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## Plant-based body lotion containing Stemona tuberosa and Aloe vera extracts enhances moisturizing property in human skin, inhibits tyrosinase activities, and Pseudomonas aeruginosa: in vitro and clinical trials

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ABSTRACT: Skin is the largest organ of the human body, serving as a protective barrier against various environmental factors, including sunlight, chemical agents, and pathogens. This study aimed to evaluate the clinical effects and moisturizing properties of a body lotion containing extracts of Stemona tuberosa and Aloe vera (BSAE) on human skin, as well as to assess the antibacterial effectiveness of the lotion against skin-infecting pathogens. A significant difference (P < 0.05) in the average water content of the epidermis was observed after volunteers used BSAE for 14 and 28 days, with values of  $84.47 \pm 1.28$  and  $100.82 \pm 1.22$ , respectively. Additionally, transepidermal water loss at day 14 and day 28 was measured at 8.31  $\pm$  1.31 and 6.83  $\pm$  0.89, respectively. The melanin content in the volunteers significantly decreased after using BSAE for 14 days, showing a value of  $269 \pm 1.89$  compared to a baseline value of  $278.43 \pm 1.03$  at day zero (before use). Furthermore, Stemona tuberosa extract demonstrated an inhibitory effect on tyrosinase activity in vitro. The BSAE also exhibited antibacterial activity against Pseudomonas aeruginosa, with a minimum inhibitory concentration (MIC) value as 16 mg/mL. GC-MS analysis identified 5-Hydroxymethylfurfural as the primary phytochemical in Stemona tuberosa extract, followed by Hexadecanoic acid. These results suggest that BSAE may enhance water retention in the epidermis, reduce transepidermal water loss and melanin content, and inhibit the growth of *P. aeruginosa*.

#### 1. INTRODUCTION

The skin is the largest organ of the human body, providing protection against various environmental factors, including sunlight, chemical agents, and pathogens such as *Pseudomonas aeruginosa*. The outermost layer of the skin, known as the stratum corneum, has selective permeability, which helps protect the skin from dryness while retaining sufficient moisture for proper function (Hoang et al., 2021). Dysfunction of the skin barrier is often indicated by a compromised stratum corneum, leading to decreased skin moisture and increased transepidermal water loss (Ribeiro et al., 2015). Hyperpigmentation, a common skin condition, occurs due to an excess deposit of melanin, resulting in darker patches on the skin (Likhitwitayawuid, 2021). Tyrosinase enzymes, found in melanocytes, are responsible for melanin produc-



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tion (Likhitwitayawuid, 2021). This enzyme catalyses the conversion of tyrosine into dihydroxyphenylalanine (DOPA), followed by the oxidation of DOPA to dopaquinone (H.Y. Chen & Yeh, 2020). To address these issues, skincare products such as body lotions are popular strategies for protecting the skin from environmental factors. These products improve a person's appearance by providing essential nutrients for healthy skin (Ribeiro et al., 2015). Additionally, incorporating plant-derived compounds into skincare formulations may serve as an alternative strategy to enhance the properties of body lotions.

Natural compounds are increasingly utilized in the formulation of modern skincare products for various purposes, including as moisturizing agents. Stemona tuberosa, a flowering plant belonging to the Stemonaceae family, is native to Asia, including Thailand (G. Chen et al., 2019). This herb has been used in traditional medicine since ancient times in China and certain South Asian countries (Xu et al., 2022). Research has shown that plants in the genus Stemona exhibit insecticidal, anti-inflammatory, and antimicrobial properties (Liu et al., 2021). Aloe vera L., a member of the Liliaceae family, has long been valued for its therapeutic benefits. The gel derived from Aloe vera is a mucilaginous, translucent substance found in the collenchyma and thin-walled parenchyma cells of its leaves (Kumar et al., 2022). Aloe vera extract possesses antiinflammatory, antibacterial, antioxidant, and wound-healing properties (Arsene et al., 2022). Notably, Aloe vera gel is wellknown for its moisturizing capabilities, helping to protect the skin from dryness.

