

Development of PVA/HA-dissolving microneedles containing *Acanthus ebracteatus* Vahl. stem extract for antioxidant activity

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ABSTRACT

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Received: 13 May 2025
Revised: 24 August 2025
Accepted: 2 September 2025
Published: 25 May 2026

Citation:
Tansathien, K., Sobharaksha, P., Wongtrakul, P., Neimkhum, W., Opanasopit, P., & Suriyaamporn, P. (2026). Development of PVA/HA-dissolving microneedles containing *Acanthus ebracteatus* Vahl. stem extract for antioxidant activity. *Science, Engineering and Health Studies*, 20, 26050006.

Acanthus ebracteatus Vahl. has been reported in Thai herbal medicine for treating various ailments, such as skin diseases, and for skin health promotion. This study aimed to determine the antioxidant efficacy of the extract and to develop dissolving microneedles. Stems and leaves were extracted, and their antioxidant activities (DPPH assay and total phenolic content) were evaluated. Characterization of the dissolving microneedles was then performed. The results indicated that the % yield of the stem and leaf extract was 8.02 ± 1.34 % and 6.04 ± 0.13 %, respectively. The stem contained a higher total phenolic content than the leaf, showing 19.75 ± 1.33 and 15.02 ± 2.07 mg of gallic acid equivalent per gram, respectively. The concentration of the extracts required to inhibit 50% of DPPH radicals (IC_{50}) was 130.28 ± 2.29 μ g/mL for the stem and 254.23 ± 3.52 μ g/mL for the leaf. These extracts were non-toxic to keratinocytes. The optimal ratio of PVA and HA in the dissolving microneedles was 8:2. The height of the needles was 449.36 ± 4.89 μ m, and the base width was 224.04 ± 2.81 μ m, which indicates that they could completely penetrate the first layer of Parafilm. Moreover, the mechanical strength indicated that the PVA:HA (8:2) microneedles experienced a lower percent height reduction compared to others, while showing rapid dissolution. Finally, the PVA/HA-dissolving microneedles containing the extract show potential for transdermal delivery.

Keywords: *Acanthus ebracteatus* Vahl.; antioxidant; dissolving microneedle; hyaluronic acid; polyvinyl alcohol

1. INTRODUCTION

The subspecies of *Acanthus ebracteatus* Vahl. is a plant species belonging to the genus *Acanthus* and the family *Acanthaceae*, which was found to be distributed in many tropical and subtropical regions such as South Asia,

Southeast Asia, East Asia, Australia, and the western Pacific Islands (Bora et al., 2017). Reports indicate that Thais use all parts of *A. ebracteatus* Vahl. for medicinal purposes. The stem was boiled to relieve the pain of the human body, and the leaf was pounded with alcohol and applied for treatment of knee pain. Moreover, the

whole plant was used as a tonic (Somprasong et al., 2014).

Various solvents, such as water, various concentrations of ethanol, and hexane, have been employed to isolate bioactive compounds from this herb, directly influencing its resulting pharmacological activities. Aqueous extracts primarily consist of polysaccharides, whereas organic extracts yield total phenolic compounds and flavonoids. (Hokputsa et al., 2004; Olatunji et al., 2022). Ethanol, in particular, is widely reported for the maceration of this species. The plant has demonstrated several health benefits, including antioxidation, anti-tyrosinase, anti-aging, anti-inflammation, wound healing, anti-hair loss, antimicrobial, and anticancer properties (Olatunji et al., 2022; Wisuitiprot et al., 2022; Kanlayavattanukul et al., 2023; Kanlayavattanukul et al., 2024). UHPLC-ESI-QTOF-MS profiling of the ethanolic extract revealed that the majority of constituents in the stem and leaf are glycosidic, specifically flavonoids and phenolic compounds. Identified simple phenolics and phenolic glycosides include hydrojuglone glucoside, 4-glucogallic acid, verbascoside, chlorogenic acid, and caffeic acid. Flavonoids identified include apigenin, disometin, luteolin, saponarin, vestitone 7-glucoside. Certain phytochemicals such as quinic acid, echinacoside, dihydromelilotoside, amorphigenol O-vicianoside are presented in the stem and root, but absent in the leaf extract (Olatunji et al., 2022).

In the 21st century, Thailand is experiencing an unprecedented demographic transformation as its population ages rapidly. driven by declining birth rates and increasing life expectancy (Anantanasuwong, 2021). Aging is characterized by a progressive decline in physiological function, involving complex systemic changes at the molecular, cellular, and organ levels. These changes impair the body's ability to maintain homeostasis, resist disease, and repair damage (Osareme et al., 2024). In response, regenerative cosmetics aim to restore the skin's natural function and structure using biocompatible technologies. Key modalities for addressing age-related skin changes include microneedling, hyaluronic acid filler, and the delivery of active biological ingredients, (Pleguezuelos-Beltrán et al., 2024).

The skin serves as the protective barrier against physical, chemical, and mechanical insults. It is composed of three main layers: the epidermis, dermis, and hypodermis. The epidermis, the outermost layer of the skin, contains the stratum corneum as its primary barrier. The cellular composition of the epidermis is predominantly keratinocytes, alongside melanocytes, Langerhans cells, and Merkel cells (Yousef et al., 2025). While active ingredients like flavonoids and phenolic compounds offer significant therapeutic potential for skin disorders, their efficacy is often limited by poor percutaneous penetration and low stability. Various strategies, such as dendrimers, lipid-based nanotechnologies, have been employed to enhance transdermal delivery (Cheng et al., 2020; Costa et al., 2021; Gugleva et al., 2021). However, even these nanocarriers often fail to sufficiently overcome the skin barrier. An innovative alternative is the use of microneedles, which can bypass the stratum corneum, to facilitate efficient permeation of active compounds into the deeper skin layers (Gowda et al., 2024).

