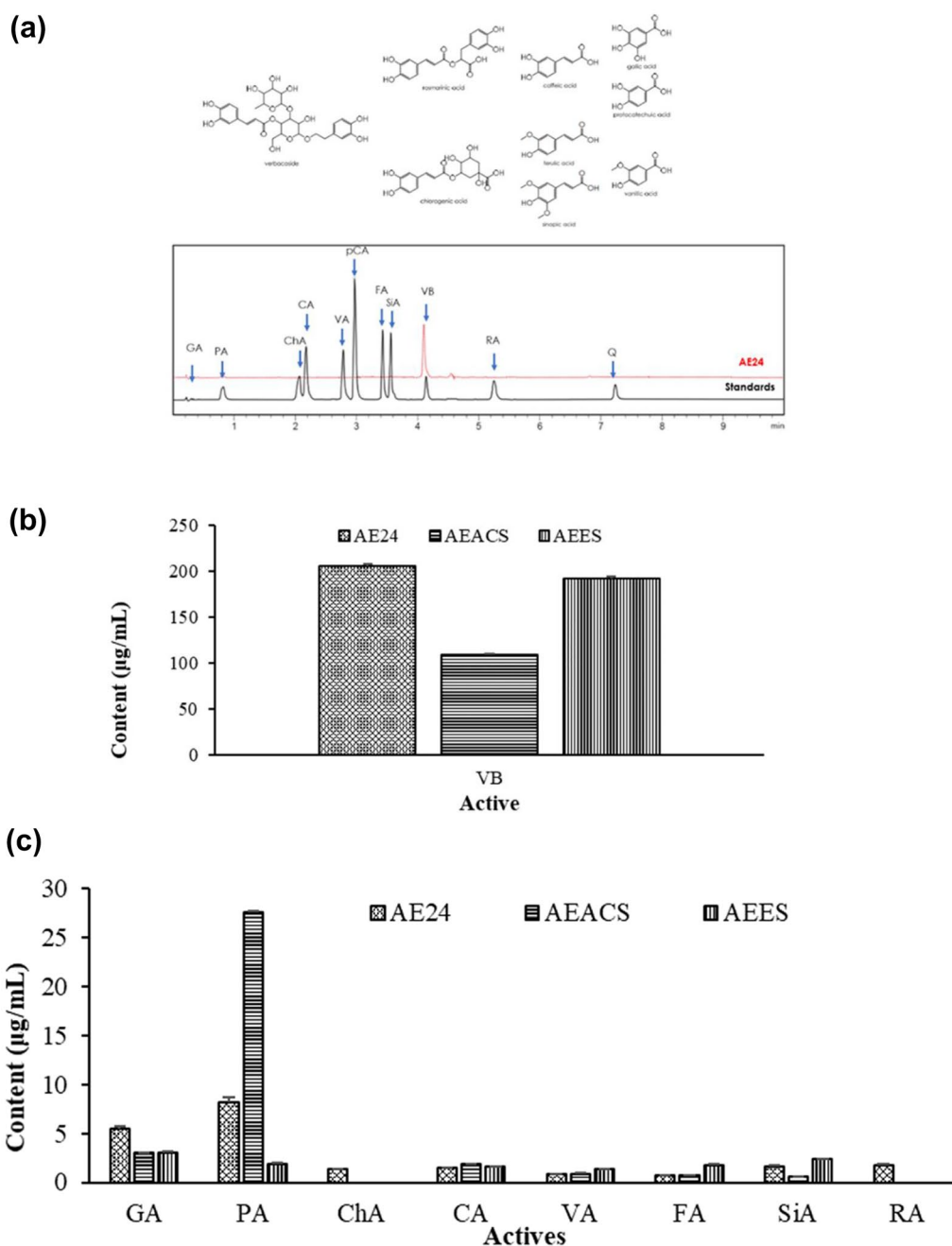
Fig. 2 Sample of UPLC chromatogram of AEL extract and the standards (**a**), verbascoside (**b**) and additional phenolics (**c**) contents of AEL extracts

effects [1]. The phenolics profiles of AEL extracts were therefore studied.

