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Original Article

In vitro biological activities of a combination of Ha-rak remedy, *Piper betle,* and *Garcinia mangostana* for the treatment of atopic dermatitis

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Abstract

Background and purpose: Ha-rak (HR), an equal-proportion combination of roots from *Capparis micracantha* DC., *Clerodendrum petasites* S. Moore., *Ficus racemosa* L., *Harrisonia perforata* (Blanco) Merr., and *Tiliacora triandra* (Colebr.) Diels, *Piper betle* L. (PB), and *Garcinia mangostana* L. (GM) are commonly used in traditional Thai medicine to treat skin diseases, including atopic dermatitis (AD). Combining three medicines in adjusted proportions can improve efficacy, reduce toxicity, and reduce medication. This study aimed to evaluate the anti-allergic, anti-inflammatory, antimicrobial, and cytotoxic activities of the ethanolic extracts of different combinations to analyze the relationship among elements, medicinal tastes, and biological activities.
Experimental approach: The biological activities (anti-allergic, anti-inflammatory, and anti-microbial activities and cell viability) of ethanolic extracts of plants and their combinations in various proportions were evaluated, as well as the chemical content of the developed remedies using the HPLC technique.
Findings/Results: HMB-123 was the most significantly effective combination for AD. HMB-123 reduced β-hexosaminidase release from RBL-2H3 cells to a greater extent than chlorpheniramine. HMB-123 significantly inhibited nitric oxide and TNF-α production in LPS-stimulated RAW 264.7 cells. HMB-123 demonstrated antibacterial activity against all tested bacteria and antifungal activity against *Candida albicans*. For single extract, PB exhibited the highest anti-fungal activity, while GM exhibited the highest anti-bacterial activity.
Conclusion and implications: The combined extracts showed potential as an optimized remedy for AD. HMB-123 demonstrated the highest anti-allergic, anti-inflammatory, and antimicrobial activities, making it a promising development candidate for AD treatment. To confirm safety and efficacy, further pre-clinical and clinical testing is necessary.

Keywords: Keywords: Anti-allergic; Anti-inflammatory; Antimicrobial; Atopic dermatitis; Cytotoxicity; Ha-rak remedy; *Piper betle* L.; *Garcinia mangostana* L.

INTRODUCTION

Atopic dermatitis (AD) is a common, recurrent, chronic skin condition that affects 10 to 20% of Thai children, with higher rates in industrialized countries. In 2018, the Institute of Dermatology in Bangkok reported that AD was the second most common skin disease, with 14,155 patients seen (1). The AD symptoms include inflamed and dry skin that is extremely itchy, red, swollen, cracked, scaled, webby, and crusted (2).

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AD is caused by type I immunological reactions mediated *via* immunoglobulin (Ig) E, produced by B cells and other plasma cells. These antibodies attach to mast cells or basophil surface membranes, causing them to degranulate and produce inflammatory mediators and allergy-related cytokines such as nitric oxide, histamine, and β-hexosaminidase (3,4). Furthermore, bacterial and dermatophyte fungal infections can exacerbate AD (5,6), with *Staphylococcus aureus, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa* being the most common opportunistic pathogens associated with AD (7,8).

The main treatment for AD is currently topical corticosteroid cream, which has many undesirable side effects such as skin atrophy, secondary infections, folliculitis, hypertrichosis, acneiform rash, hypopigmentation, dermal maceration, striae, and miliaria (2). In addition, several other drugs, including antihistamines and antibiotics, are used to treat AD patients (5).

Thai traditional medicine (TTM) is a knowledge system passed down from generation to generation and is influenced by Buddhism. TTM is based on the Dhatu theory, which states that the human body consists of four elements of earth, water, wind, and fire, with different characteristics and functions that must be in equilibrium for optimal health. Earth represents the body's physical organs, while water represents all liquids flowing and absorbed into the body, like blood and lymph. Wind controls respiration and blood circulation, while fire warms and heats the metabolism and immune system. Humans are healthy when all elements are balanced. Out of balance, these elements can cause illness. Atopic dermatitis generates red, itchy, irritated skin. An imbalance between the fire element (representing the immune system) and the water element (representing blood) could result in TTM. When fire increases, it affects other components. The imbalance can cause inflammation (redness), allergic reactions (itchiness), and skin infections (defined by distinct lesions based on the most afflicted part of each person).

In TTM treatment, medication is prescribed based on the taste of the drugs, which depend on the elements that have abnormalities. The physician compounds the remedy with a taste suitable for the patient's dhatu, which is imbalanced (9).

When prescribing traditional medicines, TTM doctors use "adjusted proportion of drug (APD)" to increase therapeutic efficacy and decrease adverse effects or toxicity. Each traditional remedy contains at least three functional groups of ingredients: main, secondary supportive, and supplementary ingredients (10).

Thai traditional medicine uses three well-known herbal topicals to treat skin diseases, including AD. Ha-rak remedy (HR), a bitter drug, is known to support blood and decrease heat in the body, which is associated with inflammatory symptoms and soothes burning skin by combining equal parts of roots from *Capparis micracantha* DC., *Clerodendrum petasites* S. Moore., *Ficus racemosa* L., *Harrisonia perforata* (Blanco) Merr., and *Tiliacora triandra* (Colebr.) Diels. The *Piper betle* L. (PB) leaves are known for their pungent taste, carminative properties, and ability to enhance wind dhatu and maintain blood circulation. It can also relieve itching caused by allergic reactions. *Garcinia mangostana* L. (GM) pericarps are sweet-astringent and have been found to possess wound-healing properties, increase body strength, and regulate the fire and wind elements related to infection, thereby protecting the body from microorganisms (11). We selected the ethanolic extract of the three substances. In addition, the markers, or active compounds of each plant (hydroxychavicol, eugenol, pectolinarigenin, and α-mangostin) dissolve well in organic solvents such as ethanol; this solubility resulted in the selection of ethanolic extracts in this research.

Consequently, this study analyzed the causes of AD using Thai traditional medicine, investigated the relationship between related ingredients, medicinal tastes, and biological activities, and developed a potential treatment cure by combining the TTM medications above using the APD technique. We also tested the remedy's *in vitro* anti-allergy, anti-inflammatory, antimicrobial, and cytotoxic properties and determined its chemical contents

using high-performance liquid chromatography (HPLC) to create a chemical fingerprint of the combined ethanolic extracts.