Microneedling technology utilizes sharp tips to penetrate the stratum corneum and reach the dermis, creating microchannels for substance transfer (Ramadon

et al., 2022). Compared to traditional transdermal drug delivery systems, microneedles offer advantages such as being painless, avoiding first-pass metabolism, improving patient compliance, and allowing for self-administration (Waghule et al., 2019). Dissolving microneedles (DMNs) represent a key category, fabricated by encapsulating active compound within water-soluble or biodegradable polymers such as hyaluronic acid (HA) and polyvinyl alcohol (PVA)). The DMN mechanism is characterized by "poke and release" action. Recent literature has favored DMNs due to their use of safe, biocompatible and mechanically robust materials that maintain the efficacy of encapsulated compounds (Chi et al., 2022; Oh et al., 2022; Sartawi et al., 2022).

To overcome the limitations of conventional delivery, this study developed DMNs to load *A. ebracteatus* Vahl. extract, which has not been previously explored. The objective was optimize the PVA/HA DMNs formulation for the delivery of antioxidant-rich *A. ebracteatus* Vahl extract.

2. MATERIALS AND METHODS

The whole plant of *A. ebracteatus* Vahl. was collected from the Bang Duan subdistrict, Samut Prakan province, Thailand. Polyvinyl alcohol (PVA 117) was purchased from Chem-Supply, Australia. Hyaluronic acid (10⁶ Daltons) was obtained from Chemipan Corporation Co., Ltd., Bangkok, Thailand. The 2,2-dimethyl-1-picrylhydrazyl radical (DPPH) was sourced from Sigma-Aldrich, St. Louis, MO, USA. All reagents used were of analytical grade.

2.1 Preparation of *A. ebracteatus* Vahl. crude extract

Both fresh stems and leaves of *A. ebracteatus* Vahl. were cleaned and dried to completion in a hot air oven at 60°C for 72 h. The dried samples were then pulverized into a fine powder using a blender and kept at -20°C before use.

The dried powdered stems and leaves were macerated in 95% ethanol at a ratio of 1:10 (w/v) with continuous agitation using a magnetic stirrer at room temperature for three days. The extracts were filtrated and concentrated to dryness crude extract using a rotary evaporator and a steam bath. The extractive yield was calculated as a percentage according to Equation 1. The resulting dried extracts were kept at 4°C until further testing.

$$\% \text{ Yield} = \frac{W_1}{W_0} \times 100 \quad (1)$$

where W_0 represents the dried weight of the powder before extraction, W_1 represents the weight of the dried extract after evaporation.

2.2 Determination of antioxidant activity

The antioxidant activity was evaluated using the DPPH radical scavenging assay. A working solution of the stable free radical of 2,2-dimethyl-1-picrylhydrazyl (DPPH•) was dissolved in 95% (v/v) of ethanol to a final concentration of 0.0066% (v/v). Briefly, 200 μ L of the DPPH• working solution was mixed with 50 μ L of extract samples at various concentrations (0.125, 0.25, 0.50, 1, 2, and 5 mg/mL) in a 96-well plate. The mixture was incubated in the dark at room temperature for 30 min. The absorbance was measured at 520 nm using a UV-Vis spectrophotometer. The antioxidant results were expressed as the half-maximal inhibitory concentration

(IC50) compared with ascorbic acid as a standard. The DPPH scavenging activity was computed according to Equation 2:

$$\% \text{Scavenging activity} = \frac{\text{Absorbance of control} - (\text{Absorbance of sample} - \text{blank of sample})}{\text{Absorbance of control}} \times 100 \quad (2)$$

2.3 Determination of bioactive compound

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method. Briefly, a serial dilution of gallic acid, as a standard, was prepared to create a standard calibration curve. Fifty microliters (50 μL) of sample were mixed with 50 μL of deionized (DI) water and 195 μL of Folin-Ciocalteu reagent in a 96-well plate in the dark for 10 min, then, 20% (w/v) Na_2CO_3 was added, and the absorbance was recorded at 755 nm using a UV-Vis spectrophotometer. The results were computed from the standard curve of gallic acid and reported as g of gallic acid equivalents per g of dry weight extract (g GAE/g extract).

2.4 Safety assessment of *A. ebracteatus* Vahl. extract on keratinocyte cells (HaCaTs)

HaCaTs were cultured in DMEM supplemented with 10% (v/v) of FBS, 1% (v/v) of GlutaMax™, 1% (v/v) of non-essential amino acids, and 1% (v/v) penicillin-streptomycin under a 5% CO_2 /95% humidified atmosphere at 37°C.

Briefly, HaCaTs (10,000 cells per well) were seeded in a 96-well culture plate and incubated in an incubator for 48 h. After that, the culture medium was removed, and both stem and leaf samples at various concentrations of 0.003125, 0.00625, 0.0125, 0.025, 0.05, and 0.1 % w/v, including the control (untreated), were added to treat the HaCaTs overnight. The 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay determined the cell viability. The test samples were discarded, and then the cells were exposed to 100 μL of MTT solution (1 mg/mL) for 3 h. The MTT solution was then replaced with 100 μL of dimethyl sulfoxide (DMSO) to dissolve the formazan crystals of living cells. Absorbance was quantified at 550 nm using a microplate reader. The cell viability was calculated using Equation 3:

$$\% \text{Cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \times 100 \quad (3)$$

2.5 Preparation of PVA/HA DMNs

The PVA/HA DMNs were fabricated using a centrifugation method. Firstly, 10% (w/v) of PVA was blended with 5% (w/v) of HA in five different ratios of 2:8, 4:6, 5:5, 6:4, and 8:2. Then, 1 g of the polymer mixture was added to the molds (10 x 10 arrays of rubber molds fabricated via 3D printing). The molds were centrifuged at 5,000 rpm for 15 min to fill the mold cavities with no bubbles. The molds were placed in an incubator at 40°C overnight and kept at room temperature to dry for approximately 2 days prior to removal from the molds.