Verbascoside, the active of an AEL [7, 22], is a phenylpropanoid derivative that is biosynthesized in the shikimic acid pathway together with its phenolic homologues, which were reported to be the constituents of *Acanthus* spp. [7], and are applicable for topical products, including cosmetics [1]. The 11 phenolics including verbascoside, were successively separated (Fig. 2a) by the analytical condition [15]. Verbascoside was the major phenolic (Fig. 2b), followed by protocatechuic and gallic acids with additional cutaneous beneficial phenolics, as depicted in Fig. 2c. However, *p*-coumaric acid and quercetin were undetectable in the present study. It should be noted that AE24 and AEES extracts were high in verbascoside content, while AEACS was high in
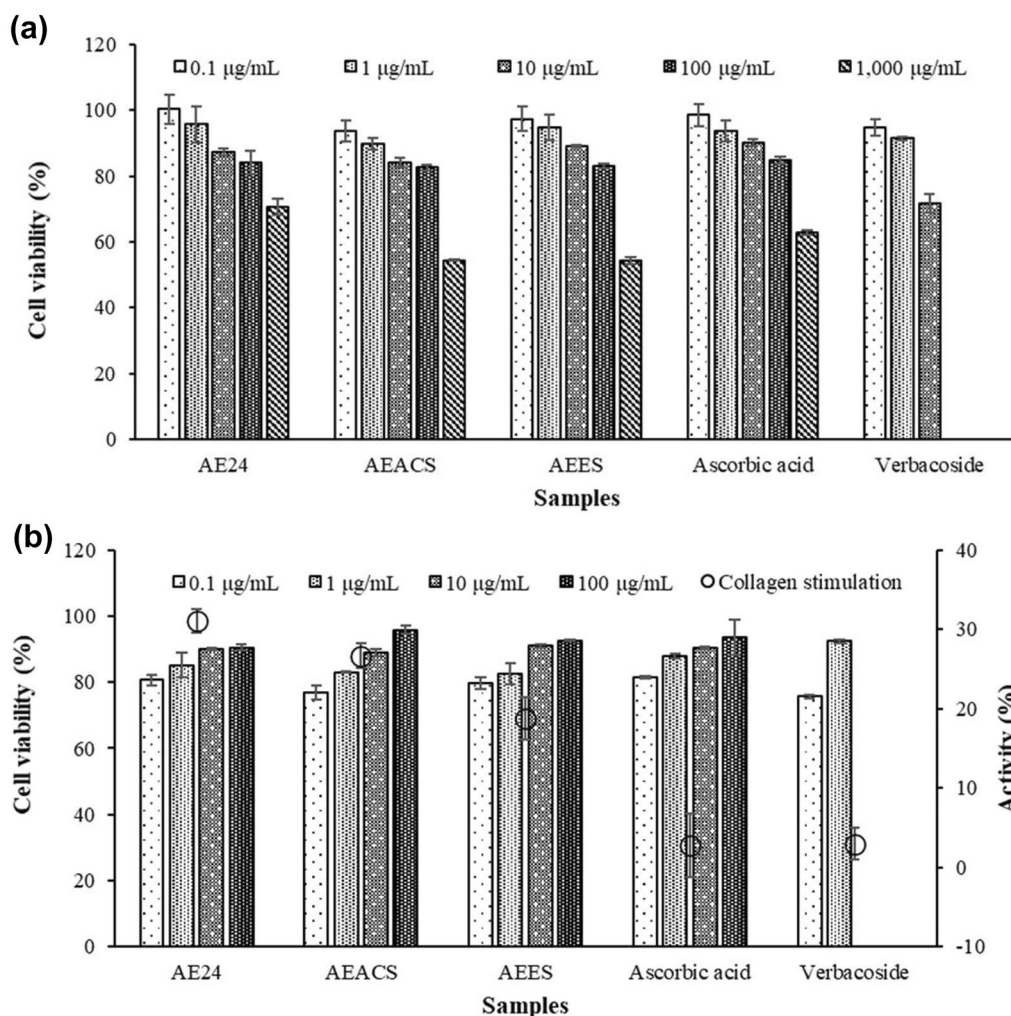
Fig. 3 Safety (**a**), antioxidant and collagen-stimulating activities (**b**) of AEL extracts and the standards in HDF

protocatechuic acid. The in vitro antioxidant activities of the AEL extracts (Fig. 1c) should be more sensitive to the small active phenolics, i.e., protocatechuic and gallic acids, than high molecular weight phenolics, i.e., verbascoside. In addition, in vitro mushroom tyrosinase inhibition may be ruled more by sinapic, ferulic, vanillic and caffeic acids in addition to verbascoside as AEES was the best anti-tyrosinase (Fig. 1d).