MATERIALS AND METHODS

Chemicals and reagents

The rat basophilic leukemia cell line (RBL-2H3) and the murine macrophage cell line (RAW 264.7) were acquired from the American Type Culture Collection (ATCC1 CRL-2256TM, VA, USA). Monoclonal Anti-DNP IgE, anti-dinitrophenylated bovine serum albumin (DNP-BSA), p-nitrophenyl-N-acetyl-b-D-glucosaminide (PNAG), albumin bovine fraction V powder, D- (+)-glucose, chlorpheniramine, lipopolysaccharide (LPS), sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, phosphoric acid, thiazolyl blue tetrazolium bromide (MTT), and prednisolone were purchased from Sigma-Aldrich Inc. (MO, USA). Calcium chloride dehydrates, citric acid monohydrate, magnesium chloride $6H_2O$, potassium chloride, sodium carbonate, and sodium bicarbonate were obtained from Merck (Darmstadt, Germany). Fetal bovine serum (FBS), minimum essential medium (MEM), penicillin-streptomycin, trypan blue, RPMI 1640 medium, Dulbecco's modified eagle medium (DMEM), and trypsin-EDTA were purchased from Gibco BRL Life Technologies (NY, USA). Phosphate-buffered saline (PBS) and piperazine-N, N0-bis(2-ethanesulfonic acid) were acquired from Amresco (OH, USA). Sodium chloride and sodium hydroxide were purchased from Univar (NSW, Australia). Commercial-grade ethanol was obtained from Sasol Chemical Pacific LTD (Shenton, Singapore). Water was purified using a Milli-Q water purification system from Millipore (MA, USA). Analytical-grade reagents, such as dimethyl sulfoxide, hydrochloric acid, and isopropanol, were purchased from Labscan Limited (Bangkok, Thailand). Amphotericin B solubilized ($C_{47}H_{73}NO_{17}$), and ampicillin ($C_{16}H_{18}N_3NaO_4S$) were purchased from Sigma, United Kingdom. Dimethyl sulfoxide $(CH_3)_2SO$ (DMSO) was acquired from RCL Labscan, Thailand. Mueller Hinton agar, Mueller Hinton broth, nutrient agar, and Sabouraud dextrose agar were purchased from Difco, BBL/USA. Resazurin sodium salt was obtained from Sigma, USA. *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *P. aeruginosa* (ATCC 15442), and *Candida albicans* (ATCC 10231) were purchased from the American Type Culture Collection. The normal human keratinocyte cell line (HaCaT) was obtained from CLS cell line service (No. 300493-SF) and was used as a control cell line in this study.

Plant materials and preparation of the extracts

The HER remedy is a combination of roots from *C. micracantha* DC. (Capparidaceae), *C. petasites* S. Moore. (Lamiaceae), *F. racemosa* L. (Moraceae), *H. perforata* (Blanco) Merr. (Rutaceae), and *T. triandra* (Colebr.) Diels (Menispermaceae). The plant materials were collected from Chachoengsao Province, Thailand. The leaves of *P. betle* L. (Piperaceae) were collected from Pathumthani province, Thailand, and the pericarps of *G. mangostana* L. (Clusiaceae) were purchased from the Ta-lad-Thai market in Pathumthani province, Thailand. The identification of plant materials was conducted by the Office of the Herbarium of the Southern Center of Thai Medicinal Plants at the Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand (Table 1). All plant names have been verified (http:// http://www.worldfloraonline.org) accessed July 1, 2023.

The plants were washed, thinly sliced, oven-dried at 50 °C, powdered with an electric grinder, and stored in sealed bags at room temperature. The five HR medicine plant powders were combined in equal parts. The powdered plant components were macerated three times for three days in 95% ethanol at room temperature to obtain the ethanolic extracts of the HR medicine, *P. betle* L., and *G. mangostana* L. A rotary evaporator condensed the filtrate to a constant weight under reduced pressure (45 °C) and stored at -20 °C after macerates were filtered. Table 1 shows the extract yield (% w/w). Table 2 shows the three extracts combined in three quantities.

Table 1. Voucher specimen number of Ha-rak remedy and its component plants, *Garcinia mangostana* L. and *Piper betle* L. and their percentage yields of crude extracts (%w/w).

Plant species /medicine	Family name	Common name	Thai name	Voucher specimen number	Yield (%)
***Capparis micracantha* DC.**	Capparidaceae	Caper-Thorn	Ching-Chi	SKP 391 03 13 01	-
***Clerodendrum petasites* S .Moore.**	Lamiaceae	-	Thao-yai-mom	SKP 202 03 09 01	-
***Ficus racemosa* L.**	Moraceae	Cluster fig tree	Ma-Duae-Chum-Porn	SKP 117 06 18 01	-
***Harrisonia perforata*)Blanco (Merr.**	Rutaceae	-	Khon-Tha	SKP 178 08 16 01	-
***Tiliacora triandra*)Colebr (.Diels**	Menispermaceae	-	Ya-Nang	SKP 114 20 20 01	-
Ha-rak remedy	-	-	-	-	3.18
***Garcinia mangostana* L.**	Clusiaceae	Mangosteen	Mung-Kud	SKP 214 09 13 01	21.38
***Piper betle* L.**	Piperaceae	Betle	Phlu	SKP 146 16 02 01	30.16

Table 2. The proportions of Ha-rak remedy, *Piper betle* L .and *Garcinia mangostana* L .for atopic dermatitis treatment

Code	The proportion			Taste of main drugs	Expected biological activities
	Ha-rak remedy	*Garcinia mangostana* L.	*Piper betle* L.		
HMB-321	3	2	1	Bitter	Anti-inflammation activity
HMB-231	2	3	1	Sweet, astringent	Anti-microbial activity
HMB-123	1	2	3	Pungent	Anti-allergic activity

Determination of antigen-induced β-hexosaminidase release from RBL-2H3 cells

The inhibitory effects of all extracts were evaluated using a modified method based on Tewtrakul *et al*. (12). This method measures the inhibition of released β-hexosaminidase resulting from the antigen-induced degranulation of IgE-sensitized RBL-2H3 cells. All tested samples were compared to the standard anti-allergy drug, chlorphenamine.

RBL-2H3 cells were grown in MEM media containing 15% heat-inactivated FBS, 1% penicillin, and streptomycin (10,000 units/mL penicillin and 10,000 mg/mL streptomycin). The cells were maintained in an incubator at 37 °C in 5% CO_2. The cells were seeded at a density of 2×10^5 cells/well in 24-well plates and incubated for 2 h. Cells in 24-well plates were sensitized with anti-dinitrophenyl-Ig E (anti-DNP IgE, 0.45 mg/mL) and incubated for 24 h. Then the cells were washed with 400 µL of Siraganian buffer (consisting of 119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$, 25 mM piperazine-N, N0-bis(2-ethanesulfonic acid), 0.1% BSA, and 40 mM NaOH, pH 7.2), and then incubated in 160 µL of buffer A for an additional 10 min.

The sample was dissolved in DMSO to give a 10 mg/mL stock solution. This stock solution was diluted by adding Siraganian buffer to give 100, 50, 10, and 1 µg/mL concentrations. Then, 20 µL of each concentration was added to each well (20 µL of solvent was added to the control cells) and incubated for 10 min. After that, 20 µL of antigen (DNP-BSA) was added to each well and incubated for another 20 min to stimulate the cells. The 50 µL of supernatant from each well (the solution from the 24-well plate) was transferred into 96-well plates and 50 µL of 1 mM substrate (PNAG) in 0.1 M citrate buffer (pH 4.5) was added. The 96-well plates were incubated at 37 °C in 5% CO_2 for 2 h. The reaction was stopped by adding 200 µL of a stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). At 405 nm, the absorbance was determined with a microplate reader.