After characterization of the five DMN formulations, the optimal PVA/HA DMN ratio was selected to load *A. ebracteatus* Vahl. Extract (at a final concentration of 0.2% w/w). Before loading, the extract was dissolved in propylene glycol. The fabrication method for extract-loaded PVA/HA was the same as described above.

2.6 Characterizations of PVA/HA DMNs

The surface appearance of all PVA/HA DMNs was imaged under an optical microscope. Additionally, the individual DMN's structure (e.g., height and width) was examined using the NIS-Elements software in triplicate.

2.7 In vitro insertion test of PVA/HA DMNs

According to Larrañeta et al. (2014), an alternative method using an artificial membrane (Parafilm® M) as a skin layer model of microneedle insertion was used for needle penetration testing (Larrañeta et al., 2014). A sheet of Parafilm® M (PF) was folded into eight layers (total thickness of approximately 1 mm). Briefly, the PVA/HA DMNs were placed on the layered PF and compressed by a thumb for 30 s (Saepang et al., 2024). After insertion, the DMNs were removed from the PF sheet. The number of holes in each layer was observed using an optical microscope with the NIS-Elements software. The % penetration of DMNs was computed following Equation 4 (Babapour et al., 2024):

$$\% \text{ Penetration of layer } n = \frac{\text{Number of holes created in layer } n}{\text{Total number of needles}} \times 100 \quad (4)$$

2.8 Mechanical strength determination

The mechanical strength of the PVA/HA DMNs was measured using a texture analyzer (TA.XTExpressC, Stable Micro Systems, Godalming, UK). The fabricated DMNs with varied PVA:HA ratios were attached to a flat probe using thin double-sided tape. During testing, the probe moved vertically downward under the following conditions: a force of 10 N, a triggered force of 0.049 N per 100 needles, a speed of 0.5 mm/s, and a 30-s hold time. The percentage reduction in height was calculated by Equation 5.

$$\% \text{ Height reduction} = \left[\frac{H_0 - H_1}{H_0} \right] \times 100 \quad (5)$$

where H_0 is the initial height of the DMNs and H_1 is the height of the DMNs after compression.

2.9 Dissolution analysis of DMNs

An *in vitro* dissolution test of the PVA/HA DMNs was performed based on the method by Park et al. (2016) with modifications. The study aimed to confirm the dissolution rate of the optimal PVA:HA (8:2) DMNs. Briefly, 5 mL of phosphate-buffered pH 7.4 solution was added to each vial. The 8:2 PVA/HA DMNs were introduced to the medium at room temperature, and samples were observed at specified time intervals of 0, 1, 5, 10, 15, and 20 min. The reduction in DMNs height was observed and estimated under an optical microscope using the NIS-Elements software in triplicate (n=3).

2.10 Active compound content and antioxidant of extract-loaded DMNs determination

The primary active compounds evaluated were total phenolics. DMNs containing the extract were dissolved in DI water; then the loaded active substances were evaluated for total phenolic content and antioxidant activity as described above. The percentages of total phenolic compound loading and % inhibition were computed according to Equations 6 and 7, respectively.

$$\% \text{ Total phenolic compound loading} = \left[\frac{\text{Loaded amount of sample}}{\text{Initial amount of sample}} \right] \times 100 \quad (6)$$



$$\% \text{ Inhibition} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of loaded extract})}{\text{Absorbance of control}} \right] \times 100 \quad (7)$$

2.11 Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). Statistically significant differences ($p < 0.05$) were determined by one-way ANOVA, followed by Turkey's post hoc test, or by Student's t-test (two-tailed) and paired t-test.

3. RESULTS AND DISCUSSION

3.1 Evaluation of percent yield of *A. ebracteatus* Vahl. crude extract

The collected aerial part of the Thai *A. ebracteatus* Vahl. herb, the appearance of the dried ground powder of leaves and stems, the extraction process, and the resulting crude extracts are shown in Figure 1. The stem and leaf extracts exhibited extractive percentage yields of $8.02 \pm 1.34\%$ and $6.04 \pm 0.13\%$, respectively.

3.2 Antioxidant activities and bioactive compounds

The dried powders of the stems and leaves from *A. ebracteatus* Vahl. were assessed for antioxidant activity and the total phenolic content. The ethanolic stem and leaf

extracts demonstrated antioxidant activity, as shown in Table 1. The DPPH radical scavenging activity of the stem extract was significantly higher than that of the leaf extract, with IC_{50} value of $130.28 \pm 2.29 \mu\text{g/mL}$ and $254.23 \pm 3.52 \mu\text{g/mL}$, respectively. However, ascorbic acid, used as a standard, was more potent than both extracts, exhibiting an IC_{50} of $33.60 \pm 0.99 \mu\text{g/mL}$. Consequently, these extracts exhibited moderate antioxidant activity compared to the standard. Regarding the antioxidant defense mechanism measured by DPPH assay, the active compounds served as free-radical scavengers and hydrogen donors (Gulcin & Alwasel, 2023). Phenolic compounds are major bioactive substances linked to antioxidant properties (Kalpoutzakakis et al., 2023). Total phenolic content was determined using the Folin-Ciocalteu (FC) method. The FC reaction measures antioxidant ability via electron transfer, which reflects the reducing capacity (Lamuela-Raventós, 2018). The total phenolic content in the stem extract was $19.75 \pm 1.33 \text{ mg GAE/g}$, while the leaves extract yielded $15.02 \pm 2.07 \text{ mg GAE/g}$. However, the difference in total phenolic compounds between both parts was not statistically significantly ($p > 0.05$). Suriyaporm et al. (2024) reported that ethanolic extract of *A. ebracteatus* had a total phenolic content at 22.5 mg GAE/g extract and an IC_{50} for DPPH radical scavenging of 0.24 mg/mL , which is consistent with the finding of our study (Suriyaprom et al., 2024).