This study aimed to investigate the moisturizing properties of a body lotion containing extracts of *Stemona tuberosa* and *Aloe vera* (BSAE) on human skin. Additionally, the antibacterial activity of the body lotion against skin infection pathogens was examined.

### 2. MATERIALS AND METHODS

### 2.1. Plant extract preparation

The overall experiments are described and summarized in a graphical abstract. The roots of *Stemona tuberosa* were washed twice and air-dried for 7 days. Next, 100 g of the dried plant material was soaked in 200 mL of 95% ethanol (1:2, w/v) at 25°C for 7 days, as previously described (Boripun et al., 2022). The mixture was then filtered using Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). The solvent was removed using a rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland). Any remaining solvent in the extract was evaporated by placing it in an incubator at 55°C for 3 to 5 days. The final extract was stored at 4°C. The extraction yield (%, w/w) was calculated by dividing the weight of the extract by the weight of the raw herb powder. A stock solution (40 mg/mL) was prepared in methanol and stored at 4°C until needed.

### Table 1

List of ingredients of herbal lotion of *Stemona tuberosa* and *Aloe vera* extracts

Ingredients	Quantity (%w/w)
Stemona tuberosa extract	5
Aloe vera extracts	2
Stearic acid	11
Isopropyl myristate	3
Glyceryl monostearate	3
Span-80	2.5
Methyl paraben	1.75
Tween 80	4
Natural fragrance	0.5
Water	67.25 q.s.*

\*; q.s. (quantity sufficient)

# 2.2. Preparation of plant-based body lotion containing *Stemona* tuberosa and Aloe vera

The ingredients of the body lotion containing the plant extracts are listed in Table 1. To prepare the plant-based body lotion, the oily components (methylparaben, Styrofoam, glyceryl monostearate, isopropyl myristate, and Span 80) were commercially purchased from K.S.P Octatech Company in Thailand and melted in a water bath at a temperature between 70°C and 85°C, according to the prescribed ratio. Meanwhile, the aqueous components (distilled water and Tween 80) were also mixed in the water bath. The oil and aqueous components were then combined and stirred continuously at a speed of 7,500 to 8,500 rpm using a homogenizer (Daihan Scientific, Korea) until an emulsion was formed. After reducing the temperature to between 40°C and 50°C, the extracts of *Stemona tuberosa* and *Aloe vera* were added. The mixture was stirred until it was fully blended into a smooth body lotion.

### 2.3. Evaluation of the effectiveness of the lotion on volunteers

In the present study, all participants provided informed consent to take part in the research. A total of 12 Thai women, aged between 20 and 50 years, who met the eligibility criteria, were selected for participation and required to complete a voluntary permission form. The inclusion criteria specified that participants must be in good health with a skin type IV melanin index, as previously described (Selwyn & Govindaraj, 2023). Exclusion criteria included a history of allergic reactions to chemicals or natural substances, skin disorders, blisters, or open sores on the test sites (specifically on both wrists, elbows, and arms), as well as pregnant or lactating women and individuals with severe or chronic illnesses that could interfere with the study. Participants who developed rashes or allergic reactions during the trial were subject to termination criteria. Throughout the testing period, participants were prohibited from using any other products for at least two weeks before and during the experiment.



# 2.4. Application of the herbal product (body lotion and data collection)

Before the study, volunteers were required to refrain from using body care products on their left inner forearm for a minimum of two weeks. It was emphasized that volunteers should thoroughly wash and dry their hands before applying the body lotion. Each volunteer then applied 1 g of the lotion to the left inner forearm, covering the area from the wrist to the inner elbow. The treatment involved gently massaging the lotion in circular motions for 3 minutes, both in the morning and evening, consistently throughout the 28-day duration. Measurements were taken before and after using the products on days 1, 14, and 28.