Verbascoside has been reported to reduce intracellular oxidative species [23] with anti-inflammatory activity against Toll-like receptor 2 and 4 [24] and IL-6 [25]. In addition, protocatechuic acid has been demonstrated to be a new candidate for anti-aging with antioxidant, antiinflammatory and collagen production stimulating activities, which correlated with skin aging treatment in vivo [18] in addition to its melanogenesis inhibition effects [26]. Furthermore, the presence of antioxidative gallic acid [27] with photoaging protection capability [28] in

the AEL extracts should achieve their anti-aging properties. Safety and biological activity assessments of the AEL extracts in the cell culture models relating to skin aging were thereafter examined in comparison with active verbascoside of the major AEL extracts in addition to the positive control or standard of each assay.

Safety and biological activities in HDF

The in vitro results demonstrated the attractiveness of the phenolic-rich AEL extracts for cutaneous aging treatment. However, safety and efficacy assessments in cell culture models are crucial for topical products [19]. The AEL extracts (0.1 – 100 µg/mL) were proven to be safe in HDF, in similar to the standard ascorbic acid, while the safety dose of verbascoside was narrower (0.1 – 1 µg/mL) (Fig. 3a).

The cellular antioxidant activities of the AEL extracts in this cell line were thereafter tested at safe doses. The
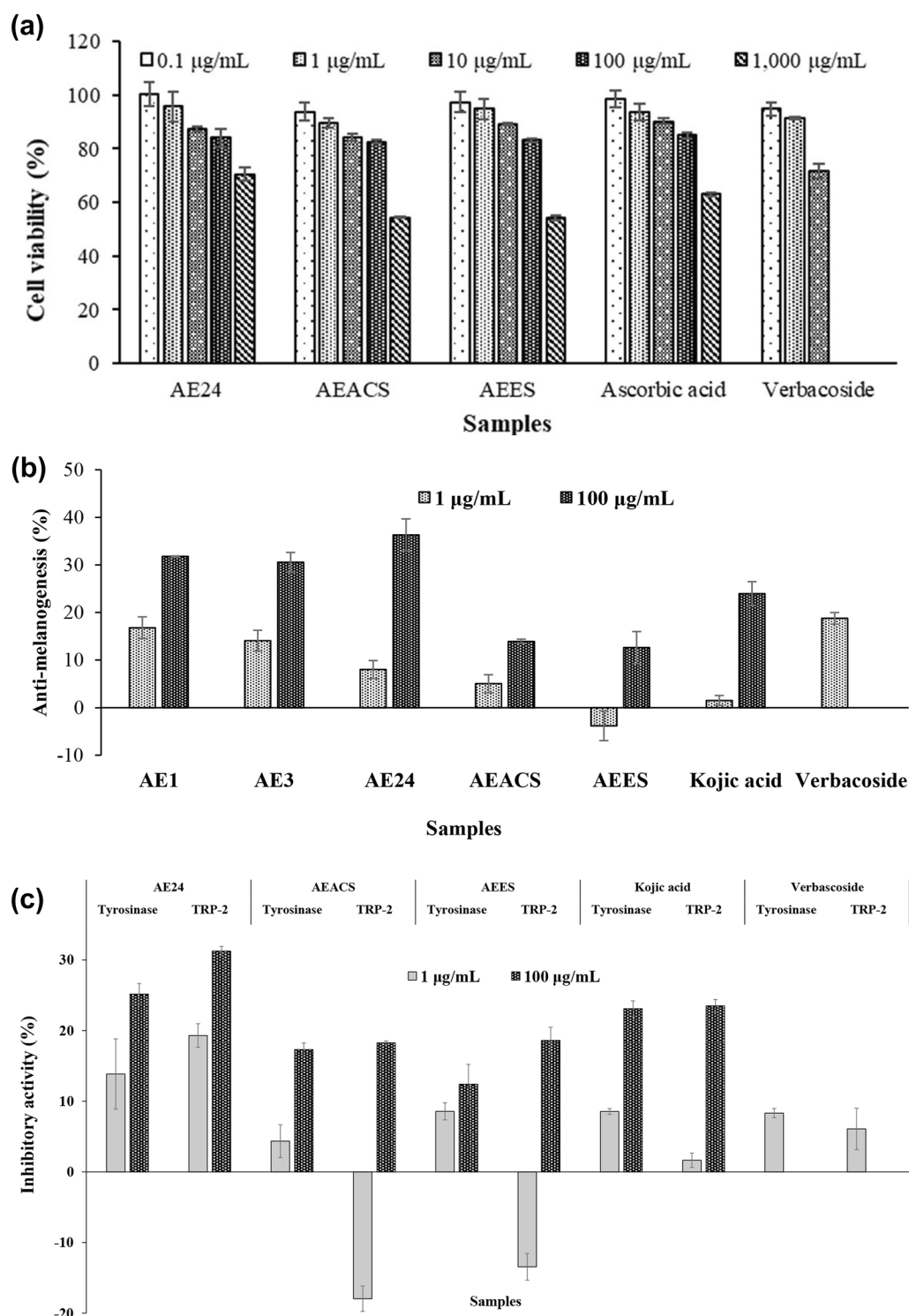
Fig. 4 Safety (**a**), activity against melanogenesis (**b**) and inhibitory activity against tyrosinase ad TRP-2 (**c**) of AEL extracts and the standards in B16F10 melanoma
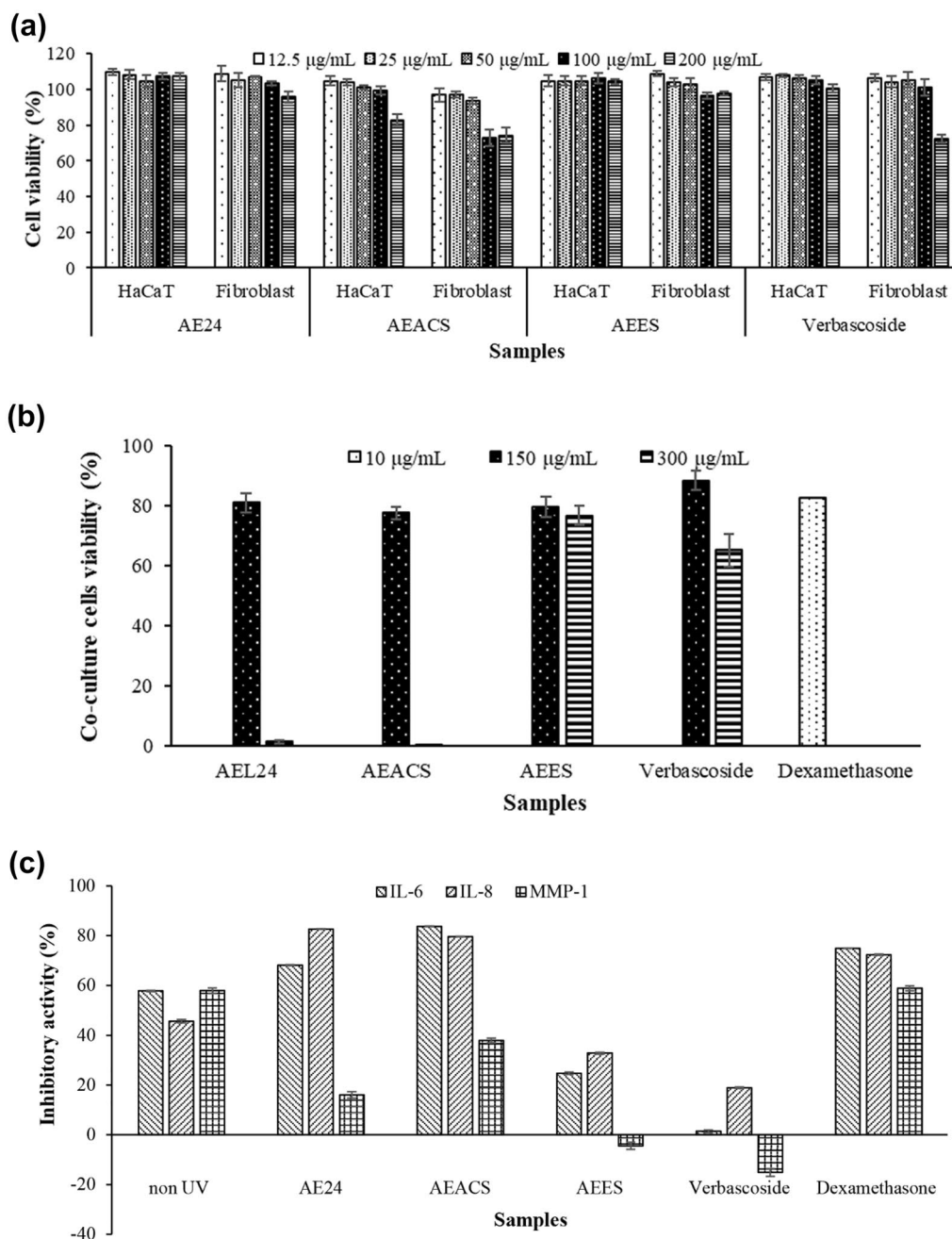
Fig. 5 Safety (**a**), protecting activity against photo-damaged (**b**), and inhibitions against IL-6, IL-8 and MMP-1 (**c**) of AEL extracts and the standards in co-culture of HaCaT and fibroblast cells