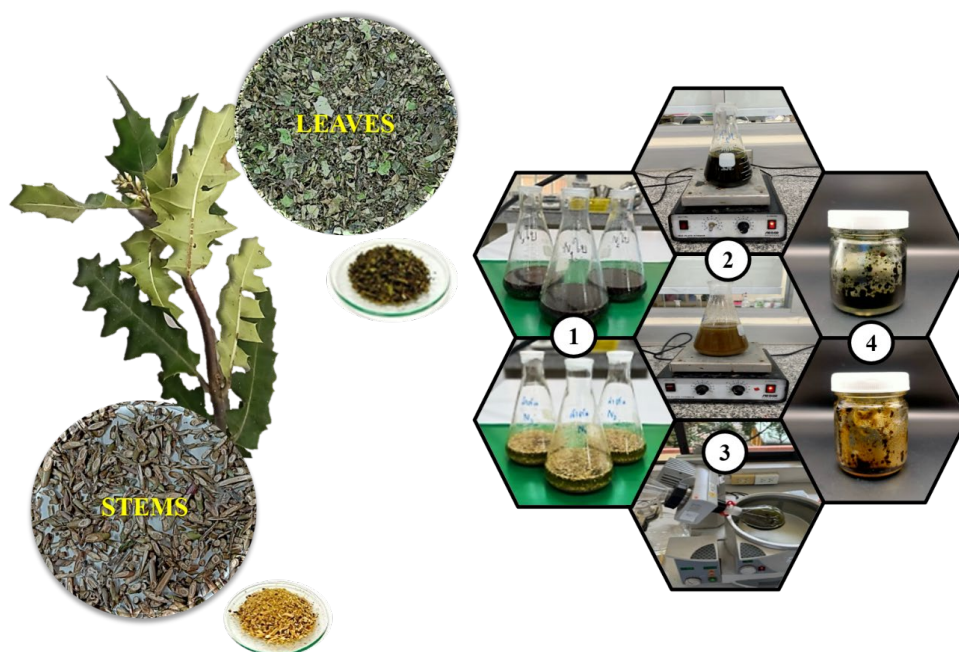


Figure 1. Botanical materials and extraction process of *Acanthus ebracteatus* Vahl.

Table 1. Total phenolic content and antioxidant activity (IC_{50}) of *A. ebracteatus* Vahl. stem and leaf ethanolic extracts

Sample	IC_{50} values ($\mu\text{g/mL}$)	Total phenolic content (mg GAE/ g extract)
Stem part	$130.28 \pm 2.29^{*,**}$	19.75 ± 1.33
Leaf part	$254.23 \pm 3.52^*$	15.02 ± 2.07
Ascorbic acid	33.60 ± 0.99	-

Note: IC_{50} and total phenolic content are presented as mean $\pm$ SD ($n=3$); * indicates a significant difference at p -value < 0.05 compared to ascorbic acid (one-way ANOVA), ** indicates a significant at p -value < 0.05 between stem and leaf extract (paired t-test).

3.3 Safety assessment of ethanolic *A. ebracteatus* Vahl. extract in HaCaTs

The safety of botanical active compounds from plant sources in a cell culture model is essential for personal care products (Antignac et al., 2011). The MTT assay was used to evaluate the viability of HaCaTs treated with various concentrations (0.003125 – 0.1 %w/v) of ethanolic stem and leaf extracts. A cell viability of 80% or more is generally considered indicative of a non-toxic profile. (López-García et al., 2014). The cell viability results for both extracts are presented in Figure 2. The results demonstrated varying degrees of cytotoxicity depending on the concentrations and plant parts. In addition, linear regression analysis was used to determine the CV₈₀ (the concentration resulting in 80% cell viability). The stem extract exhibited a higher CV₈₀ than the leaf extract, with values of 0.012 ± 0.001 % w/v and 0.007 ± 0.001 % w/v, respectively.

3.4 Fabrication and characterization of PVA/HA DMNs

Selected polymers for creating DMNs included PVA and HA, previously reported as biocompatible and biodegradable polymers (Chiellini et al., 2003; Chudzińska et al., 2024). The PVA/HA DMNs were fabricated in different ratios (2:8, 4:6, 5:5, 6:4, and 8:2) of PVA to HA in aqueous solution, which aimed to find an appropriate polymer concentration to load an extract. Figure 3 summarizes the optical microscopy observations for DMNs made from different blend ratios of polymers. The PVA blend with HA produced from the molding process was confirmed to have a

favorable overall shape, and the needles were well-formed in F1 to F5. All needles in F1-F5 showed a tip the height between 398 and 449 µm with a base width in the range of 221 to 243 µm, as shown in Table 2. In this study, hyaluronic acid was extracted from *Tremella fuciformis* Sporocarp, which has an average molecular weight of 10⁶ Daltons. HA is a natural linear chain polymer belonging to a group of heteropolysaccharides, known as glycosaminoglycans (GAGs). The main structure contains a repeating disaccharide, which consists of *N*-acetyl-D-glucosamine and D-glucuronic acid connected by β-(1→3)-glycosidic linkage. Two HA molecules are connected by hydrogen bonds, which contribute to rigidity during production. In the previously published work, the network depends on the molecular weight and concentration of HA, such that high molecular weight native HA (>10⁶ Da) forms an extended network at a concentration of 1 µg/mL (Fallacara et al., 2018). HA is recommended for the fabrication of DMNs; specifically, HA can help skin by stimulating the proliferation of fibroblasts, inducing cell migration, and producing collagen (Choi et al., 2017). Thus, HA-based microneedles have been widely used in the pharmaceutical and cosmetic industries for applications such as destroying skin cell architecture, stimulating elastin and collagen production, and drug delivery (Saha & Rai, 2021). PVA as the matrix polymer of the DMNs is not only biocompatible, but it also has excellent mechanical properties and processability (Zhang et al., 2021). When PVA and HA were combined to make the DMNs, it was found that they provided a skin hydration or moisturizing effect by reducing transepidermal water loss (TEWL) (Kitsongsermthong et al., 2021).