### 2.5. Measurement of skin condition

To measure the parameters of the volunteers' skin, they rested in a temperature-controlled room at 25°C for 30 minutes to stabilize their skin conditions. Subsequently, a probe was placed on the skin of the left inner forearm, positioned 4 centimetres from the left antecubital fossa. Each measurement was repeated three times, with each session taking approximately 1 to 2 minutes. Four distinct skin measurements were conducted during this process: the stratum corneum water content was assessed using a Corneometer® CM825 to evaluate the moisture content of the skin (Roggenkamp et al., 2021); transepidermal water loss (TEWL) was measured with a Tewameter® TM300 to assess the skin's moisture retention ability (Dabrowska & Nowak, 2021); firmness and elasticity of the skin were evaluated using a Cutometer® MPA 580; and the melanin index and erythema index were measured with a Mexameter® MX 18 to evaluate skin brightness and erythema levels (Gabe et al., 2022).

i) The stratum corneum water content was evaluated using a Corneometer<sup>®</sup> CM825, which assesses the moisture content of the skin based on its electrical properties. The keratin protein in the skin exhibits weak conductive properties, and when the skin is moist, it conducts electricity more effectively. The electrical properties of the skin are directly related to its water content. To take a measurement, the probe is placed on the abdomen of the arm, pressed slightly, and a reading is taken. This process is repeated three times, with a total measurement time of about 1 to 2 minutes (Dąbrowska & Nowak, 2021).

ii) Transepidermal water loss (TEWL) was measured using a Tewameter<sup>®</sup> TM300 to evaluate the skin's moisture retention ability and the cosmetic product's effectiveness in preventing water barrier evaporation. The probe is pressed slightly against the skin, and readings are taken consecutively five times, with a total measurement time of approximately 1 to 2 minutes (Schoenfelder et al., 2023).

iii) The firmness and elasticity of the skin were assessed using a Cutometer<sup>®</sup> MPA 580, which evaluates the mechanical properties of the skin by suctioning it into small openings. The probe (with an opening diameter of 2 mm) is applied with constant pressure for a specified duration (about 2 to 5 seconds). When the suction is released, the skin attempts to return to its original state (preferably measured over 2 to 4 seconds). This measurement is repeated three times on the inner arm, taking about 1 to 2 minutes. Due to the small size of the probe, it does not damage the underlying structures of the skin (dermis and hypodermis) (Jufri et al., 2021).

iv) The melanin index and erythema index were measured using a Mexameter<sup>®</sup> MX 18. These parameters evaluate skin pigmentation and redness, respectively. The measurement process follows similar principles as described for the Cutometer<sup>®</sup>, with a probe that has an opening diameter of 2 mm. The probe applies constant pressure for about 2 to 5 seconds, and upon release, the skin's response is observed over a preferred duration of 2 to 4 seconds. This measurement is also repeated three times on the inner arm and takes approximately 1 to 2 minutes. Again, due to the small size of the probe, it does not damage the structure of the inner skin (dermis and hypodermis) (Jufri et al., 2021).

### 2.6. Effects of Stemona tuberosa extract on tyrosinase activity

Anti-tyrosinase activity was investigated as previously described, with minor modifications (Yucharoen et al., 2023). Kojic acid, a known tyrosinase inhibitor, was used as the standard. Briefly, 20  $\mu$ L of kojic acid at various concentrations was mixed with 140  $\mu$ L of phosphate buffer (pH 6.8) and 20  $\mu$ L of tyrosinase (Sigma Aldrich, USA). The mixture was incubated at room temperature for 10 minutes in the dark. After incubation, 20 µL of L-DOPA (L-3,4-dihydroxyphenylalanine) was added, and the sample was further incubated in the dark for an additional 10 minutes. Inhibitory activity was measured at an absorbance of 492 nm. The percent inhibition of kojic acid was plotted as a standard curve and calculated using the appropriate equation. To determine the effects of Stemona tuberosa extract on tyrosinase activity, the extract at a concentration of 5 mg/mL was used instead of the standard compound and tested as described above. The absorbance of the sample was then calculated using the same equation.

