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A Randomized Controlled Trial on the Moisturizing Effects of *Amomum schmidtii* (K. Schum.) in Working-Age Adults and its Effects on Gene Expression in HaCaT Keratinocytes

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Abstract

Allergic contact dermatitis commonly affects working-age adults due to repeated allergen exposure, leading to skin barrier damage, water loss, and oxidative stress. *Amomum schmidtii* (K. Schum.) (*A. schmidtii*) is traditionally used in Thai medicine to improve skin hydration. This study aimed to compare the effects of *A. schmidtii*, coconut, and cold creams on skin moisture, oiliness, and skin creases in working-age adults. Moreover, antioxidant activity and potential mechanisms were investigated by analyzing skin moisture-related gene expression in keratinocyte cells. A double-blind, randomized controlled trial involving 45 volunteers was conducted. Participants applied *A. schmidtii*, coconut, and cold creams to the dorsal hands. Skin moisture, oiliness, and creases were assessed using the Corneometer® CM820/CM825, Sebumeter® SM815, and stereomicroscopic image analysis, respectively. The results exhibited that skin moisture and oiliness of *A. schmidtii* cream-treated groups significantly increased compared to bare skin ($p < 0.05$). In addition, the distance between skin creases of *A. schmidtii* cream-treated group was significantly decreased after application compared to bare skin ($p < 0.05$). Interestingly, the effects of *A. schmidtii* cream-treated group on these skin test parameters were practically similar to coconut and cold cream. Additionally, *A. schmidtii* oil demonstrated antioxidant activity as assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The oil showed a notable antioxidant capacity, with an IC_{50} value of 7.26 compared to 1.56 µg/mL for the standard antioxidant, Trolox. Moreover, after treating human keratinocyte (HaCaT) cells with *A. schmidtii* oil for 24 h, there was an increase in the synthesis of hyaluronic acid by upregulating the *HAS2* gene, along with an increase in the expression of genes encoding tight junction proteins (*TJP1* gene) and filaggrin (*FLG* gene). These results provide evidence that *A. schmidtii* could be used as a moisturizing agent to prevent dry skin in working adults and could upregulate the gene-related skin moisture expression.

Keywords: *Amomum schmidtii* (K. Schum.), Skin oiliness, Skin moisture, Skin creases, Gene expression, Working-age adults

Introduction

Based on epidemiological studies, the most common occupational skin diseases affecting working-age adults are irritants and allergic contact dermatitis. Although occupational skin diseases may not be a major concern for all workers, they were nevertheless ranked among the top 5 health conditions in terms of incidence per 100,000 population in Thailand [1]. The necessary treatment of contact dermatitis is avoidance of physical and chemical hazards in the office. However, working in an air-conditioned room in the office or surrounding cold weather, including a co-factor of atopic dermatitis, still worsens these problems [2]. Itchy and dry skin were the criteria of dermatitis and carried the burdens of these symptoms that impact the quality of individuals' life [3]. Most Thai working-age adults have experienced dry skin because they might not avoid cold surroundings in air-conditioned offices. The environment might cause dry skin, and xerosis might be a chronic problem for some people. Skin irritation can be prevented by controlling environmental exposure and using personal protective equipment. Therefore, applying a great emollient on the skin is suitable for primary prevention or early prevention in healthy working adults. Human skin moisture should be maintained by the moisturizer in terms of cream, lotion, and oils. Moisturizing cream or emollient will hydrate skin, and improve the quality of softening and soothing skin by repairing the linkage of skin barrier function [4]. Healthy skin can be improved by applying moisturizer cream daily, which is crucial in enhancing these skin functions. To support this role, the use of natural plant-based ingredients in moisturizers has gained increasing interest, especially those with antioxidant and barrier-repairing properties. Among these, traditional Thai medicinal plants such as *A. schmidtii* have shown potential for improving skin quality.

The root and leaf of *A. schmidtii* plant, which is classified in family Zingiberaceae have an attractive smell, and their properties can improve skin texture, as Thai folks mentioned [5]. *A. schmidtii* oil was applied to massage and spa because plants in the Zingiberaceae genus exhibit antioxidant and anti-inflammatory properties through the inhibition of nitric oxide and prostaglandin E2, and they have also shown anticancer potential by targeting the tumor-associated enzyme PHGDH [6]. In addition, some studies have demonstrated that *A. schmidtii* oil also exhibits an antibacterial effect, as seen in the case of *Staphylococcus aureus* [7].

Key genes related to skin hydration and barrier function include *HAS2*, which is responsible for hyaluronic acid synthesis, helping to retain moisture [8]; *TJP1*, which encodes tight junction proteins that prevent water loss and maintain cell cohesion [9]; and *FLG*, which is essential for skin barrier structure and hydration[10]. Monitoring the expression of these genes provides insight into the molecular mechanisms of moisturizers on skin health.

There is no evidence about the moisturizing effect of *A. schmidtii* oil in working-age adults. This study aimed to compare the effect of *A. schmidtii* cream, coconut cream, and cold cream on skin moisture, oiliness, and skin creases and the potential mechanisms underlying these effects through the expression levels of skin moisture-related component genes in human keratinocyte cells.