Table 2. Physical dimensions and formulations of PVA/HA-based DMNs

Formulation	PVA	HA	Tip height (µm)	Base width (µm)
F1	2	8	400.98 ± 15.81	222.21 ± 9.73
F2	4	6	419.17 ± 18.41	221.34 ± 14.82
F3	5	5	434.87 ± 1.99	243.80 ± 10.60
F4	6	4	398.17 ± 11.25	227.31 ± 4.13
F5	8	2	449.36 ± 4.89	224.04 ± 2.81
F6	8	2	435.52 ± 6.44	234.79 ± 8.02

Note: *Data exhibited mean ± SD (n=3)

3.5 *In vitro* insertion capabilities of PVA/HA DMNs

In the present work, the insertion ability of the PVA/HA-based DMNs into the 8-layer PF overlay demonstrated that almost all the DMN formulations without an extract (F1, F3-F5) could penetrate the 1st to 3rd PF layers after thumb-forced compression into the stack of 8-layer PF, while F2 could only reach the 2nd PF layer. The DMNs that penetrated down to the first layer of PF at 100% insertion were presented as F1, F4, and F5. Upon imaging, the micropores reached each PF layer, and these micropores were formed as depicted in Figure 4 (F1-F5). Based on the needles penetrating the PF layer at 100%, it was assumed that the DMNs could overcome the skin barrier and reach the epidermis layer. Skin is classified into 3 layers: the epidermis, the dermis, and subcutaneous tissue, which includes the skin barrier known as the stratum corneum.

In each human skin layer, the stratum corneum thickness is about 20 µm (Lee et al., 2018), while the depth of the epidermis is between 75 and 150 µm (up to 600 µm on soles or palms). In addition, the dermis is usually less than 2,000 µm (up to 4,000 µm on an adult back) (Wong et al., 2016). In the case of PF, a single layer of it exhibited a thickness of around 130 µm (Qu et al., 2016). Consequently, F5 could penetrate better than F1 and F4 because of the number of holes created in the 2nd and 3rd layers.

After interpreting the characterization and penetration capability, F5 was chosen to load *A. ebracteatus* Vahl. stem extract. The morphology of the DMNs displayed in Figure 3 (F6) showed that the needles had a conical shape with sharp tips and a length of 435.52 ± 6.44 µm. In the penetration test, it could insert down to 3rd PF layers, as shown in the results in Figure 4 (F6).

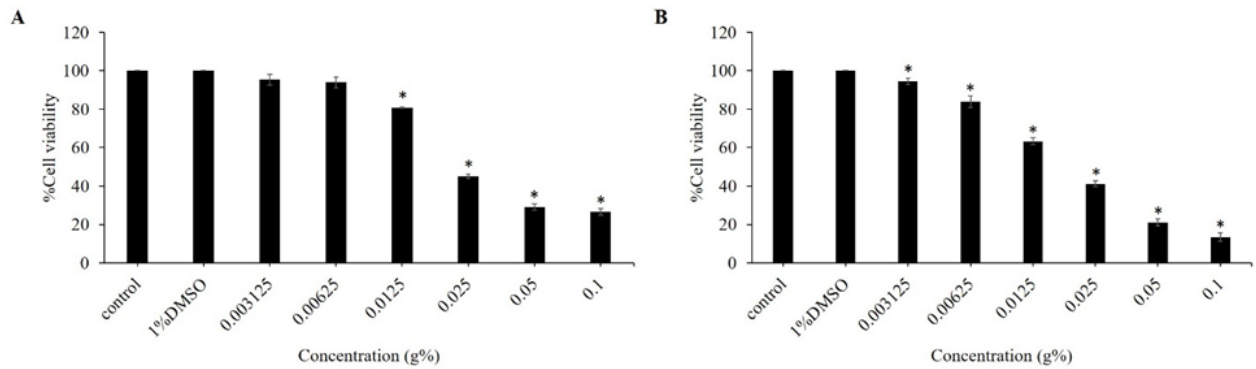


Figure 2. Cytotoxicity profile of *A. ebracteatus* Vahl. extracts on HaCaT keratinocytes. Cell viability of HaCaTs cells treated with various concentrations of ethanolic *A. ebracteatus* Vahl. (A) stem extract and (B) leaf extract for 48 h

Note: Control groups were treated with medium only. * indicates a significant difference from the control group at $p < 0.05$ (Student's *t*-test)