antioxidant activities of the AEL extracts healed HDF from cellular oxidative damage with a dose-dependent manner (Fig. 3b), but were inefficient at the lowest test concentration, 0.1 μg/mL. Antioxidant activity is associated with protection against extracellular matrix degradation and stimulates the skin fiber, i.e., collagen, production.[14] Thus, the anti-aging property of the AEL extract was additionally examined in terms of collagen

stimulation at 1 μg/mL. AE24 was the best collagen stimulator and comparable ($p > 0.05$) with AEACS, while AEES was the least efficient. Interestingly, all of the AEL extracts had better abilities to boost cellular collagen production than the benchmark ascorbic acid due to the major active constituent, i.e., verbascoside ($p < 0.01$), at the same test concentration.

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Thus, the anti-aging potency of the AEL extracts may be governed by several actions in addition to antioxidant activity. The activity on cellular melanin production, which co-clarifies anti-aging [1], should be undertaken, including additional assessments in co-culture and ex vivo human skin models for a better understanding of the anti-aging potency of the AEL extracts.

Safety and biological activities in B16F10melanoma

The activities of AEL extracts towards cellular melanogenesis were tested in accordance with the revealed in vitro anti-tyrosinase activity. This assay is worth examining because skin hyperpigmentation or skin dullness is the crucial mechanism in skin aging [1].

The preliminary, safety of the extracts was tested. It should be noted that the safe concentrations of the AEL extracts followed the same trend as exhibited in HDF (Fig. 4a), as per the standard kojic acid. In addition, verbascoside's safety range was narrower. Anti-melanogenesis of AEL extracts was therefore examined at the maximum safety concentration, i.e., 100 μg/mL (Fig. 4b). AE24 was found to be the most potent cellular melanin inhibitor ($p < 0.01$), and it was noted to have superior potency over the standard kojic acid at the same test concentration ($p < 0.01$). To monitor the performance of the major active compound, verbascoside, this active constituent was additionally tested at its maximum safety concentration of 1 μg/mL. In addition, the AEL extracts and kojic acid were assessed at this concentration in parallel. Interestingly, at lower concentrations, verbascoside suppressed melanin production better than kojic acid and the AEL extracts.