### 2.7. Antibacterial activity

The antibacterial activity of BSAE against *S. aureus* and *P. aeruginosa* was evaluated using a broth microdilution assay, as previously described (Mitsuwan et al., 2020). Each type of bacteria was cultivated in Mueller Hinton broth (MHB) (Difco, Claix, France) and incubated at 37°C for 3 to 5 hours. A total of 20  $\mu$ L of the serially diluted BSAE was mixed with 80  $\mu$ L of the medium and 100  $\mu$ L of a bacterial suspension (1 × 10<sup>6</sup> CFU/mL) in a 96-well microtiter plate. The mixture was then incubated at 37°C for 18 hours. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were defined as the lowest concentrations of the extract that inhibited observable growth and killed the bacteria, respectively.

### 2.8. Phytochemical constituents in Stemona tuberosa extract

Gas chromatography-mass spectrometry (GC-MS) analysis was employed to detect the phytochemical constituents in *Stemona tuberosa* extract. The analysis was conducted using an Agilent Technologies 7890 B gas chromatograph equipped



with a 5977A Mass Selective Detector (Agilent Technologies, USA). A VF-WAXms capillary column measuring 30 m  $\times$  250  $\mu$ m  $\times$  0.25  $\mu$ m was used, with helium gas as the carrier at a flow rate of 1 mL/min. The column temperature was programmed to start at 60°C and increase to 160°C at a rate of 10°C per minute. It was then raised to 250°C at a rate of 2.5°C per minute and maintained at this temperature for 15 minutes. Operating in electron ionization mode, the mass spectrometer continuously scanned from 35 to 500 m/z at a source temperature of 230°C and an electron energy of 70 eV. The identification of phytochemical components in the extract was performed using the mass spectral data in conjunction with the Wiley library.

#### 2.9. Statistical analysis

Descriptive statistics were used to interpret the results. Triplicate results are presented as mean  $\pm$  SD. All data were recorded and entered using the statistical software package SPSS (SPSS Inc., Chicago, IL, USA). A one-way ANOVA and paired sample t-test (P < 0.05) were conducted to assess statistical significance. Differences were considered significant at p  $\leq$ 0.05.

### 3. RESULTS AND DISCUSSION

### 3.1. Efficacy of BSAE on the volunteer's skin

The human skin serves as the first line of defence and a barrier against many diseases that can affect humans and spread through the skin. Healthy, moisturized skin, whether from synthetic or naturally derived body lotions, is popular among people worldwide (Ahuja et al., 2021). Additionally, having fair skin without dark spots or hyperpigmentation is highly valued among people living in Asia, including Thailand. To enhance various properties of body lotions, some formulations include plant extracts (Nizioł-Łukaszewska et al., 2018). This study focused on the extracts of *Stemona tuberosa* and *Aloe vera*, which act as moisturizing agents in a newly formulated body lotion. The efficacy of this body lotion (BSAE) on the volunteers' skin was measured.

# 3.1.1 BSAE increased the amount of water accumulated in the epidermis

The effects of BSAE on the amount of water accumulated in the epidermis of the volunteers were investigated over different time periods. As shown in Figure 1, a significant increase in the average water content of the epidermis was observed after 14 days of using BSAE, with a value of  $84.47 \pm 1.28$  (P < 0.05). Furthermore, after 28 days, the average water content in the epidermis increased to  $100.82 \pm 1.22$ .

### 3.1.2 BSAE reduced the transepidermal water loss

Based on the increased water accumulation in the epidermis of the volunteers, the effects of BSAE on transepidermal water loss were assessed. The average transepidermal water loss measured at the initial stage (before use) was  $11.95 \pm 1.72$  (Figure 2). After 14 days of using the lotion containing the plant



Figure 1. Effects of BSAE on the amount of water accumulated in the epidermis of the volunteers at different time periods.

extracts, there was a significant reduction in transepidermal water loss among the volunteers (P < 0.05). The amounts of transepidermal water loss at days 14 and 28 were  $8.31 \pm 1.31$  and  $6.83 \pm 0.89$ , respectively.