Each sample's inhibition (%) of β-hexosaminidase release was determined using equation (1), and IC_{50} values were obtained from the GraphPad Prism program.

$$\text{Inhibition (\%)} = \left[1 - \frac{T - B1 - B2}{C - B1 - B2}\right] \times 100 \quad (1)$$

Where C (Control) is DNP-BSA and substrate (PNAG) without test sample; T (Test) = DNP-BSA with the substrate (PNAG) and test sample; B1 (Blank 1) = substrate (PNAG) only; B2 (Blank 2) = substrate (PNAG) and test sample.

The MTT assay measured cell viability after supernatant transfer. MTT solution (500 µL at 0.5 mg/mL) was added to each well and incubated in 24-well plates for 2 h. After removing the media from each well, 400 µL of DMSO was added to dissolve the formazan dye in the cells. A microplate reader measured 570 nm absorbance. The samples were cytotoxic if their optical density was less than 70% of the control groups. The inhibition percentage was calculated using equation (2):

$$\text{Inhibition (\%)} = \left[\frac{OD_{control} - OD_{sample}}{OD_{control}}\right] \times 100 \quad (2)$$

Determination of LPS-induced NO production from RAW 264.7 cells

The inhibitory effects of all extracts were evaluated using the method of Tewtrakul *et al.* (13). RAW 264.7 cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% penicillin, and streptomycin (10,000 units/mL penicillin and 10,000 mg/mL streptomycin). The cells were seeded in 96-well plates at a density of 1×10^5 cells/well and allowed to adhere for 24 h at 37 °C in 5% CO_2. Thereafter, the medium was removed, and 100 µL of LPS (2 ng/mL) in the fresh medium was added to each well. The cells were subsequently treated with various concentrations of samples, which were dissolved in DMSO (100 µL/well) and incubated. After 24 h, NO production was determined using the Griess reagent. One-hundred µL of supernatant from each well was transferred to 96-well plates and mixed with the same volume of Griess reagent (1% sulfanilamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% phosphoric acid). A microplate reader was used to measure the absorbance at 570 nm.

The MTT assay measured cell viability after supernatant transfer. MTT solution (10 µL, 5 mg/mL) was added to each well of 96-well

plates and incubated for 2 h. After removing the media from each well, 100 L of isopropanol with 0.04 M HCl was added to dissolve the formazan dye in the cells. A microplate reader measured 570 nm absorbance. Each well contained 0.2% DMSO. The samples were cytotoxic if their optical density was less than 70% of the control groups. The inhibition percentage was calculated using the equation (2).

Determination of anti-microbial activity

The anti-microbial activities of *P. betle, G. mangostana*, the HR remedy, and their combinations were investigated against four causative agents of skin infections. The microorganisms tested were *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), and *P. aeruginosa* (ATCC 15442), which were grown on nutrient agar for 18-24 h at 37 °C under aerobic conditions. *C. albicans* (ATCC 10231) was grown on Sabouraud dextrose agar for 18-24 hours at 37 °C under anaerobic conditions.

Determination of minimal inhibitory concentration

The microdilution technique was used to measure the extracts' minimum inhibitory concentrations (MICs) (14). First, the extracts were dissolved in DMSO at a concentration of 400 mg/mL, and then they were diluted 2-fold with broth medium. Next, 50 µL of the diluted extracts were added to a 96-well plate. The turbidity of *S. aureus* and *S. epidermidis* cultures was then adjusted to the 0.5 McFarland standard and diluted with sterile Mueller-Hinton broth at 1:200 for a final concentration of 5×10^5 CFU/mL. Then, 50 µL of the inoculum was added to the 96-well plates and covered with plastic wrap. The samples were mixed thoroughly using a plate shaker before incubating at 37 °C.

Add 10 µL of 1 mg/mL resazurin solution (blue dye) to the samples in the 96-well plate after 24 h and incubate at 37 ℃ for 2 h. Resazurin (blue) turning to resorufin (pink) is irreversibly proportionate to aerobic respiration. The MIC was the final blue well. Positive, negative, and viable controls were used in all triplicate experiments.

Determination of minimal bactericidal concentration

To determine the minimal bactericidal concentration (MBC), 100 µL aliquots were collected from blue-no-bacterial-growth wells and streaked over Mueller Hinton agar media. The plates were incubated at 37 °C for 24 h. The MBC was the lowest concentration that prevented bacterial colony growth. To ensure accuracy, the experiment was performed repeatedly in triplicate using positive and negative controls.

***Determination of cytotoxic activity* (*MTT assay*)**

This study evaluated the inhibitory effects of all extracts using the method described by Tewtrakul *et al.* (13). The normal human keratinocyte cell line (HaCaT) was used in this study, which was purchased from CLS cell line service (No. 300493-SF). HaCaT cells were cultured in a DMEM medium containing 10% heat-inactivated FBS and 1% penicillin and streptomycin (10,000 units/mL penicillin and 10,000 mg/mL streptomycin). The ethanolic extracts were initially dissolved in sterile DMSO and prepared at 50 µg/mL. The cells were seeded in 96-well plates at a density of 2.5×10^5 cells/well and allowed to adhere for 24 h at 37 °C in 5% CO_2. Then, the medium was removed, and fresh medium was added to each well (100 µL/well). Subsequently, the cells were incubated for 24 h with varied concentrations of samples dissolved in DMSO (100 µL/well). After incubation, the supernatant was removed and 10 µL of MTT solution was added, followed by a 2-h incubation. After removing the supernatant, the precipitate of insoluble formazan was dissolved in 100 µL of DMSO. At 570 nm, the absorbance was measured. When the optical density of the sample group was greater than 70% of that of the control group, the samples were considered viable. The percentage of viability was calculated using the following equation, and IC_{50} values were calculated using the Prism program.

$$\text{Servival (\%)} = 100 - \left[\left(\frac{ODcontrol - ODsample}{ODcontrol}\right) \times 100\right] \quad (3)$$

Determination of drug content analysis by HPLC technique

HPLC was used for drug content analysis. Four compounds, pectolinarigenin from HR, α-mangostin from GM, eugenol and hydroxychavicol from PB, were employed as markers for the combination extract.

An Agilent® XDB-C18 column, dimension 250 × 4.60 mm, 5 µm, with a photodiode array detector (DAD) was used. The mobile phase system was 0.1% orthophosphoric acid in water-acetonitrile gradient elution with an A:B ratio of 95:5 at 0 min. At 30 min, the A:B ratio was 5: 95. At 35 min, the A:B ratio was 95:5. All samples were diluted in methanol to 10 mg/mL and filtered through a 0.45 µm Nylon filter. At 284, 317, and 331 nm, pectolinarigenin, α-mangostin, hydroxychavicol, and eugenol were identified, respectively.

Statistical analysis

Results represent the mean ± SEM of three independent, triplicate trials. The IC_{50} values were calculated using Prism. The statistical study used one-way ANOVA followed by Wilcoxon rank-sum with Bonferroni correction post hoc test with a significance level of $P < 0.05$.

RESULTS

Medicinal combination with TTM technique

Compounding a novel drug requires the combination of several medicinal compounds. The main, secondary, and supplementary components of a medication combination exist. APD compounding is established in Thai Traditional Medicine and Ayurveda is applied. It increases the efficacy of pharmacological combinations and reduces adverse effects.