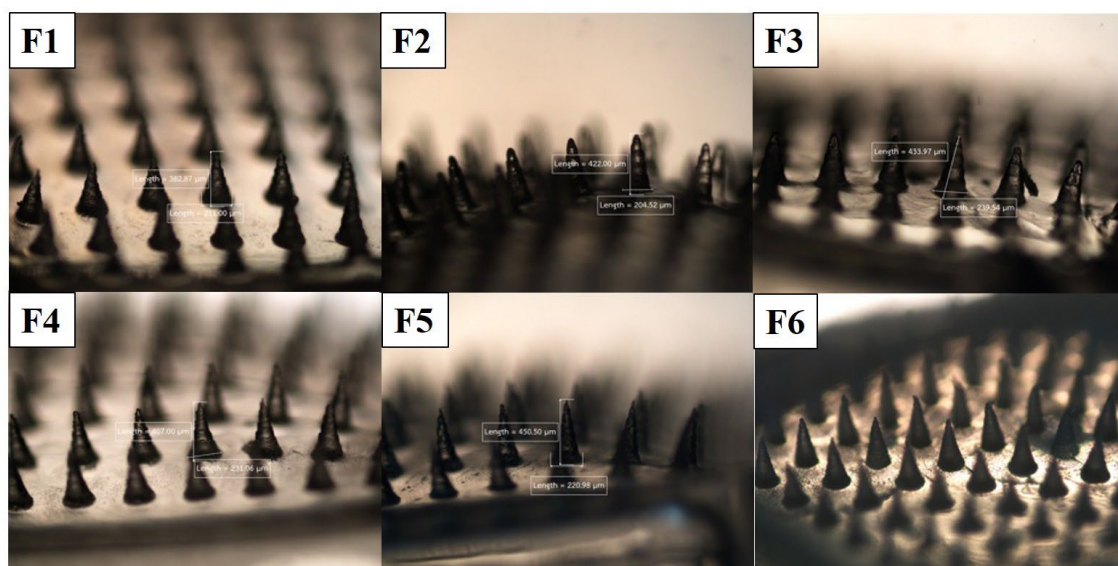


Figure 3. Representative optical microscope images (4× magnification) of PVA/HA DMNs fabricated with various polymer blend ratios; F1-F5 represent PVA:HA ratios of 2:8, 4:6, 5:5, 6:4, and 8:2, respectively F6 represented 8:2 the PVA: HA ratio loaded with *A. ebracteatus* Vahl. stem extract

3.6 The mechanical strength of DMNs

DMNs require sufficient mechanical strength to pierce the skin effectively without breaking or bending during application. Generally, a texture analyzer is used to assess the relationship between movement and strength of DMNs over time to estimate the force at which the DMNs' tips bend or break. Currently, the minimum force required to penetrate skin is 0.058 N/needle (Kim et al., 2013). Some reports revealed that microneedles can withstand forces up to 14 N per whole array; however, the microneedle strength depends on several factors, including material, geometry, and loading conditions (Harieth Alrimawi et al., 2024). In the case of PVA-based DMNs, the compression force can be adjusted to 34 N when the number of needles is increased to 16×16 needles (Ando et al., 2024). Figure 5 in this study displays the percentage height reduction of DMNs fabricated with various ratios of PVA and HA under axial force. The % height reduction of DMNs indicated that formulation F5 was the best candidate for

loading *A. ebracteatus* Vahl. stem extract. F5 showed a % height reduction less than the others because the content of PVA and HA affected the mechanical strength of the DMNs. However, the % height reduction of each formulation was not significantly different between groups at a p -value < 0.05 . These results suggest that the amount of water in the base materials of PVA-based DMNs is very important for achieving the best mechanical strength (Ando et al., 2024). Likewise, HA has strong hygroscopic properties and a variety of molecular weights, which exhibit different mechanical properties. It was reported that the mechanical strength of microneedles decreased with increasing molecular weights of HA (Chi et al., 2022). Consequently, F5 had the best penetration and the lowest height reduction after withstanding mechanical stress. After the *A. ebracteatus* Vahl. stem extract (F6) was loaded in F5, the percentage of height reduction slightly increased, but there was no significant difference.

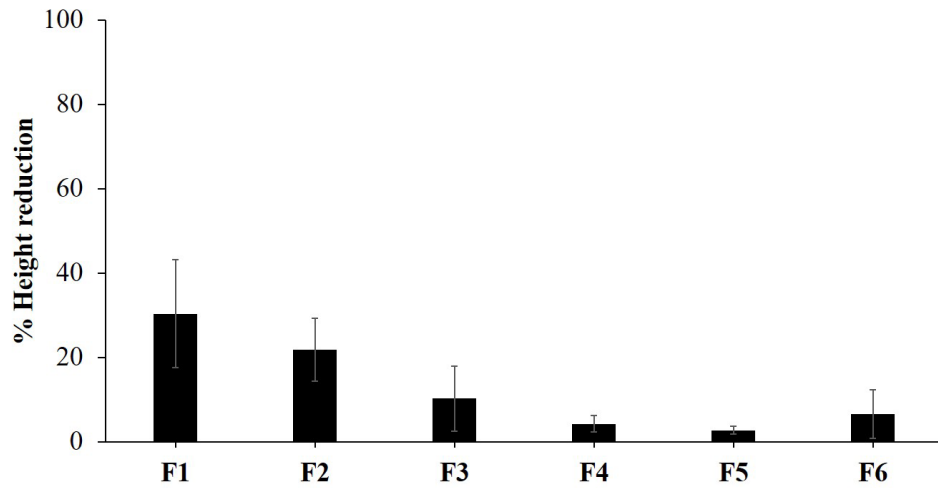


Figure 4. Representative optical microscopy images (4× magnification) demonstrating the penetration capabilities of blank PVA/HA DMNs (F1-F5) and *A. ebracteatus* Vahl. stem extract-loaded DMNs (F6) through multiple layers of Parafilm M®