The mechanism against melanogenesis was thereafter observed in terms of tyrosinase and TRP-2 inhibitory activities (Fig. 4c). At the maximum safety dose (100 μg/mL), AEL extracts were clearly active against both melanin producing enzymes, especially TRP-2. Moreover, the enzyme inhibitions were in harmony with the cellular melanin production. AE24 was the most potent extract ($p < 0.01$) and performed better than kojic acid ($p < 0.05$) at the same tested concentration. It should be noted that the AEACS and AEES extracts insufficiently inhibited TRP-2 at low concentrations. Verbascoside's inhibitory activity was less sensitive to tyrosinase and TRP-2 in contrast with melanogenesis. Thus, the anti-melanogenesis effects of verbascoside may be ruled by a different mechanism in addition to the melanin producing enzyme, i.e., α-melanocyte-stimulating hormone or α-MSH [1]. It should be noted that the cellular anti-tyrosinase activity differed from the in vitro result that AEES was the best mushroom tyrosinase inhibitor (Fig. 1d).

### Biological activities in co-culture of HaCaT and HDF cells

To verify the cutaneous aging treatment potency of the AEL extracts, additional assessment in the co-culture model was taken into account. The co-culture of HaCaT and HDF cells was examined in the present study.

The safety of the AEL extracts was examined in HaCaT and HDF cells (Fig. 5a) to guide the concentration that will be used in the co-culture cells. The cells were noncytotoxic treated with AE24, AEACS and verbascoside in the range of 0–100 μg/mL, while the range of AEES was 0–50 μg/mL. These concentrations were thereafter guided for the safety assessment in the co-culture model. Cytotoxicity of the co-culture was initiated by UV radiation. Cell viability was compared with the control groups, medium-treated and non-UV exposure. The AEL extracts (150 μg/mL) were proven to protect against UVA and UVB photoaging (Fig. 5b) as per verbascoside treatment. In addition, the positive control dexamethasone exhibited protective efficacy against photoaging at 10 μg/mL in the co-culture of HaCaT and fibroblast cells.

Accordingly, the protective mechanism against photoaging was revealed in terms of suppression of inflammatory mediators. Verbascoside was reported to suppress IL-6 activity in HaCaT [25]. Thus, IL-6 suppression activity was objectively examined in accordance with the presence of verbascoside. Activity against IL-8 was conducted in parallel with IL-6 in because they are cytokines involved in skin aging [12, 29]. AEACS and AE24 were significantly ($p < 0.01$) better than AEES per the co-culture cells that were not exposed to UV. It should be noted that AEACS was more potent than the benchmark, dexamethasone, and verbascoside. The IL-6 and IL-8 suppression were comparable in the cells treated with AEACS, while AE24 and AEES were more sensitive to IL-8. To confirm the performance against collagen degradation, the inhibitory effect against MMP-1 was examined. MMP-1 inhibition was in accordance with collagen stimulation-producing activity (Fig. 3b). All of the AEL extracts promoted collagen production better than verbascoside. AEACS was the best extract ($p < 0.01$) among the AEL extracts at inhibiting MMP-1. Thus, AEL extracts were proven to protect against photoaging by means of anti-inflammatory and anti-MMP actions. The anti-aging potency of the AEL extracts was additionally examined ex vivo.

Safety and biological activities in ex vivo human skin

Senescent cells have been shown to be involved in skin aging with impaired dermal cellular activities as a result of proliferation, which accumulates due to oxidative stresses and inflammation, including UV-induced

(a)

(b)