Materials and methods

Plant collections, authentication, and chemical tests

Fresh samples of *A. schmidtii* (roots and trunk) were purchased from the local market in Wangnamkiew district, Nakhonratchasima, Thailand. The voucher specimen (SOI0943U) was authenticated by Dr. Santi Wattana, a lecturer and plant biologist at the Institute of Science, Suranaree University of Technology and deposited at Suranaree University of Technology (SUT) herbarium flora. The whole parts of *A. schmidtii*, especially the roots and trunk, were washed thoroughly, air-dried briefly to reduce surface moisture, and chopped into small pieces using a mechanical chopper. Approximately 30 kg of the prepared plant material was placed in a stainless-steel distillation unit and subjected to water distillation. A total of 180 L of deionized water was added. The mixture was heated, and distillation was conducted at 130 °C for 6 h. The essential oil typically began to accumulate after several hours of heating. The average yield was 30 mL of clear yellow oil. The oil was stored in amber glass bottles at room temperature until further use. For cream formulation, *A. schmidtii* oil was incorporated into a base cream at a final concentration of 1 %w/w. To prepare 100 g of cream, 1 mL of *A.*

schmidtii oil was mixed with 9.56 g of white Vaseline, 17.22 mL of liquid paraffin, 0.96 g of cetyl alcohol, 2.87 g of stearyl alcohol, 0.48 g of sodium lauryl sulfate, 2.87 mL of glycerin, 0.1 mL of paraben in propylene glycol, and 65 mL of water via a melting process. Coconut cream (1% coconut oil) and cold cream were prepared using the same preparation method.

Gas chromatography/mass spectrometry (GC-MS) analysis

GC-MS was used to evaluate the active chemical components in the oil of *A. schmidtii*. Agilent Gas Chromatography Model 7890A with Agilent 7000B was used to conduct the GC-MS analysis. A column HP-5 capillary column (30 m×0.25 mm×0.25 μm) was used to separate the chemicals. Injector temperatures were maintained at 230 °C, and the injector volume was 1 μL. Helium was the carrier gas (1 mL/min). The following temperature program was configured with 2 ascending stages between 40 and 250 °C. The temperature of the column oven was first set at 40 °C for 5 min, and then it was raised to 180 °C at a rate of 3 °C/min. In the last stage, the temperature of the column oven was raised to 250 °C at a rate of 10 °C/min. It ran for 68 min in total. The compounds were identified by comparing them with the NIST Mass Spectral Library.

Human skin test

Study design and participants

All participants were enrolled from August 2021 to April 2022 and completed a consent form before participating in the study. The trial was registered with the Thai Clinical Trials Registry, identifier: TCTR20210702006, approved on July 2, 2021. Participants were Thai adults aged 18 to 60 years who met the inclusion criteria of working in air-conditioned office environments for more than 4 h daily and having no underlying skin disorders, diabetes mellitus, chronic kidney disease, thyroid dysfunction, or history of hypersensitivity reactions. The exclusion criteria included individuals with a history of hypersensitivity to any components of the cream formulations or *A. schmidtii* oil, those diagnosed with contagious skin conditions, or those with chronic medical disorders that could affect skin function or physiological response. All participants were free to withdraw at any time during the study. The investigators and the participants were blinded during skin tests with the creams. The environment was controlled at 25 ± 0.5 °C of room temperature and 40% ± 10% of air humidity. The participants must stay in the room for 3 h until the examination was completed. All participants were tested for all interventions; *A. schmidtii* cream, coconut oil cream, and cold cream were compared to the control (i.e., no cream applied to individuals' bare skin). Participants were enrolled by the research assistants and assessed for eligibility by the researcher of this study. The researcher labeled stickers on each cream container and kept the note of original random allocation sequences of numbered containers in a locked file on a computer with limited access. The investigators and participants were blinded to the types of samples. The assessment of efficacy was analyzed by the researcher. The safety and complications were evaluated by a doctor after the examination.

The human skin test was evaluated. Coconut cream and cold cream were selected as comparison groups due to their established moisturizing properties. Coconut oil is a plant-based emollient shown to reduce transepidermal water loss and improve skin hydration in clinical studies [11]. Cold cream, a traditional water-in-oil emulsion, is widely used as a benchmark moisturizer for dry and sensitive skin [12]. The skin on both dorsal hands was cleaned with a gentle skin cleanser before testing. Four small stickers on the dorsal part of both hands were prepared for *A. schmidtii* cream, coconut cream, and cold cream, including bare skin comparisons, respectively. One sticker was penetrated 2 squares and 1 circle, as shown in **Figure 1**, in which each box was examined for 3 primary outcomes: 1) Skin moisture or skin hydration; 2) Skin oiliness or skin sebum; 3) Distance between skin creases or skin length.

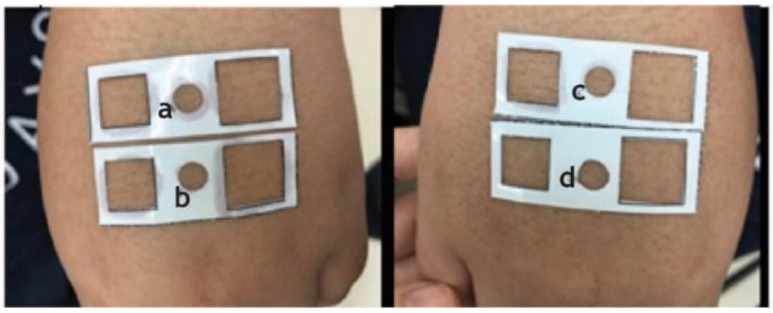

Figure 1 Human skin test. One box was penetrated 2 squares (1.2 cm^2 for hydration measurement and 1.5 cm^2 for sebum measurement) and 1 circle (the diameter 0.5 cm^2 for skin creases photo by stereomicroscope 6.5×), which 4 small boxes on the dorsal part of both hands were prepared for (a) *A. schmidtii* cream, (b) the coconut cream, (c) the cold cream, and (d) bare skin comparing tests.