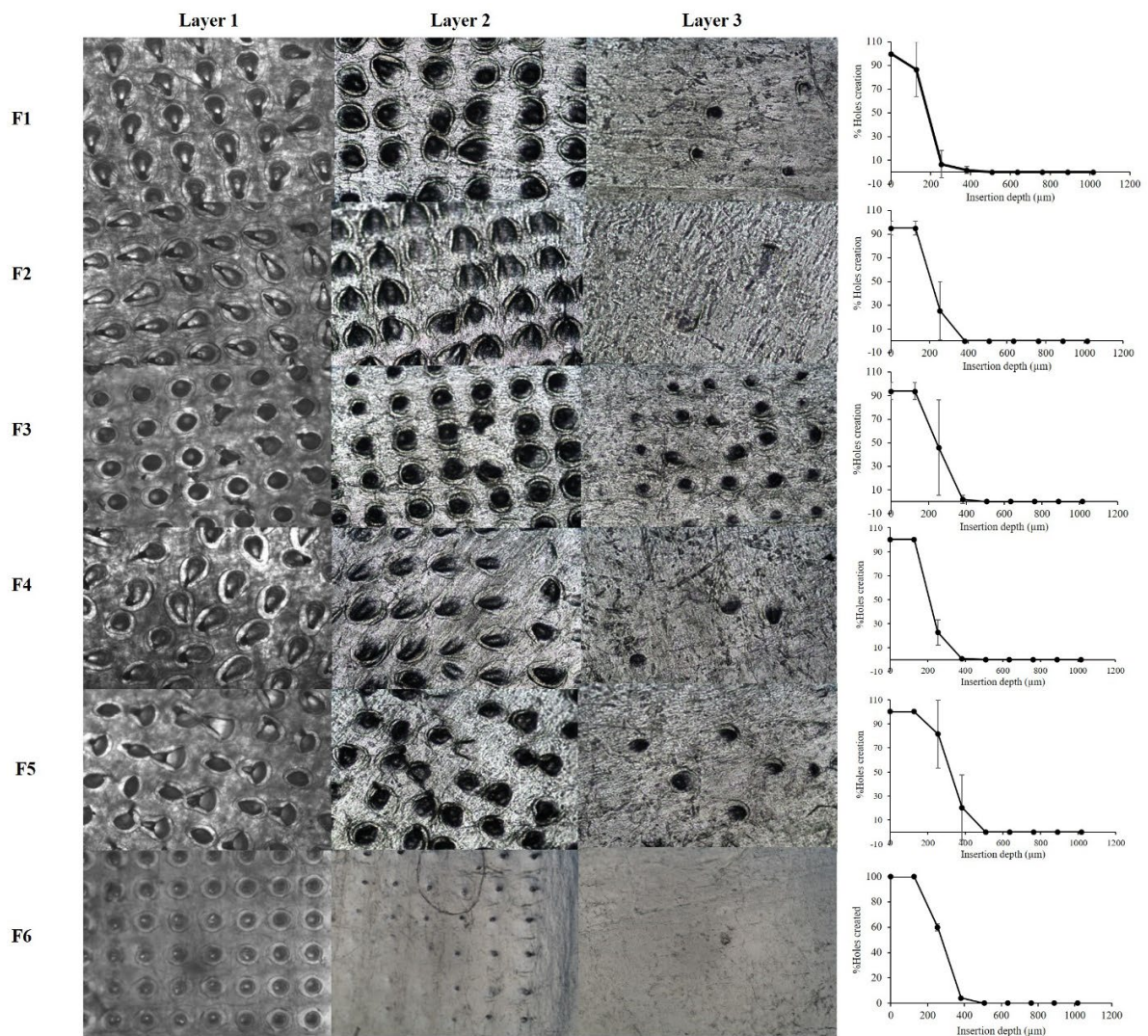


Figure 5. The percentage height reduction of F1-F6 (N=3)

3.7 Dissolution rate of PVA/HA-fabricated DMNs

For the *in vitro* dissolution study, the 8:2 PVA/HA DMNs (F5) was chosen based on their morphologies. The dissolution rate study monitored the change in microneedle structure over time at 0, 1, 5, 10, 15, and 30 min, as presented in Figure 6. As shown in the results, the DMNs demonstrated rapid dissolution; the needles began to visually bend after 1 min and height was slightly reduced in a time-dependent manner. As per the previous results, the PVA/HA DMNs at a ratio of 8:2 were selected to study for delivery of active ingredients with immediate release. In the current report, the performance of PVA-based microneedle arrays revealed that PVA microneedles with a length of about 500 μm had previously shown a dissolution

rate that decreased with time, dissolving completely in about 40–60 min (Abdelghany et al., 2022; Nasiri et al., 2022). For hyaluronic acid-based DMNs, HA is an excellent water-soluble polymer; the HA-based microneedles presented rapid dissolution and sustained release. However, the different molecular weights of HA affected the dissolution rate. The dissolution behavior illustrated that low molecular weight HA dissolved faster than high molecular weight HA. Meanwhile, the dissolution time for high molecular weight was longer than for low molecular weight (Miura et al., 2025). In this study, the use of high molecular weight HA might have affected the dissolution time, as the profile showed a trend of rapid dissolution and sustained release.