(c)
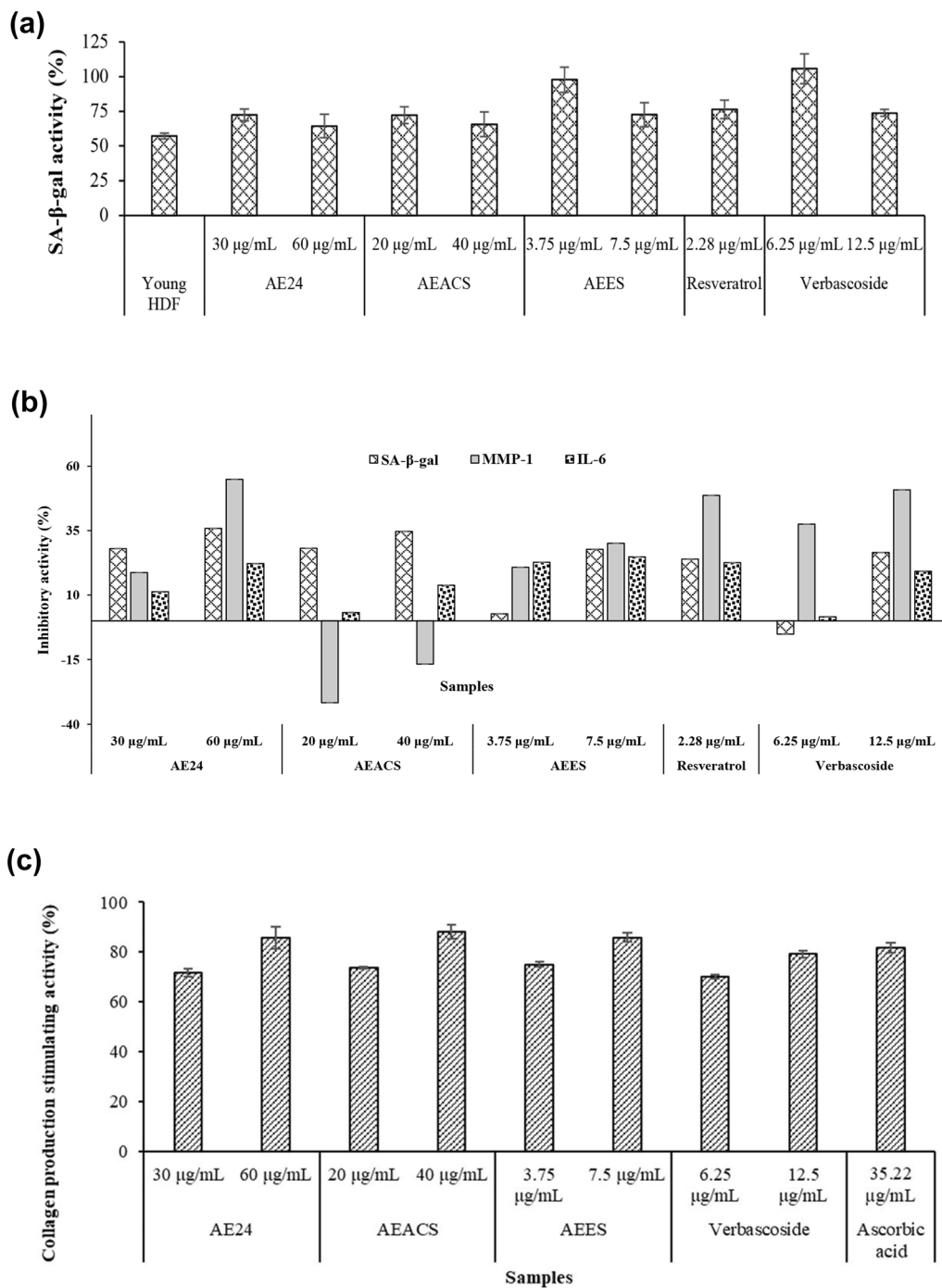
Fig. 6 Ex vivo activity against SA-β-gal (**a**), inhibitory activity against SA-β-gal, IL-6 and MMP-1 (**b**) collagen production stimulating activity (**c**) of AEL extracts

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aging, i.e., photoaging. SA-β-gal has been identified as the key biomarker of skin senescence [29]. A study on the potency of AEL extracts against skin senescence aging was additionally revealed in an ex vivo human skin model.