The primary components are selected based on taste and biological activity to generate therapeutic responses. For example, if the main ingredient has a pungent taste, its function is to balance the circulatory system, thus relieving allergic symptoms such as itching. If the main ingredient has a sweet-astringent taste, it can promote faster wound healing. When the formulation has a bitter taste, it can alleviate inflammation and burning symptoms. Based on the medicinal taste and APD technique, a proportionate amount of each ingredient is expected to achieve the required biological activity, as mentioned in Table 3.

Determination of antigen-induced β-hexosaminidase release from RBL-2H3 cells

The MTT assay was also used to evaluate cell vitality to confirm that the inhibitory effect of antigen-induced β-hexosaminidase release from RBL-2H3 cells was not caused by cell death (cell viability > 70%). The ethanolic crude extract from *P. betle* exhibited the most potent anti-allergic activity with IC_{50} values of 13.33 ± 1.14 µg/mL with no significant difference from the control (chlorphenamine), as shown in Table 4. On the other hand, the extracts of *G. mangostana* and the HR remedy demonstrated considerably lower anti-allergic activity, with IC_{50} values exceeding 100 µg/mL. Among the combined extracts, HMB-123 showed the strongest anti-allergic activity, which was significantly better than the standard drug chlorphenamine (followed by HMB-231 and HMB-321 with no significant difference from the control (chlorphenamine).

Determination of LPS-induced NO production from RAW 264.7 cells

The results of the inhibition of NO production by the tested extracts and the standard anti-inflammatory drug, prednisolone, are presented in Table 5. The MTT assay was also used to evaluate cell vitality to confirm that the inhibitory effect of NO production was not caused by cell death (cell viability > 70%). The ethanolic extract from *P. betle* exhibited the most potent anti-inflammatory activity, followed by *G. mangostana*. The HR remedy showed much lower activity with IC_{50} > 100 µg/mL. All single extracts showed significant differences compared with the positive control, prednisolone. Among the combinations, all combination extracts indicated notable differences from the positive control, prednisolone. HMB-123 exhibited the strongest anti-inflammatory activity, followed by HMB-231 and HMB-321.

Table 3. The relationship between taste and biological activities related mechanism of atopic dermatitis according to traditional Thai medicine.

Botanical name	Thai name	Part of use	Taste of drug	Use in traditional Thai medicine	Supported biological activities
Ha-rak remedy	Ha-rak remedy	Root	Bitter	Relieve the sensation of burning skin due to its soothing properties (Foundation of Thai Traditional Medicine and Ayurvedathamrong school, 2007)	Anti-inflammatory activities (inhibited lipopolysaccharide-stimulated NO-production) (28). Anti-allergic activities (inhibition of β-hexosaminidase release) (28). Anti-microbial activity (*Staphylococcus aureus*, *Streptococcus pyrogenes*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Acinetobacter buamannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis*) (29)
***Garcinia mangostana* L.**	Mung-Kud	Pericarp	Sweet, astringent	Heal both acute and chronic wounds (National List of Essential Medicines, 2016)	Anti-inflammatory (inhibited lipopolysaccharide-stimulated NO- production and PGE_2 production) (21) Anti-allergic activities (blocked the histaminergic and serotonergic response) (21) Anti-microbial activity (*Propionibacterium acnes* and *Staphylococcus epidermidis*) (22)
***Piper betle* L.**	Phlu	Leaf	Pungent	Relieves itchy skin and skin inflammation (National List of Essential Medicines, 2016)	Anti-inflammatory (inhibited IL-8 secretion in a TNF-α and IL-4) (30-31) Anti-allergic activities (decreased histamine produced) (21) Anti-microbial activity (*Candida albicans*, *Streptococcus mutans*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*) (34-36)

Table 4. Inhibition of β-hexosaminidase release (%) and cell viability (%) at various concentrations (μg/mL) of ethanolic extracts in RBL-2H3 cells. Data represent mean ± SEM, n = 3. *$P < 0.05$ indicates significant differences compared with the positive control (chlorpheniramine).

Crude extract	Inhibition of β-hexosaminidase release (%) Cell viability (%)				IC_{50} (μg/mL)	*P*-value
	1 μg/mL	10 μg/mL	50 μg/mL	100 μg/mL		
Ha-rak remedy	-	-	-	1.22 ± 3.56 (94.03 ± 0.67)	> 100*	< 0.001
***Garcinia mangostana* L.**	-	-	-	33.14 ± 2.75 (82.46 ± 2.51)	> 100*	< 0.001
***Piper betle* L.**	-12.27 ± 0.52 (79.07 ± 0.05)	37.49 ± 8.08 (87.4 ± 0.41)	68.33 ± 2.44 (84.52 ± 2.85)	86.65 ± 7.31 (88.49 ± 0.38)	13.33 ± 1.14	0.778
HMB-321	1.33 ± 1.02 (83.09 ± 0.08)	37.84 ± 3.21 (84.10 ± 1.08)	67.86 ± 3.42 (83.85 ± 0.41)	80.71 ± 4.91 (82.08 ± 0.05)	14.10 ± 1.13	0.967
HMB-231	12.52 ± 8.33 (79.65 ± 1.96)	49.59 ± 10.51 (85.33 ± 1.41)	75.79 ± 6.14 (84.20 ± 1.06)	81.88 ± 5.53 (79.33 ± 1.51)	10.91 ± 2.84	0.111
HMB-123	19.73 ± 4.38 (81.08 ± 0.62)	58.68 ± 6.34 (81.45 ± 0.94)	74.93 ± 7.62 (77.95 ± 1.21)	86.69 ± 2.12 (82.87 ± 0.75)	8.01 ± 1.56*	0.003
Chlorpheniramine	-5.24 ± 5.72 (79.02 ± 0.08)	32.94 ± 4.61 (76.22 ± 0.98)	72.34 ± 3.23 (75.50 ± 0.88)	91.28 ± 1.01 (75.69 ± 1.36)	15.74 ± 1.63	

Table 5. Inhibition of LPS-induced NO release (%) and cell viability (%) at various concentrations (μg/mL) of ethanolic extracts on RAW 264.7 cells. Data are expressed as mean ± SEM, n = 3. **P* < 0.05 indicates significant differences compared with the positive control (prednisolone).