**Figure 2.** Effects of BSAE on the transepidermal water loss atdifferent time periods.

#### 3.1.3 Effects of BSAE on the melanin content of the volunteers

The effects of BSAE on the melanin content of the volunteers were assessed over different time periods. The results showed a significant reduction in melanin content after 14 days of using BSAE, with a value of  $269 \pm 1.89$  (Figure 3). Additionally, after 28 days of using BSAE, the melanin content was measured at  $257.22 \pm 1.54$ . In comparison, the initial melanin content before use (day 0) was  $278.43 \pm 1.03$ .

### 3.1.4 Effects of BSAE on the firmness of the skin of volunteers

The effects of BSAE on the firmness of the skin of the volunteers were evaluated over different time periods. The average firmness values at days 14 and 28 were both 0.27  $\pm$  0.01, which was the same as the value recorded on day 0. However, no significant difference in skin firmness was detected among the volunteers (Figure 4).





**Figure 3.** Effects of BSAE on the melanin content of the volunteers at different time periods.



**Figure 4.** Effects of BSAE on the firmness of the skin of volunteers at different time periods.

The results revealed that the water content in the epidermis of the volunteers increased after 14 days of using BSAE. Aloe vera extract is widely used as a moisturizing agent in cosmetic formulations due to its polysacchariderich composition (Dal'belo et al., 2006). Studies have shown that formulations containing Aloe vera extract at concentrations of 0.25% and 0.50% (w/w) significantly increased the water content of the stratum corneum, as measured after a single application and after a 2-week treatment period, respectively (Dal' belo et al., 2006). Additionally, hydration of the skin was observed with the use of Aloe vera and Aloe marlothii gel, even after a single application (Fox et al., 2014). Gel products made from *Aloe vera* and Aloe ferox have also been found to reduce cutaneous erythema (Fox et al., 2014). However, it has been noted that the polysaccharide components from certain Aloe species can lead to skin dehydration after repeated applications (Fox et al., 2014). In particular, extracts from these Aloe species were prepared at a concentration of 3% w/v, which may be too high and could result in skin dehydration (Fox et al., 2014). In this study, a concentration of 2% Aloe vera was used to prepare the body lotion, which demonstrated an increase in water accumulation in the epidermis as well as a reduction in transepidermal water loss.

In addition to moisturizing the skin, moisturizers and body lotions offer several other benefits, including anti-inflammatory, antipruritic, antimitotic, and wound healing effects. The present study demonstrated that the melanin content in the volunteers was significantly reduced after 14 days of using BSAE. Melanin pigments are responsible for the colour of skin and hair. Hyperpigmentation occurs when there is an excess production of melanin in the skin, leading to dark spots that are often perceived as less radiant or bright. Research has shown that *Aloe vera* leaf extract and its pure compounds, aloin and aloesin, can help lighten skin pigmentation (Ali et al., 2012).

### 3.2. Anti-tyrosinase activity

To determine anti-tyrosinase activity, a standard curve was established using the inhibitor, kojic acid. The standard curve ( $R^2 = 0.9832$ ) is presented in Figure 5. Based on this standard curve, the tyrosinase inhibition by Stemona tuberosa extract was found to be 35.06  $\pm$  1.71. The tyrosinase enzyme, which is involved in melanin production, is present in melanocytic cells. This enzyme converts tyrosine into dihydroxyphenylalanine (DOPA), followed by the oxidation of DOPA to dopaquinone (H.Y. Chen & Yeh, 2020; Likhitwitayawuid, 2021). The present study demonstrated that the extract of Stemona tuberosa inhibited tyrosinase activity in vitro. This finding supports the potential of BSAE to influence melanin content in volunteers over different time periods.



Figure 5. A standard curve of tyrosinase inhibition using kojic acid as the standard.