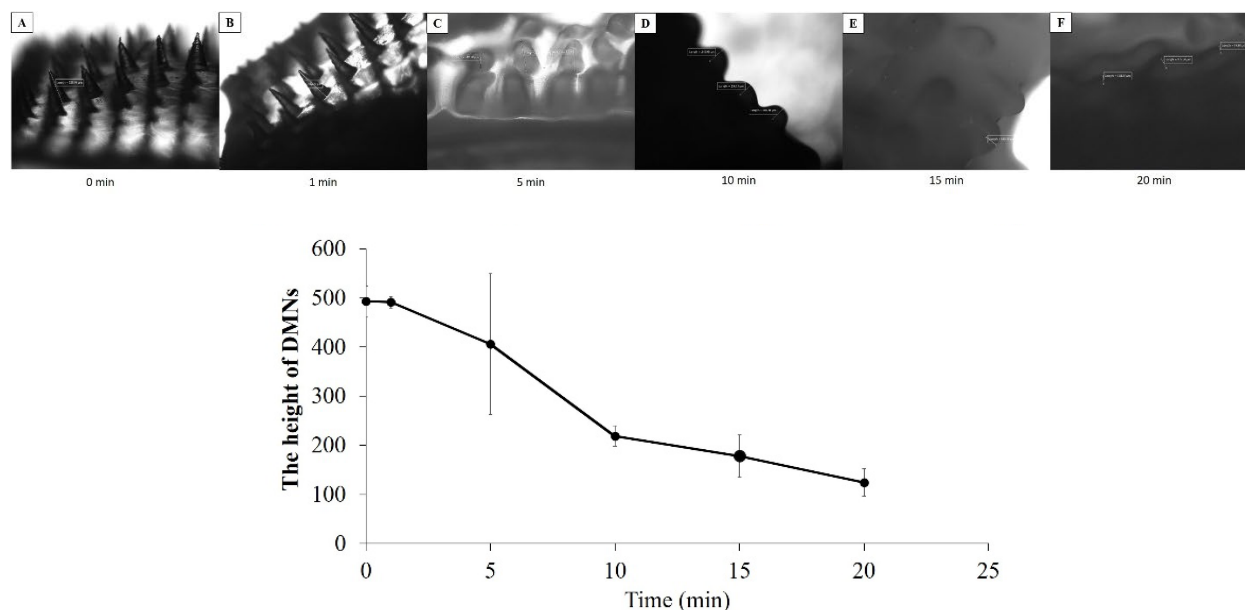


Figure 6. In vitro dissolution kinetics of optimized *A. ebracteatus* Vahl. stem extract-loaded DMNs (F6); (A–F) representative optical microscopy images illustrating the morphological changes and dissolution of the 8:2 PVA/HA DMNs at specific time intervals: 0, 1, 5, 10, 15, and 30 min, respectively, (G) quantitative dissolution profile depicting the relationship between microneedle height reduction and time

3.8 Active compound loading and antioxidant of the extract-loaded DMNs

The total phenolic content and antioxidant activity of the *A. ebracteatus* Vahl. stem extract that was placed onto the DMNs were assessed to determine whether the active substance was effectively encapsulated. After 0.2% (w/w) of extract was loaded into the PVA/HA (8:2) fabricated DMNs, the active compounds, measured as total phenolic content, were reported to be $10.41 \pm 2.02\%$ of the total phenolic compound loading when compared with the 0.2% (w/w) of initial extract at 100% before loading. In addition, the antioxidant activity of active compounds loaded DMNs showed $19.12 \pm 3.04\%$ inhibition when compared with the initial extract before loading, which showed $92.38 \pm 0.83\%$ inhibition. In this study, the DMNs manufacturing method used a micro-molding process, which limits drug loading capacity. Generally, the needle geometry, such as the number of needle tips, the base width, and the tip height, are determinant factors of the drug loading capacity of DMNs (Aldawood et al., 2021; Oliveira et al., 2024). The microneedle arrays in this study were configured with 100 needle points, a base width of $234.79 \pm 8.02 \mu\text{m}$, and a tip

height of $435.52 \pm 6.44 \mu\text{m}$. As a result, the encapsulation of the *A. ebracteatus* Vahl. stem extract was diminished by approximately 80–90% in the PVA/HA (8:2)-fabricated DMNs when compared to the initial extract prior to loading.

4. CONCLUSION

In summary, the study provided the scientific basis of ethanolic *A. ebracteatus* Vahl. extract. The extract had total phenolic compounds, which act as the active ingredients. Bioactivity assay of both stems and leaves demonstrated antioxidant activity, with the stem extract exhibiting higher values than the leaf extract. Moreover, in human skin cell safety assessments using keratinocytes, both plant parts were found to be safe and non-toxic at low concentrations. However, the stem extract was less toxic than the leaf extract, based on cell viability results CV_{80} . Consequently, the stem extract was selected for incorporation into DMNs made from mixed hydrophilic polymers: PVA and HA. After the DMNs were fabricated in

various ratios of PVA and HA, the 8:2 ratio was determined to be optimal for loading the extract after evaluating the characterization, *in vitro* insertion studies, dissolution profiles, and mechanical strength properties. The fabrication of PVA/HA-based DMNs containing *A. ebracteatus* Vahl. extract resulted in an excellent appearance, characterized by a conical shape and a complete needles array, while maintaining antioxidant activity. In addition, the DMNs could penetrate 100% of the first layer of PF (approximately 127 μm); when compared to human skin thickness, this indicates the needles might reach the epidermis layer with sufficient mechanical strength, followed by rapid dissolution and sustained release. Therefore, these PVA/HA DMNs are a viable platform for loading *A. ebracteatus* Vahl., offering significant potential for transdermal drug delivery.

AUTHOR CONTRIBUTIONS

K. T. conceived of and designed the model and wrote the manuscript with support from P.S., K.T., P.S., W.N., and P.W. carried out the experiments, analyzed the data, and contributed to the interpretation of the results and discussion. P.O. and P.S. supported the instruments to investigate the experiments. All authors provided critical feedback and helped shape the final manuscript.

ACKNOWLEDGMENTS

We acknowledge the Thailand Science Research and Innovation-Fundamental Fund (Grant No. 208156/680000000070) for the financial support. We are grateful to the Cosmetic and Herbal Innovation Center (CHIC) and the Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Huachiew Chalermprakiet University for supporting the scientific facilities and teamwork. Additionally, we appreciate the Pharmaceutical Development of Green Innovations Group (PDGIG), Department of Industrial Pharmacy, Faculty of Pharmacy, Silpakorn University for their lab collaboration.

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