Crude extract	Inhibition of NO release (%) Cell viability (%)							IC_{50} (μg/mL)	*P*-value
	0.01 μg/mL	0.1 μg/mL	1 μg/mL	10 μg/mL	30 μg/mL	50 μg/mL	100 μg/mL		
Ha-rak remedy	-	-	-	-	-	21.61 ± 1.22	48.69 ± 1.33	> 100*	< 0.001
***Garcinia mangostana* L.**	-7.30 ± 1.32 (107.88 ± 3.17)	-1.78 ± 0.68 (109.18 ± 8.24)	12.57 ± 1.36 (105.86 ± 11.33)	24.25 ± 4.57 (125.83 ± 7.31)	51.99 ± 0.82 (125.18 ± 2.04)	-	-	25.82 ± 2.10*	< 0.001
***Piper betle* L.**	-10.51 ± 1.95 (97.31 ± 10.07)	2.27 ± 1.15 (88.11 ± 7.28)	23.15 ± 7.34 (89.85 ± 6.25)	54.95 ± 1.98 (93.67 ± 7.71)	-	-	-	9.67 ± 0.17*	0.004
HMB-321	-3.37 ± 8.18 (109.95 ± 3.58)	0.85 ± 7.64 (128.49 ± 2.00)	1.59 ± 8.27 (119.86 ± 6.25)	34.60 ± 14.92 (125.82 ± 6.69)	-	89.73 ± 1.49 (87.31 ± 5.60)	-	11.15 ± 0.93*	0.001
HMB-231	-0.77 ± 1.74 (110.76 ± 10.85)	3.49 ± 6.02 (118.38 ± 2.56)	0.51 ± 1.80 (117.95 ± 5.12)	38.74 ± 9.96 (126.23 ± 9.36)	-	90.70 ± 1.35 (90.23 ± 2.51)	-	10.58 ± 2.02*	0.001
HMB-123	-10.91 ± 4.37 (109.95 ± 3.58)	4.85 ± 1.82 (107.35 ± 4.09)	18.96 ± 4.13 (110.93 ± 2.86)	71.28 ± 7.46 (114.86 ± 5.73)	-	95.29 ± 0.72 (105.79 ± 0.92)	-	9.18 ± 0.33*	0.006
Prednisolone	-0.99 ± 3.72 (108.82 ± 5.97)	51.44 ± 5.95 (87.61 ± 5.48)	65.10 ± 4.68 (76.87 ± 3.06)	72.10 ± 3.10 (81.45 ± 2.35)	-	84.52 ± 1.14 (74.04 ± 1.79)	-	0.10 ± 0.01	

Table 6. MIC and MBC data obtained by the broth microdilution method of ethanolic extracts against 4 different micro-organisms.

Crude extract	*Staphylococcus aureus*		*Staphylococcus epidermidis*		*Pseudomonas aeruginosa*		*Candida albicans*	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Ha-rak remedy	5	5	1.25	1.25	> 5	> 5	> 5	> 5
***Garcinia mangostana* L.**	0.009	0.009	0.019	0.15	0.625	0.625	> 5	> 5
***Piper betle* L.**	0.312	0.312	0.31	0.31	2.5	2.5	0.078	0.078
HMB-321	0.625	0.625	0.15	0.15	> 5	> 5	> 5	> 5
HMB-231	0.312	0.312	0.078	0.15	1.25	1.25	5	> 5
HMB-123	1.25	2.5	0.62	0.62	2.5	2.5	0.312	0.312
Ampicillin	0.195 μg/mL	0.195 μg/mL	19 μg/mL	19 μg/mL	2.5 μg/mL	2.5 μg/mL	-	-
Amphotericin B	-	-	-	-	-	-	0.195 μg/mL	0.195 μg/mL

MIC, Minimum inhibitory concentrations; MBC, minimal bactericidal concentration.

Determination of MIC

Table 6 shows the results of the antimicrobial activity of the tested extracts and standard drugs, ampicillin and amphotericin B. *G. mangostana* exhibited the most potent inhibition against *S. aureus, S. epidermidis,* and *P. aeruginosa*, respectively. However, it was not effective against *C. albicans* even at concentrations > 5 mg/mL. *P. betle* was the most potent inhibitor of *C. albicans*, followed by *S. aureus, S. epidermidis,* and *P. aeruginosa*. The HR remedy exhibited the robust inhibition against *S. epidermidis*, followed by *S. aureus*. However, it was ineffective against *P. aeruginosa* and *C. albicans* at concentrations > 5 mg/mL.

Of the combination extracts, HMB-231 exhibited the robust antimicrobial activity against *S. aureus, S. epidermidis, P. aeruginosa,* and *C. albicans*. HMB-123 was the robust inhibitor of *C. albicans,* followed by *S. epidermidis, S. aureus*, and *P. aeruginosa*. HMB-321 exhibited robust antimicrobial activity against *S. epidermidis* and *S. aureus*, while *P. aeruginosa* and *C. albicans* were not inhibited at concentrations > 5 mg/mL. The antimicrobial activities of the combination extracts were the same as those of the individual extracts.

***Determination of minimal bactericidal concentration* (*MBC*)**

Table 6 presents the results of the antimicrobial activity of the tested extracts and Ampicillin. *G. mangostana* exhibited robust bactericidal activity against *S. aureus, S. epidermidis,* and *P. aeruginosa*, while *P. betle* was most active against *C. albicans*. The combined herbs showed some inhibitory activity but less than that of *G. mangostana* and *P. betle*. The combination HMB-231 had the lowest MBC against *S. aureus, S. epidermidis,* and *P. aeruginosa*. HMB-123 had the same MBC values as MIC values for bacteria and fungi except for *S. aureus*. The MBC values of the HR remedy and HMB-321 were the same as their respective MIC values.

Determination of cytotoxic activity

The results of the MTT assay testing cytotoxic activity of the extracts against the normal human keratinocyte cell line (HaCaT) are presented in Table 7. *G. mangostana* extract was found to be cytotoxic to HaCaT cells, followed by *P. betle*, HMB-231, HMB-123, and HMB-321. The HR remedy showed much lower cytotoxicity with an IC_{50} greater than 100 µg/mL.

Study the chemical fingerprint and drug content analysis by HPLC technique

The compounds in the combination extracts were analyzed by HPLC to study the chemical fingerprint. The four compounds (hydroxychavicol and eugenol found from *P. betle* Linn., pectolinarigenin found from HR remedy, and α-mangostin determined in *G. mangostana* Linn.) were found at different retention times. Retention times of hydroxychavicol, eugenol, pectolinarigenin, and α-mangostin were 15.96, 21.46, 19.86, and 31.51, respectively (Fig. 1). The resultant chromatogram of the combination extracts that showed the presence of these markers in the HMB extract (Fig. 2), standard equations with R^2 values, and superimposed spectra of markers from the combination extracts are shown in Figs. 2-4. The quantitative analysis of each marker was undertaken at different wavelengths that provided the best selectivity, i.e., hydroxychavicol and eugenol at 284 nm; α-mangostin at 317 nm; and pectolinarigenin at 331 nm. It was determined that hydroxychavicol was the major compound in all extracts, followed by α-mangostin and eugenol. While pectolinarigenin was not detected in all extracts at both wavelengths. Therefore, hydroxychavicol, α-mangostin, and eugenol were used as the markers for standardization of all combination extracts (Table 8).

Table 7. The effect of various concentrations of the ethanolic extracts (µg/mL) on HaCaT cells viability. Data are expressed as mean ± SEM, n = 3. $P < 0.001$ indicates significant differences compared with the untreated control.