First, the safety of the AE24, AEACS and AEES extracts in ex vivo human skin was tested (3.08–250 or 10–200 μg/mL, 3.9–125 or 8–100 μg/mL and 1.25–40 or 2.5–40 μg/mL) in comparison with verbascoside (1–30 μg/mL). The safe doses of the AE24, AEACS and AEES extracts were determined to be 30–60, 20–40, 3.75–7.5 and 6.25–12.5 μg/mL, respectively (data not shown). Thereafter, biological activities were assessed at these safe doses in comparison with the benchmarks, resveratrol (2.28 μg/mL) or ascorbic acid (35.22 μg/mL). The aged cells treated with AEL extracts were shown to suppress SA-β-gal activity in a dose-dependent manner (Fig. 6a). Thereafter, the inhibitory activity was calculated relative to that of aged cells. AE24 and AEACS were comparable against SA-β-gal activity and better than AEES and verbascoside at their maximum and safe doses (Fig. 6b). It should be noted that the AEL extract activity was better than that of resveratrol. Moreover, the anti-senescence activity was proven by IL-6 and MMP-1 suppression (Fig. 6b). AE24 and AEES were superior to AEACS upon IL-6 suppression as per MMP-1 inhibition. Although AEACS was able to suppress SA-β-gal, its inhibitory effect against IL-6 was moderate and insufficient to inhibit MMP-1. The positive control was resveratrol, which was the best inhibitor. It should be noted that the MMP-1 inhibition of verbascoside delineated the potency against the collagen degradation enzymes of AE24 and AEES, which contained more verbascoside than AEACS (Fig. 2b). In addition, the ex vivo collagen-stimulating efficacy was examined to confirm the results observed in the cell lines (Fig. 3b). The activity increased with the treatment concentration (Fig. 6c). AEACS at the maximum test concentration stimulated the highest collagen production ($p < 0.05$) and was better than ascorbic acid.

Conclusions

Phenolic-rich extracts of the white sea holly flower of *A. ebracteatus* were successively prepared. The antioxidative AEL extracts are presented with their phytochemical profiles. Safety and cellular activities appointed for cutaneous aging treatment, i.e., photoaging, are evidenced by antioxidant, anti-inflammatory and anti-MMP activities. Pharmacological activities are facilitated by verbascoside, the major constituent, and the additional phenolic constituents especially protocatechuic and gallic acids. The anti-aging activity of AEL extracts is dominantly ruled by verbascoside, and co-contributed with the new candidate anti-aging phenolics for protocatechuic and gallic acids. Sea holly is therefore highlighted as a potential source for verbascoside production to meet with the increasing demand for this natural active, including the cutaneous benefit of phenolics. This phytomedicine is therefore found to be as a sustainable and natural source for anti-inflammatory and anti-aging products. The need for the development of new industrial uses of *A. ebracteatus* in addition to traditional recipe applications is clear. AE24 was indicated as the efficient extract based on its active constituents, biological activities and safety profiles. The extract preparation process is the most feasibly for industrial practices. Innovative products development in accordance with its phytochemical and pharmacological profiles is encouraged.

Abbreviations

ABTS	2,2′-Azino-bis(3-ethylbenzothaiazoline)-6-sulfonic acid)
AcCN	Acetonitrile
AcOH	Acetic acid
AEL	*A. ebracteatus* Leaves
CA	Caffeic acid
ChA	Chlorogenic acid
DPPH	1,1-Diphenyl-2-picrylhydrazyl
EC	Equivalent concentration
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
EtOAc	Ethyl acetate
FBS	Fetal bovine serum
FA	Ferulic acid
FRAP	Ferric reducing ability of plasma
GA	Gallic acid
gGAE	G of gallic acid equivalents
HDF	Human dermal fibroblasts
HPLC	High-performance liquid chromatography
IC$_{50}$	Inhibitory concentration at 50%
IL	Interleukin
MMP-1	Matrix metalloproteinase-1
MSH	Melanocyte-stimulating hormone
NHF	Normal human dermal fibroblast
*p*CA	*p*-Coumaric acid
Q	Quercetin
RA	Rosmarinic acid
SA-β-gal	Senescence-associated β-galactosidase
SASP	Senescence-associated secretory phenotype
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SiA	Sinapic acid
SRB	Sulforhodamine B
TPC	Total phenolic contents
TPTZ	2,4,6-Tri(2-pyridyl)-*S*-triazine
TRP-2	Tyrosinase related proteins-2
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet

Author contributions

MK: conceptualization, methodology, project administration, funding acquisition, investigation, writing—reviewing and editing. PC: investigation, formal analysis, data curation, writing—original draft preparation. MK: investigation, formal analysis, data curation. TI: investigation, formal analysis, data curation. NL: investigation, writing—original draft. All authors read and approved the final manuscript.

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Declarations

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Not applicable.

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Competing interests
The authors declared that there is no competing interest.

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