# 3.3. Antibacterial activity of BSAE against *S. aureus* and *P. aeruginosa*

The antibacterial activity of BSAE against *S. aureus* and *P. aeruginosa*, representative pathogens of skin infections, was assessed using broth microdilution methods. BSAE demonstrated antibacterial activity against *P. aeruginosa*, with a minimum inhibitory concentration (MIC) of 16 mg/mL (Table 2 and Figure 6). However, the minimum bactericidal concentration (MBC) was found to be greater than 64 mg/mL. BSAE did not exhibit antibacterial activity against *S. aureus*.

Previous studies have reported that dihydrostilbene 8 and other compounds isolated from *Stemona tuberosa* possess antibacterial properties against bacteria such as *Bacillus pumilus* (Lin et al., 2008). Additionally, an aqueous extract



### Table 3

Chemical composition of the crude extract of Stemona tuberosa extract

Component RT	Percentage of total (%)	Formula	Compound name
6.8791	0.87	C <sub>5</sub> H <sub>7</sub> N	1H-Pyrrole, 1-methyl-
11.6181	0.71	$C_6H_{12}O$	Cyclohexanol
12.3508	1.31	$C_2H_4O_2$	Acetic acid
12.5381	2.52	$C_5H_4O_2$	2-furan-carboxaldehyde
13.2280	0.82	$C_7H_7Cl$	Benzene, (chloromethyl)-
14.0357	2.24	$C_6H_6O_2$	2-Furancarboxaldehyde, 5-methyl-
15.3675	0.57	$C_5H_6O_2$	2(5H)-Furanone, 5-methyl-
16.7528	2.89	$C_5H_5NO_2$	2-acetyloxazole
17.4107	1.71	$C_7H_{11}NO$	Pyridine, 1-acetyl-1,2,3,4-tetrahydro-
21.0998	1.55	$C_6H_4O_3$	2,5-Furandicarboxaldehyde
25.9205	1.92	$C_3H_6O_3$	L-Lactic acid
26.0382	1.29	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol
27.0812	0.58	$C_4H_8O_3$	Butanoic acid, 3-hydroxy-
27.2577	2.37	$C_7H_{15}NO$	1-Methyl-2-piperidinemethanol
27.4396	3.46	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
27.5465	0.57	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
31.9111	22.08	$C_6H_6O_3$	5-Hydroxymethylfurfural
32.8571	2.14	$C_8H_8O_2$	Benzene acetic acid
33.1948	0.60	$C_6H_8O_3$	2,5-Di(hydroxymethyl)-furan
35.6605	0.81	$C_{14}H_{22}N_2O$	Lidocaine
35.7889	0.56	$C_6H_5NO_2$	3-Pyridinecarboxylic acid
38.4953	8.85	$C_{16}H_{32}O_2$	Hexadecanoic acid
41.5976	1.04	$C_{18}H_{36}O_2$	Octadecanoic acid
42.0950	3.18	$C_{18}H_{34}O_2$	cis-13-Octadecenoic acid
42.2020	1.30	$C_{18}H_{34}O_2$	cis-Vaccenic acid
43.0631	5.04	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z, Z)-
44.5019	0.51	$C_{18}H_{30}O_2$	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-
45.6840	1.07	$C_6H_{10}O_5$	beta-D-Glucopyranose, 1,6-anhydro-
46.8928	3.30	$C_8H_8O_4$	Vanillic acid
52.7710	1.40	$C_{10}H_{12}O_4$	beta-(4-Hydroxy-3-methoxyphenyl) propionic acid



**Figure 6.** Antibacterial activity of BSAE against *S. aureus* and *P. aeruginosa* as determined by a broth microdilution assay. The blue colour wells exhibited the MIC of the agent against the bacteria. While, the pink wells indicated no inhibition of the bacteria at the tested concentration.