Crude extract	Cell viability (%)					IC_{50} (µg/mL)	*P*-value
	0.1 µg/mL	1 µg/mL	10 µg/mL	50 µg/mL	100 µg/mL		
Ha-rak remedy	100.04 ± 2.68	101.47 ± 3.30	106.75 ± 3.70	128.21 ± 9.14	147.02 ± 11.06	> 100	
***Garcinia mangostana* L.**	101.33 ± 0.45	92.24 ± 2.20	90.21 ± 2.34	15.11 ± 2.60	10.15 ± 1.48	39.52 ± 0.41	
***Piper betle* L.**	101.28 ± 0.93	96.06 ± 1.98	88.56 ± 6.61	51.96 ± 0.82	40.47 ± 4.93	51.92 ± 1.08	< 0.001
HMB-321	98.02 ± 0.36	87.00 ± 2.35	69.07 ± 3.95	46.60 ± 1.71	10.96 ± 2.11	93.89 ± 1.18	
HMB-231	96.78 ± 0.09	94.87 ± 1.23	84.17 ± 4.82	67.51 ± 3.24	9.86 ± 1.39	69.66 ± 3.00	
HMB-123	99.53 ± 1.25	94.02 ± 2.08	83.25 ± 3.23	78.43 ± 1.92	26.56 ± 0.46	79.04 ± 2.78	

Table 8 The contents of four compounds in the combination extracts. Data are expressed as mean ± SEM.

	Content (mg/g)			
Sample	**Hydroxychavicol**	**Pectolinarigenin**	**Eugenol**	**α-Mangostin**
HMB-321	72.15 ± 0.75	Not detected	29.39 ± 0.65	74.25 ± 1.26
HMB-231	48.87 ± 1.58	Not detected	16.67 ± 1.03	93.44 ± 3.60
HMB-123	108.45 ± 1.21	Not detected	39.15 ± 0.73	73.46 ± 1.79

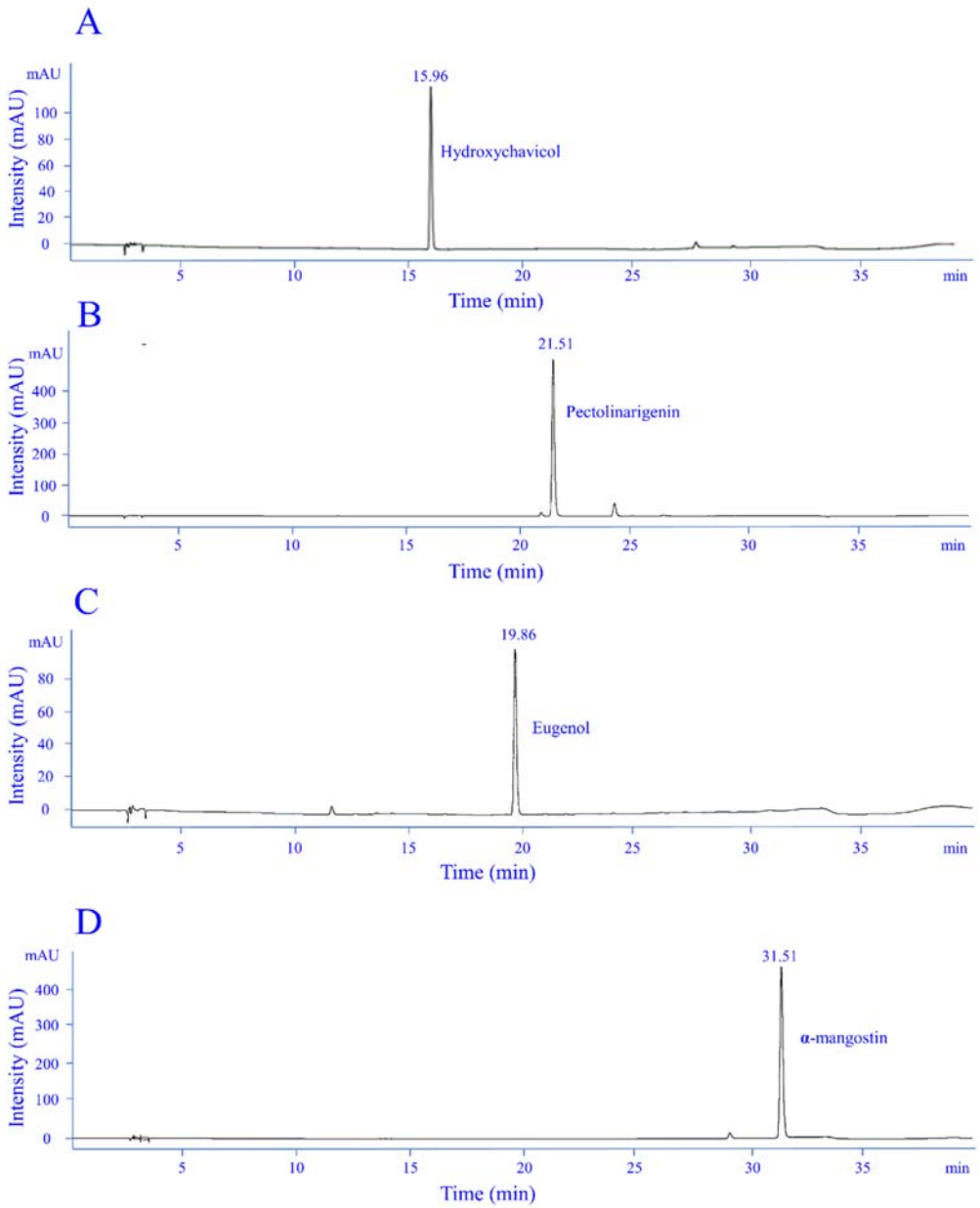
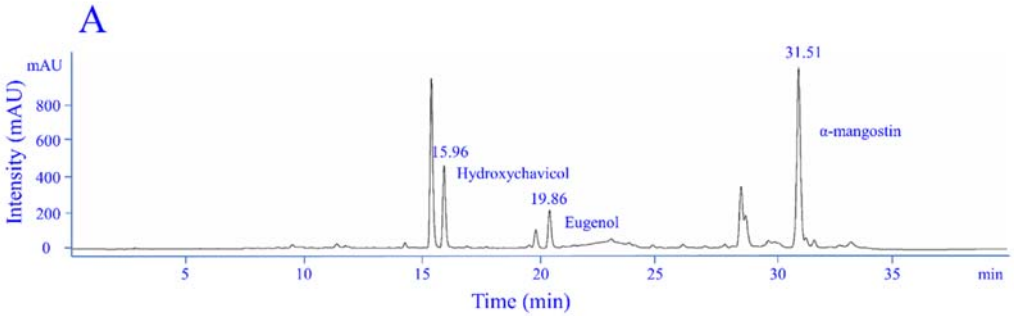


Fig. 1. HPLC chromatograms of (A) hydroxychavicol, (B) eugenol, (C) pectolinarigenin, and (D) α-mangostin. HPLC, High-performance liquid chromatography.