Sample size calculation

The sample size calculation of the primary outcome was determined by using G*Power 3.1 software. A sample size of 45 was calculated for the primary outcome with an effect size of 0.25, α error of 0.05, power of 0.95, 4 groups, 6 times of measurements, and 20% of participants probably drop out.

Skin moisture or skin hydration

Skin hydration measurement was performed 3 times to get the average of stratum corneum conductance. The skin moisture was tested by the Corneometer® CM820 or CM825 (Courage + Khazaka electronic GmbH, Germany), which is measured by dielectric capacitance as previously described [13]. The skin sensor was calibrated before the skin test. Dry skin was determined if the average measurement value was less than 30 - 40 arbitrary units (AU) or electrical conductance units. In contrast, moisturizing skin was determined if the average measurement value was higher than that. The data were collected at 0, 1, 10, 30, 60, and 120 min and were recorded via computer analysis by code.

Skin oiliness or skin sebum

Oily skin was tested by the Sebumeter® SM 815 following the method of Cheng *et al.* [14] with minor modifications. Dry skin was determined if the measurement value was less than 6 µg sebum/cm^2 and normal skin was detected as higher than that. The corneometer and the sebumeter were connected with the multi-probe adapter (MPA) system, and data were analyzed by the software CK-MPA-Multi Probe Adapter FB Version: 2.4.5.1/220/2018. The data were collected at 0, 1, 10, 30, 60, and 120 min and recorded via computer analysis by code.

Distance between skin creases or skin length

Distance between skin creases was evaluated using a method developed by Bielfeldt *et al.* [15] with minor modifications. The skin texture at the dorsal part of the hands was photographed with a 6.5× magnification of a stereomicroscope (Zeiss version Stemi 2000). Then, the distance between skin creases was applied for image analysis using the Image J program. Three points at the left, right, and middle sides on a diameter line of 1 skin photo were analyzed for the average distance between skin creases. The data were collected at 0 and 120 min and were recorded by computer analysis by code.

DPPH scavenging assay

The antioxidation scavenging activity was tested by DPPH scavenging assay [16]. Briefly, DPPH 3.154 mg was prepared with 100 mL of absolute ethanol, 0.05 g of the standard Trolox, and diluted with 10 mL of absolute ethanol (AR grade). The 5,000 µg/mL stock solution was diluted to 1, 5, 10 and 20 µg/mL. *A. schmidtii* oil at 0.5 g was prepared in a similar process to the standard Trolox; as a result, the concentrated solutions of 7.06, 14.13, 28.25, 56.50, 113, and 226 µg/mL were obtained. These *A. schmidtii* oils were dropped into a microwell plate and mixed with DPPH 100 µL at 30 °C, then the absorption was measured at 515 nm. Each concentration test was performed in triplicate. The percentage of antioxidant scavenging to DPPH was calculated as 50% inhibitory concentration (IC_{50}).

Cell culture

HaCaT cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in culture flasks containing 4 - 5 mL of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were incubated at 37 °C in a humidified incubator with 5% CO_2.

Cytotoxic assay

The cytotoxic effect of *A. schmidtii* oil on HaCaT cell proliferation was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, HaCaT cells were seeded in a 96-well plate at a density of 1.5×10^4 cells per well. The cells were incubated at 37 °C for 24 h, after which they were treated with culture medium containing various concentrations of *A. schmidtii* oil, ranging from 7.81 to 125 µg/mL. Following another 24-hour incubation, the effect on cell proliferation was evaluated. The culture medium was then removed, and 0.5 mg/mL of MTT reagent was added. The cells were incubated again for 4 h at 37 °C. Viable cells converted the MTT reagent into formazan crystals, which were subsequently dissolved in DMSO. The absorbance was measured at 570 nm using a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Japan).

Gene expression analysis by quantitative reverse transcription PCR (RT-qPCR)

The expression of genes associated with skin functions, including Hyaluronan Synthase 2 (*HAS2*), Filaggrin (*FLG*), and Tight Junction Protein 1 (*TJP1*), was analyzed by RT-qPCR using the primer set as listed in **Table 1**. Briefly, HaCaT cell lines (4.5×10^5 cells/well) were seeded in a 6-well plate and incubated at 37 °C for 24 h. The cells were subsequently treated with *A. schmidtii* oil at concentrations of 7.8125, 15.625, and 31.25 µg/mL for 18 h, followed by RNA isolation using a GF-1 Total RNA Extraction Kit (Vivantis Technologies, Malaysia). The contaminated genomic DNA was eliminated by DNase digestion prior to reverse transcription into cDNA using the Viva cDNA Synthesis Kit (Vivantis Technologies, Malaysia). A total of 50 ng cDNA was used as a template in a real-time polymerase chain reaction using the LightCycler® 480 SYBR Green I Master Mix (Roche Diagnostics, USA). The polymerase chain reaction thermocycling was programed as follows; initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 56 °C for 20 s and 72 °C for 30 s, followed by melting curve analysis over a temperature range of 65 - 95 °C. Relative fold-changed mRNA expression was calculated by the 2-ΔΔCt method and normalized with the ACTB gene [17].