### Table 2

MIC and MBC values of body lotion from *Stemona tuberosa* and *Aloe vera* extracts against skin infectious pathogens

Antimicrobial agents	MIC/MBC (mg/mL)		
	Pseudomonas aeruginosa	Staphylococcus aureus	
Body lotion	16/>64	>64/>64	
Ceftriaxone	0.001/0.002	NA	
Vancomycin	NA	0.001/0.002	
NA=Not applicable			

of *Stemona tuberosa* was shown to decrease nitric oxide production by inhibiting cyclooxygenase-2 expression and inducing nitric oxide synthase protein in RAW 264.7 cells (Lim et al., 2015). Treatment of RAW 264.7 cells with this extract also reduced the secretion of inflammatory cytokines (Lim et al., 2015). Furthermore, a pure compound known as tuberostemonine N, extracted from the root of *Stemona tuberosa*, prevented the secretion of pro-inflammatory cytokines and chemokines in bronchoalveolar lavage fluid from mice (Jung et al., 2016). The anti-inflammatory activity of compounds present in *Stemona tuberosa* extract may play a significant role





Figure 7. Structures of the mainphytochemicals presented in *Stemona tuberosa* extract as detected by GC-MS analysis.

in its potential applications, warranting further investigation before any definitive conclusions can be drawn. Recently, our research group reported on the antioxidant activities of *Stemona tuberosa* extract (Laohaprapanon et al., 2024).

### 3.4. Phytochemicals in Stemona tuberosa extract

The phytochemicals in *Stemona tuberosa* extract were investigated using GC-MS analysis. As shown in Table 3, a total of 30 compounds were detected in the crude extracts. The results revealed that 5-hydroxymethylfurfural was the predominant compound in *Stemona tuberosa*, followed by hexadecanoic acid and 9,12-octadecadienoic acid (Z,Z). Additionally, the main phytochemicals detected in the extract are presented in Figure 7. The isolation of alkaloids such as tuberostemonine K, tuberospironine, and tuberostemonine has been documented (Jiang et al., 2006); however, these compounds were not detected by GC-MS in this study.

### 4. LIMITATION AND RECOMMENDATION

To further evaluate the efficacy of BSAE, in vivo studies involving men and individuals from different age groups is recommended. Additionally, the anti-inflammatory activity of BSAE should be assessed *in vitro*, along with its cytotoxicity. Investigating the antibacterial activity of BSAE against other species of skin infectious pathogens would also be valuable. Furthermore, an analysis of the market potential for BSAE, including a business startup evaluation, should be conducted to predict the product's viability in the market.

### 5. CONCLUSION

This study demonstrates that BSAE, prepared from extracts of *Stemona tuberosa* and *Aloe vera*, exhibits moisturizing properties on human skin. A significant increase in the average water content of the epidermis was observed after the volunteers used BSAE for 14 days. Additionally, the amount of transepidermal water loss at day 14 was measured, and during the same period, the melanin content in the volunteers significantly decreased following the use of BSAE. The extract of *Stemona tuberosa* was found to inhibit tyrosinase activity *in vitro*. Furthermore, BSAE exhibited antibacterial activity against *P. aeruginosa*, with the MIC value of 16 mg/mL. These results suggest that BSAE may enhance water accumulation in the epidermis, reduce transepidermal water loss and melanin content, and inhibit the growth of *P. aeruginosa*.

### **CONFLICTS OF INTEREST**

The authors declare that they have no competing interests.

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### **ETHICAL APPROVAL**

This study was performed in accordance with the principles outlined in the Declaration of Helsinki. All procedures involving volunteers were approved by Walailak University's Human Research Committee (WUEC-21-273-02). Additionally, all experiments involving bacteria were approved under the biosafety regulations for scientific research at Walailak University, Thailand (WU-IBC-66-020).

### AUTHOR CONTRIBUTIONS

SL and WM conceived and designed the experiments. SL, SJ, and WM conducted the experiments, while SL, SJ, and WM analysed and interpreted the data. SL and WM performed the statistical analyses. VN contributed reagents, materials, analytical tools, and data. SL, VN, MLP, SV, and WM drafted the manuscript. All authors have read and approved the final version of the manuscript.

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