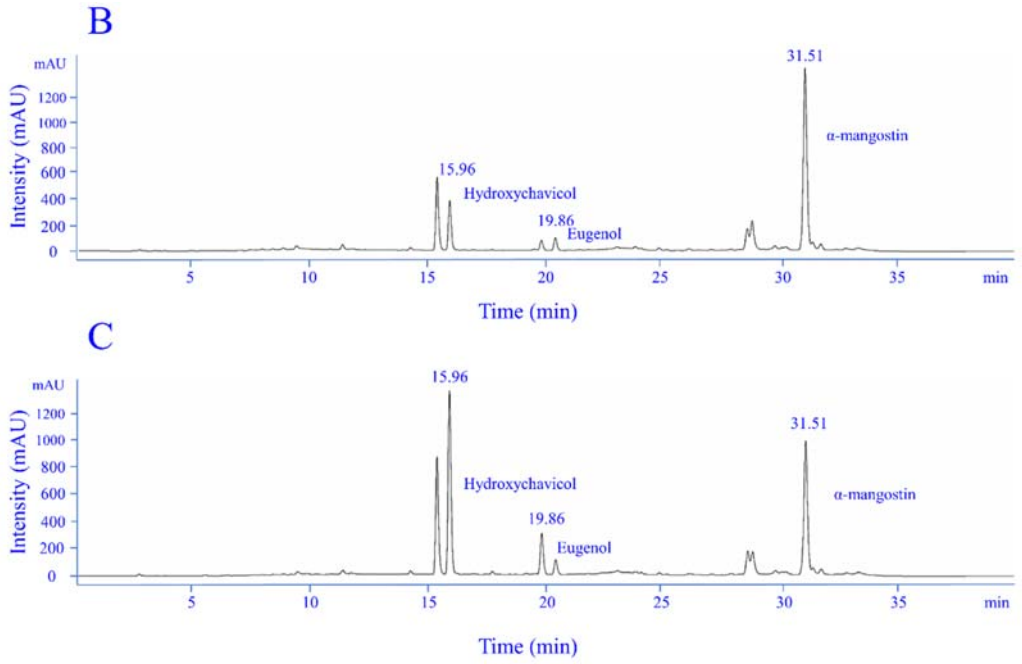


Fig. 2. HPLC chromatograms: (A) HMB-321, (B) HMB-231, (C) HMB-123. HPLC, High-performance liquid chromatography.

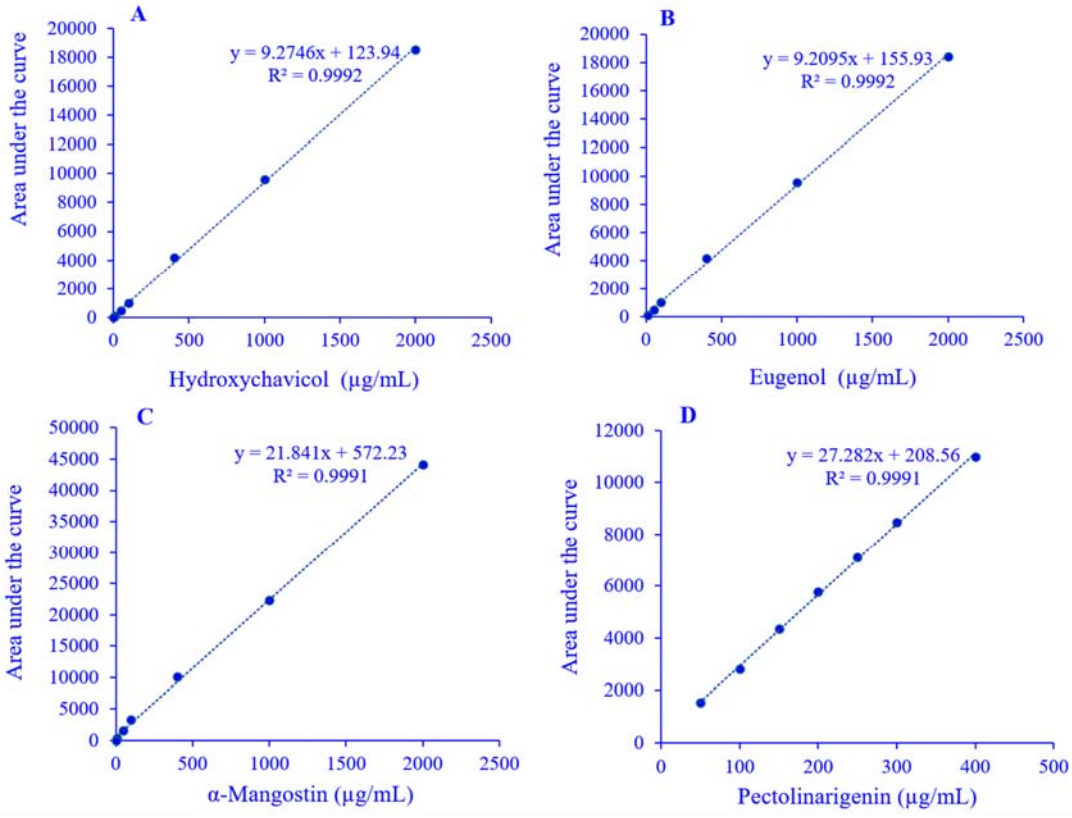


Fig. 3. The standard curve of (A) hydroxychavicol, (B) eugenol, (C) α-mangostin, and (D) pectolinarigenin.