Table 1 Primer sequences for detection of *HAS2, TJP1* and *FLG* gene.

Gene	Forward (5' to 3')	Reverse (5' to 3')
HAS2	CTCTTTTGGACTGTATGGTGCC	AGGGTAGGTTAGCCTTTTCACA
TJP1	ACCAGTAAGTCGTCCTGATCC	TCGGCCAAATCTTCTCACTCC
FLG	GGACAGGAACAATCATCGGGG	CAACCTCTCGGAGTCGTCTG
ACTB (β-actin)	AAATCGTGCGTGACATCAAAGA	GCCATCTCCTGCTCGAAGTC

Statistical analysis

The paired t-test was used for the pre- and post-test distance between skin creases analysis. Moreover, the 1-way analysis of variance (ANOVA) with Turkey's HSD post hoc was employed to analyze human skin test, DPPH scavenging assay, cytotoxic assay, and gene expression analysis. $p < 0.05$ was considered a statistically significant difference between the groups.

Results

Chemical identification of *A. schmidtii*

The GC-MS analysis of the *A. schmidtii* oil is presented in **Figure 2** and **Table 2**. The results revealed a complex mixture of 47 identified compounds. The abundant primary compounds were Benzene, 1-(1-butenyl)-4-methoxy-, trans-, (+)-2-Bornanone, and with %area at 61.31%, 7.28% and 6.51%, respectively.

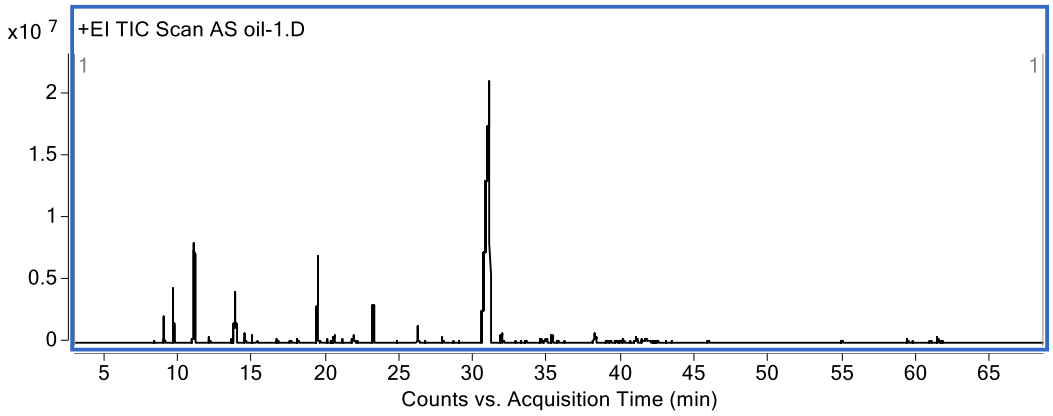

Figure 2 GC-MS Chromatogram of compounds in *A. schmidtii* oil.

Table 2 Compounds in *A. schmidtii* oil that were analyzed by GC-MS analysis.

Compounds	RT (min)	Area (%)
Tricyclene	8.389	0.07
α-Thujene	8.769	0.02
1R-α-Pinene	9.000	1.40
Camphene	9.659	3.08
β-Pinene	11.078	6.51
β-Myrcene	12.073	0.28
α-Phellandrene	12.508	0.03
(+)-4-Carene	13.151	0.02
o-Cymene	13.560	0.20
D-Limonene	13.764	1.24
Eucalyptol	13.861	2.62
trans-β-Ocimene	14.476	0.46
cis-β-Ocimene	14.967	0.41
γ-Terpinene	15.320	0.13
Fenchone	16.644	0.15
2-Carene	16.766	0.08
β-Linalool	17.615	0.16
Fenchol	18.042	0.21
(+)-2-Bornanone	19.447	7.28
Camphene hydrate	19.584	0.09
Isoborneol	20.043	0.16
Pinocarvone	20.274	0.06
endo-Borneol	20.521	0.43
Terpinen-4-ol	21.095	0.20
α-Terpineol	21.811	0.38
Myrtenol	22.048	0.05
Fenchyl acetate	23.176	2.02
Benzaldehyde, 4-methoxy-	24.728	0.16

Compounds	RT (min)	Area (%)
(-)-Bornyl acetate	26.173	0.90
α-Terpineol acetate	29.008	0.07
Benzene, 1-(1-butenyl)-4-methoxy-, trans-	31.052	67.31
β-Caryophyllene	31.845	0.54
α-Santalene	31.983	0.10
Epi-β-Santalene	33.060	0.03
α-Caryophyllene	33.185	0.11
Alloaromadendrene	33.467	0.06
Elixene	34.934	0.27
8-Isopropenyl-1,5-dimethyl-1,5-cyclodecadiene	35.254	0.40
Aromadendrene oxide-(2)	38.130	0.62
Caryophyllene oxide	38.218	0.35
Guaiol	38.931	0.18
β-Eudesmol	39.201	0.06
Selina-6-en-4-ol	40.935	0.27
Juniper camphor	41.018	0.25
β-bisabolol	41.644	0.22
Phytol	54.860	0.09
p-Anisoin	61.327	0.28

Human skin test

Overall recruitment, adverse effects, and compliance

The 45 healthy adult volunteers were enrolled. Subject allocation is shown in the overall flow of participants assessed for eligibility in **Figure 3**. The 1,080 times were calculated from 45 participants by randomization. Although 48 volunteers applied for the human skin research, 45 met the inclusion criteria without dropping out while 3 were excluded due to working in air-conditioned environments for less than 4 h per day. No adverse events or hypersensitivity reactions were reported during or after the application of *A. schmidtii*, coconut, or cold cream.

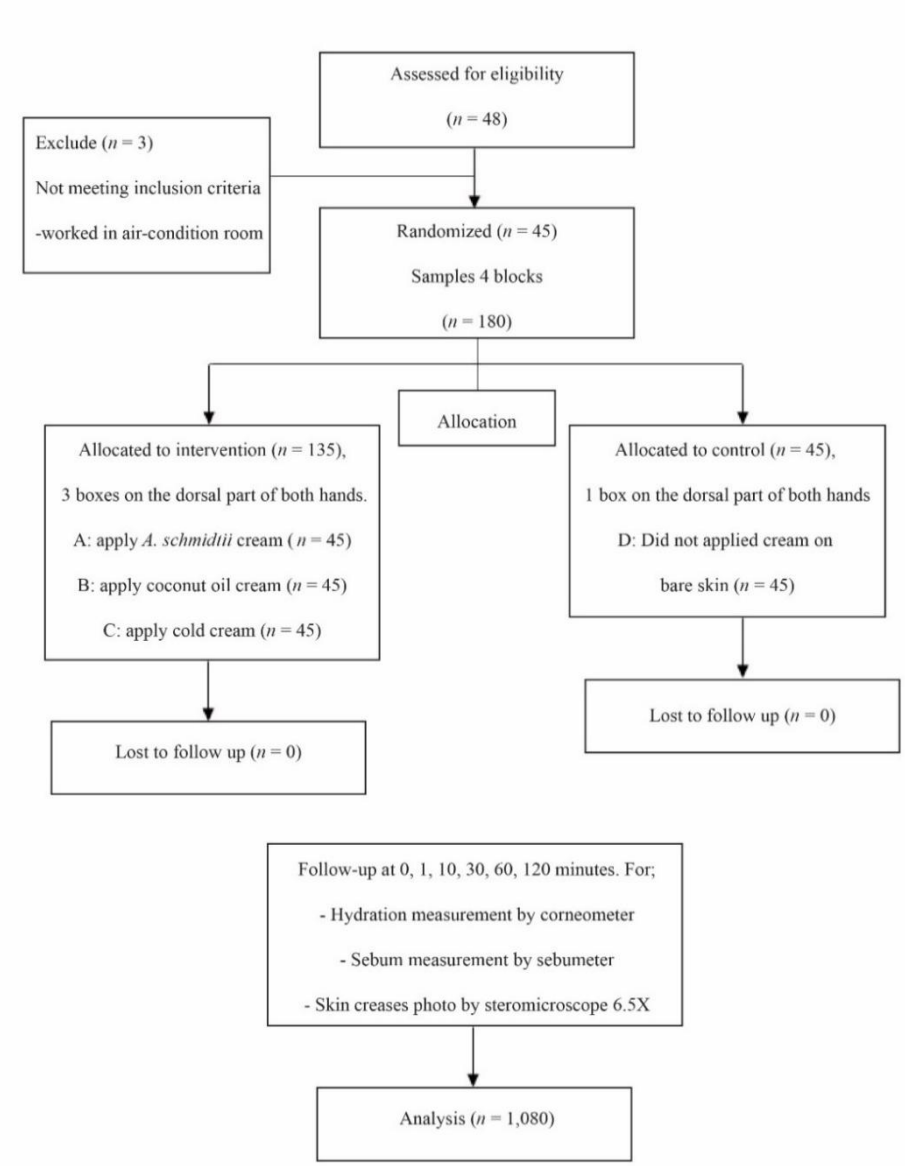

Figure 3 The overall flow of participants was assessed for eligibility.

The participants' characteristics are displayed in **Table 3**. The 45 participants were 28 females (62.22%), and 17 males (37.78%), whose average age was 40.49 ± 10.39 years old. 35 participants (77.78%) used skin cream daily due to dry skin. Prior to cream testing, 18 females (40.0%) and 8 males (17.8%) were defined as having dry skin after measuring with the corneometer. The average body mass index (BMI) of all subjects was 23.92_± 4.2 kg/m^2 (minimum value of BMI = 17.09 kg/m^2, and maximum value of BMI = 40.74 kg/m^2). The fat cell mass might relate to skin moisture and oiliness. However, there was no difference between BMI < 23 kg/m^2 and BMI > 23 kg/m^2, which was the cut-off point for Asian folks (*p*-value = 0.989 and 1.000, respectively).

Table 3 Baseline demographic and clinical characteristics for each group.

Characteristics	**Age-Groups**				
	18 - 29	**30 - 39**	**40 - 49**	**50 - 59**	**Total**
Age (y)	26.82 ± 2.86	34.82 ± 2.86	47.27 ± 2.37	52.00 ± 1.60	40.49 ± 10.39

Characteristics	Age-Groups				
	18 - 29	**30 - 39**	**40 - 49**	**50 - 59**	**Total**
Gender (M/F)	4/7	4/7	4/7	5/7	17/28
Weight (kg)	61.45 ± 10.12	67.32 ± 18.72	62.82 ± 7.47	63.58 ± 11.69	63.79 ± 12.43
BMI (kg/m^2)	22.15 ± 2.76	25.09 ± 6.87	24.08 ± 1.81	24.38 ± 3.62	23.92 ± 4.20
Occupations (persons)					
Government officer	4	7	9	12	32
Company employee	5	1	0	0	6
Freelance	2	3	2	0	7
Working hours in the air-conditioned atmosphere (persons)					
4 - 8 h	7	5	8	7	27
> 8 h	4	6	3	5	18

Three main outcomes of measurement were investigated: Skin moisture or skin hydration by using Corneometer®, skin oiliness or skin sebum, and distance between skin creases or skin length. The baseline of primary outcomes was not different in the age group.

Skin moisture or skin hydration

Skin moisture at 1, 10, 30, 60, and 120 min of *A. schmidtii* cream, coconut cream, and cold cream was significantly higher than bare skin (all comparisons showed $p < 0.05$; **Figure 4**). At 1 min, all creams exhibited $p < 0.001$; *A. schmidtii* showed $p = 0.001$ - 0.017 at later times, while coconut and cold creams remained highly significant ($p < 0.001$). *A. schmidtii* cream increased the skin moisture level from 40.58 ± 10.44 (at 0 min) to 49.17 ± 8.98 (at 120 min) (21.16%). This increase was significantly higher than bare skin throughout 120 min but not significantly different from coconut or cold cream (p ranging from 0.059 to 1.000).

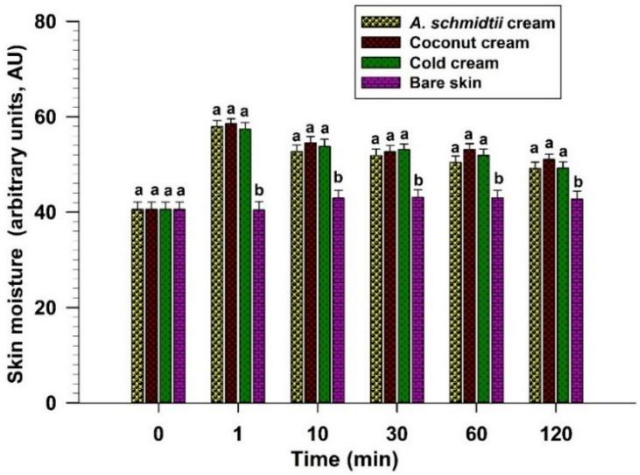

Figure 4 The effect of *A. schmidtii* cream on skin moisture measurement after treatment with each cream was measured at 0, 1, 10, 30, 60, and 120 min. Data are expressed as means ± Standard error of the mean (SEM; n = 45). A significant difference between the groups, which means sharing the different superscript letters (a,b), was compared using ANOVA and Tukey's HSD post hoc test at $p < 0.05$.

Skin oiliness or skin sebum

Figure 5 showed that *A. schmidtii* cream, coconut cream, and cold cream significantly increased skin oiliness compared to bare skin from 1 to 120 min (p ranging from < 0.001 to 0.021). *A. schmidtii* cream reduced skin oiliness by 86.98%, from 162.67 ± 43.94 to 21.18 ± 27.03 sebum/cm^2, at 1 to 120 min, respectively. Interestingly, *A. schmidtii* and coconut creams exhibited higher skin oiliness than the cold cream. In contrast, skin oiliness of the bare skin group was stable from 1 to 120 min.

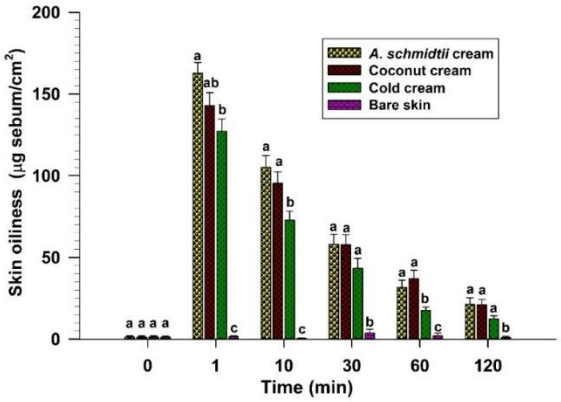

Figure 5 The effect of *A. schmidtii* cream on skin oiliness levels after treatment with each cream was measured at 0, 1, 10, 30, 60, and 120 min. Data are expressed as means ± SEM (n = 45). A significant difference between the groups, which means sharing the different superscript letters (a,b), was compared using ANOVA and Tukey's HSD post hoc test at p < 0.05.

Distance between skin creases or skin length

Figure 6 showed that *A. schmidtii* cream, coconut cream, and cold cream groups exhibited a significant decrease in skin creases, with reductions of 15.04% (p = 0.008), 11.42% (p < 0.001), and 9.46% (p = 0.032), respectively. These findings suggest that all 3 moisturizing formulations were effective in diminishing skin creases. In contrast, the bare skin control group showed no statistically significant change over the same period (p = 0.108).

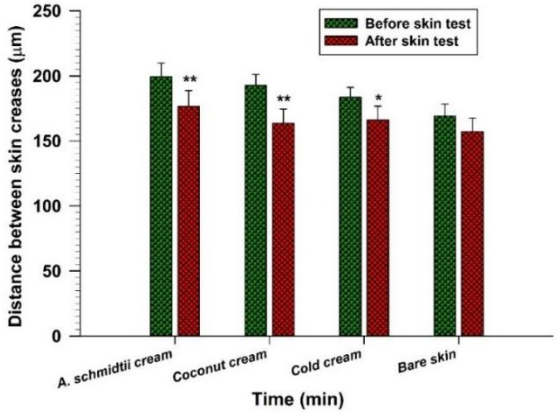

Figure 6 The effect of *A. schmidtii* cream on the distance between skin creases before (0 min) and after (120 min) the skin test. Data are expressed as means ± SEM (n = 45). The significant difference between the before and after-test in each group was compared using paired t-test at * p < 0.05 and ** p < 0.01.

The antioxidant property of A. schmidtii oil

The DPPH radical scavenging assay was employed to assess the antioxidant capacity of *A. schmidtii* oil in comparison to the standard, Trolox. The antioxidant capacity was measured spectrophotometrically at a wavelength of 515 nm. Our findings indicated that *A. schmidtii* oil exhibited an antioxidant capacity with an IC_{50} value of 7.26 µg/mL, whereas the IC_{50} value for standard Trolox was 1.56 µg/mL.

Effect of *A. schmidtii* oil on cell viability

To evaluate the cytotoxicity of *A. schmidtii* oil on HaCaT cells, a concentration-dependent assay was conducted over a 24-hour incubation period. HaCaT cells were treated with varying concentrations of the *A. schmidtii* oil, and cell viability was assessed. The results indicated that concentrations of *A. schmidtii* oil up to 31.25 µg/mL did not significantly decrease cell viability compared to the vehicle-treated control group (p ranging from 0.262 - 0.698; **Figure 7**), suggesting no observable cytotoxic effects at or below this concentration. Based on these findings, 31.25 µg/mL was selected as the highest non-cytotoxic dose and was therefore utilized in gene expression experiments.

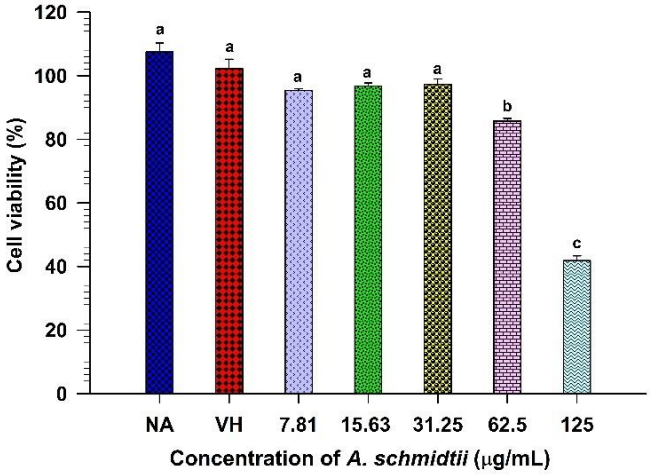

Figure 7 The effect of *A. schmidtii* oil on HaCaT cell viability was determined using the MTT assay. Data are expressed as means ± SEM (n = 6). A significant difference between the groups, which means sharing the different superscript letters (a,b), was compared using ANOVA and Tukey's HSD post hoc test at $p < 0.05$.

Gene expression analysis by RT-qPCR

The *mRNA* expression levels of *HAS2*, *TJP1*, and *FLG* genes are illustrated in **Figure 8**. Treatment with *A. schmidtii* oil resulted in a concentration-dependent upregulation of all 3 genes compared to the untreated control. The most notable increases were observed at 31.25 µg/mL, with statistically significant differences for *HAS2* (p = 0.007; **Figure 8(a)**), *TJP1* (p = 0.006; **Figure 8(b)**), and *FLG* (p = 0.005; **Figure 8(c)**). These findings suggest that *A. schmidtii* oil may enhance skin hydration and barrier function by stimulating the expression of genes involved in hyaluronic acid synthesis (*HAS2*), tight junction integrity (*TJP1*), and filaggrin production (*FLG*).

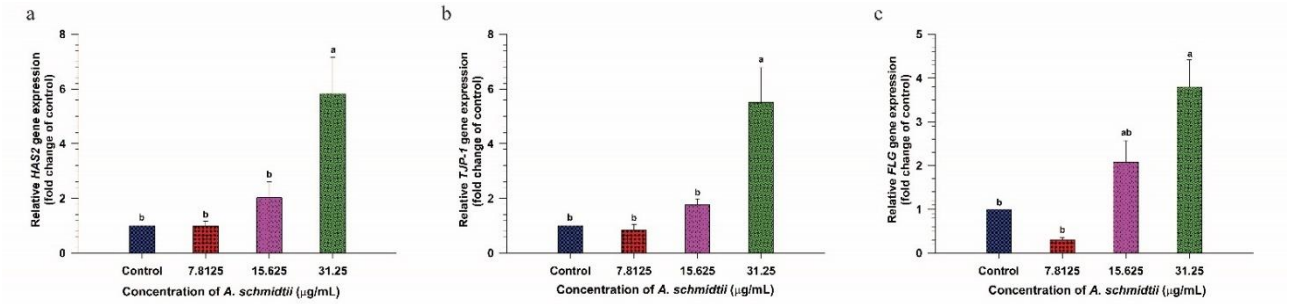

Figure 8 The effect of *A. schmidtii* on the expression of genes associated with skin functions: (a) *HAS2* gene, (b) *TJP-1* gene, (c) *FLG* gene. Data are expressed as means ± SEM (n = 3). A significant difference between the groups, which means sharing the different superscript letters (a,b), was compared using ANOVA and Tukey's HSD post hoc test at $p < 0.05$.

Discussion

A. schmidtii, a traditionally valued Thai herb from the Zingiberaceae family, contains several bioactive compounds as identified by GC-MS, including Benzene, 1-(1-butenyl)-4-methoxy-, trans-, (+)-2-Bornanone and other compounds. These findings are consistent with a previous study that *Amomum biflorum* Jack (synonym: *Amomum schmidtii* (K. Schum.), which also demonstrated the presence of benzoic acid, β-Pinene and α-Terpineol [18,19]. Furthermore, antioxidant testing via the DPPH assay confirmed notable radical scavenging activity, supporting its potential as a natural antioxidant [16].

Skin moisture, oiliness, and creases were investigated on the dorsal part of the hands, with images taken using a stereomicroscope at 6.5× magnification. The *A. schmidtii* cream, along with coconut oil and cold cream, demonstrated good skin moisture retention and could prevent dry skin for more than 2 h. Overall, these findings indicate that *A. schmidtii* cream is as effective as traditional moisturizing agents in enhancing skin hydration. Because it is derived from plants, *A. schmidtii* may offer an alternative or complementary option for dermatological and cosmetic formulations, particularly for consumers looking for natural skincare products.

Moreover, skin oiliness affects skin moisture, which helps keep the skin hydrated. Skin oiliness increased significantly after applying *A. schmidtii*, coconut, and cold cream. On the dorsal hands, skin oiliness peaked in the first minute but gradually declined after 2 h in all tested creams. These findings suggest that *A. schmidtii* cream could help control sebum levels, maintaining a balance between dryness and oiliness. This finding is consistent with the previous reports that a Zingiberaceae plant can regulate excessive sebum secretion [20,21].

Therefore, the essential oil of *A. schmidtii* may help improve skin texture without causing greasiness. Additionally, essential oil-based creams have been reported to enhance skin hydration, reduce erythematous lesions, promote wound healing and help maintain skin texture in various skin conditions, including acne [22,23].

Furthermore, the significant decrease in the distance between skin creases after application of *A. schmidtii*, coconut, and cold cream suggests improved skin smoothness and elasticity. This is consistent with recent finding showing that moisturizers can reduce wrinkle depth and enhance skin texture when assessed by advanced imaging methods [24,25]. In this study, individuals' skin photos were applied using similar pixel analysis techniques to measure the distance between skin creases, but fluorescent light was used for photo analysis. There was a difference in taking a picture with polarized light, which has been used for skin pathology analysis for a long time. The photo analysis technique might have affected the result, which showed no different interaction effect on the distance between skin creases [26].

In addition to its moisturizing and antioxidant properties, *A. schmidtii* cream was well tolerated, and no adverse events were reported during the study, indicating its safety for short-term topical use in healthy adults. The mechanism of *A. schmidtii* action on gene expression was also investigated. The results indicated that *A. schmidtii* enhances the synthesis of hyaluronic acid (HA), an essential extracellular matrix that plays a role in maintaining skin hydration and facilitating

wound healing [27]. Furthermore, the *A. schmidtii* increases the expression of a gene encoding a tight junction protein (TJP), which forms continuous fibrous connections among adjacent cells, serving as a barrier to macromolecule penetration and reducing water loss [9]. Moreover, *A. schmidtii* enhances the expression of a gene-encoded filaggrin, a gene that regulates structural barrier proteins in the skin [28]. Taken together, *A. schmidtii* improves the functions of skin cells, particularly in keratinocyte cells.

This study has limitations that should be considered. The sample was relatively small and limited to Thai office workers, which may affect the external validity and generalizability of the findings. Additionally, the short follow-up period (2 h) only allowed assessment of immediate effects and did not capture potential long-term or sustained efficacy. Future studies should include larger and more demographically diverse populations, as well as extended follow-up durations, to comprehensively evaluate the prolonged effects and broader applicability of *A. schmidtii* cream.

Conclusions

A. schmidtii cream was investigated to compare its efficacy with coconut and cold creams through a human skin test. Also, the expression of genes associated with skin functions was observed. The results provide scientific evidence that *A. schmidtii* cream significantly improves skin moisture, oiliness, and skin creases compared to bare skin by increasing the expression of *HAS2*, *TJP1*, and *FLG* genes. These effects suggest its potential role in enhancing skin barrier function and hydration. Therefore, this cream could be applied to prevent dry skin in working adults and may also benefit patients with dermatitis. In addition, *A. schmidtii* demonstrated antioxidant activity, supporting its potential as a multifunctional natural skincare ingredient. The cream also possesses a pleasant fragrance, which should be further investigated for additional dermatological benefits such as anti-hyperpigmentation and anti-wrinkle effects.

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The authors acknowledge the use of generative AI tools (e.g., QuillBot and ChatGPT by OpenAI) for language editing and grammar correction in the preparation of this manuscript. No AI was involved in content generation, data analysis, or interpretation. The authors accept full responsibility for the content and conclusions of this work.

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