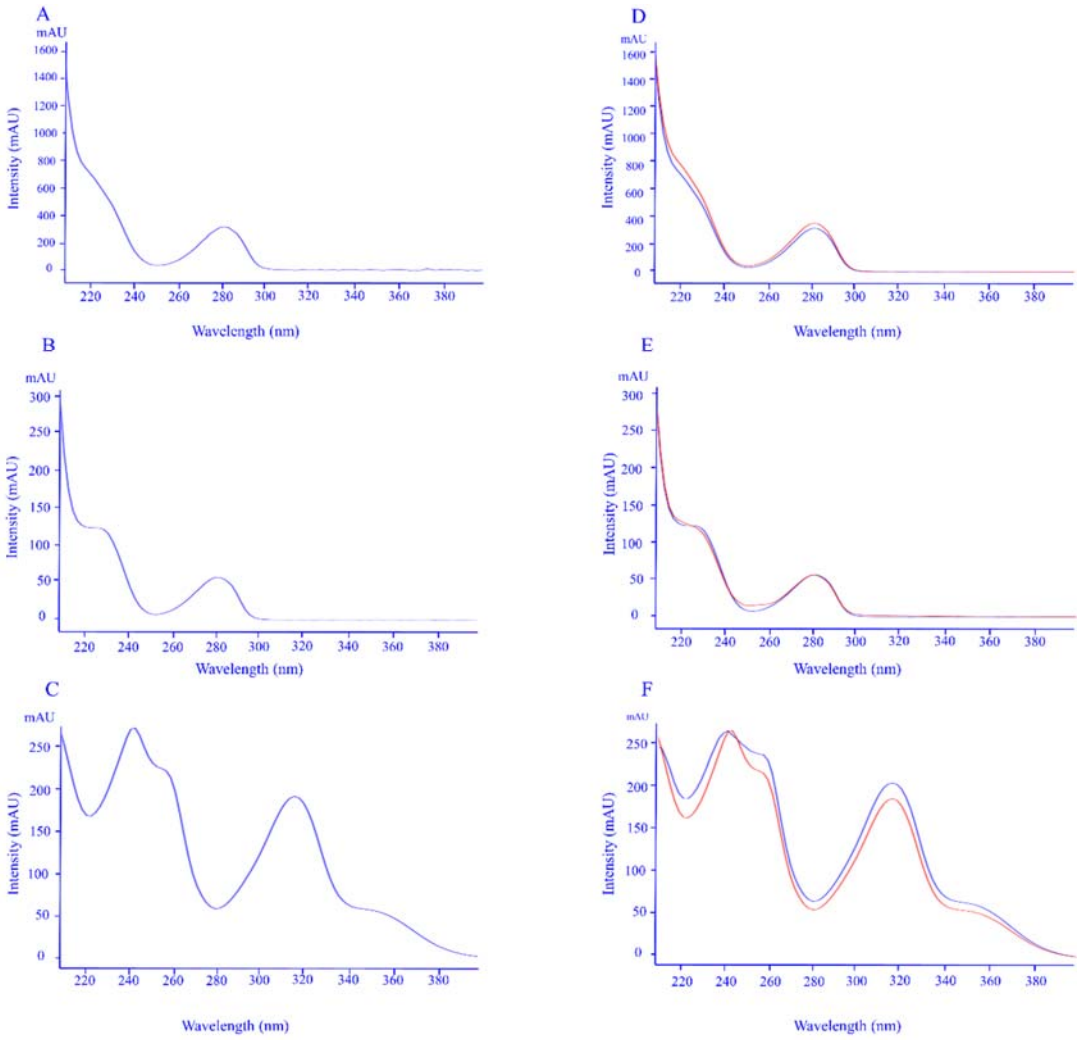


Fig. 4. The superimposed spectra of standard markers of (A) hydroxychavicol, (B) eugenol, and (C) α-mangostin and standard markers versus HMB combination: (D) hydroxychavicol and HMB combination, (E) eugenol and HMB combination, and (F) α-mangostin and HMB combination.

DISCUSSION

AD represents a type I immunological reaction, mediated *via* IgE (15,16). The cells release various inflammatory cytokines and inflammatory mediators, such as nitric oxide (4). The specific IgE receptors on the surface membranes of mast cells and basophils have a high affinity for IgE (3,17). When B cells and other plasma cells are exposed to antigens, they generate and release antigen-specific IgE antibodies, which attach to the surface membranes of mast cells and basophils. This reaction sensitizes mast cells and basophils, causing them to degranulate and release inflammatory mediators and allergy-related cytokines such as histamine, β-hexosaminidase, leukotrienes, and proteases, which cause the allergic response (3). Furthermore, bacterial and dermatophyte fungal infections exacerbate AD (5,6). Previous studies have shown that *S. aureus* and *S. epidermidis* are opportunistic pathogens that can cause significant problems when breaching the epithelial barrier on the skin (7), while *P. aeruginosa* was commonly isolated from AD skin. (8).

Conventional Western medicine treats AD with topical corticosteroids and antihistamines for skin irritation and itching. An infection can be treated using antibiotics. However, prolonged topical corticosteroid use causes skin atrophy, secondary infection, folliculitis,

hypertrichosis, acneiform, hypopigmentation, dermal maceration, striae, and miliaria (2). Patient medication abuse causes drug-resistance problems. Antifungal medicines such itraconazole, ketoconazole, posaconazole, and voriconazole may slow corticosteroids metabolism and cause adverse effects when administered combined (18). Thai traditional medicine treats and provides using Dhatu (four elements), taste, and formulation (changed drug proportion). Balance is the purpose of treatment. Synergism improves active component bioavailability, therapeutic advantages, and drug toxicity (19,20).

In TTM, HR treatment, *P. betle* L. leaves, and *G. mangostana* L. pericarps are popular herbal remedies. These three medications help treat AD symptoms and were combined using the above methods and biological activities examined. The sweet-astringent drug *G. mangostana* L. (mangosteen) has been found to possess wound-healing properties, increase body strength, and regulate the fire and wind elements related to infection, thereby protecting the body from microorganisms. This study aimed to test the biological activities of GM. The results showed that GM exhibited strong antibacterial activity against all tested bacteria, including *S. aureus, S. epidermidis,* and *P. aeruginosa*. GM also demonstrated potent anti-inflammatory activity. Consistent with the study by Chen *et al*. (21), the two active compounds in GM, α- and γ-mangostins, significantly inhibited lipopolysaccharide-stimulated NO production in RAW 264.7 cells. Additionally, these compounds significantly reduced prostaglandin E_2 production in lipopolysaccharide-activated RAW 264.7 cells, with IC_{50} values of 11.08 and 4.5 μM, respectively. GM also demonstrated anti-microbial activity against *Propionibacterium acnes* and *S. epidermidis* (22). Tannins are frequently found in common astringent plants. It is known that plants containing tannins are commonly used for healing wounds and can stop infection while they continue to rapidly heal the wound internally (23). This is consistent with studies that found tannins possess antimicrobial activities (24,25).

The bitter drug HR remedy is known to support blood and decrease heat in the body, which are associated with inflammatory symptoms. The present study revealed that HR exhibited no significant impact on NO production or β-hexosaminidase release. Despite this lack of influence on these inflammatory markers, HR demonstrated anti-microbial activity against all evaluated bacterial strains (*S. aureus* and *S. epidermidis*). Previous reports have shown that 95% ethanolic extract of HR possesses anti-inflammatory and anti-allergic activities, with IC_{50} values of 40.36 and 39.78 μg/mL, respectively. Pectolinarigenin, present in HR and bitter polyphenols common in several plant families is biologically active (26,27). Pectolinarigenin inhibited β-hexosaminidase release from RBL-2H3 cells more effectively, with an IC_{50} of 6.3 μg/mL, than the chlorpheniramine positive control (IC_{50} of 16.2 μg/mL) (28). Additionally, the study by Nuaeissara *et al*. (29) demonstrated that HR has anti-microbial activity against *S. aureus, S. pyrogenes, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Acinetobacter buamannii, P. aeruginosa, Klebsiella pneumoniae,* and *Bacillus subtilis*. Bitter phytocompounds have also shown a higher probability of exhibiting anti-inflammatory activity than other phytocompounds (30).

P. betle L. (betel) is a plant known for its pungent taste, carminative properties, and ability to enhance wind dhatu and maintain blood circulation. It can also relieve itching caused by allergic reactions. According to research, ethanolic extracts of PB are effective at inhibiting β-hexosaminidase release, more so than the standard antihistamine, chlorpheniramine. PB has demonstrated strong anti-inflammatory activity and has shown potent anti-bacterial activity against all tested bacteria and fungi including *S. aureus, S. epidermidis, P. aeruginosa,* and *C. albicans*. Moreover, 95% ethanolic extract of PB significantly decreases histamine production in IgE-mediated hypersensitivity reactions and inhibits IL-8 secretion in TNF-α and IL-4-induced allergic reactions (31). The intensely pungent aroma comes from the betel leaves because the essential oil includes a high concentration of terpenes and phenols (32). Hydroxychavicol, the major component in PB, also possesses anti-inflammatory activity,

inhibiting TNF-α expression in LPS-stimulated cells at 2.5, 5, and 10 µg/mL. All tested concentrations of hydroxychavicol significantly lowered TNF-α expression compared to the control (rolipram) (33). Finally, PB has been shown to have antimicrobial activity against *C. albicans, S. mutans, S. aureus, K. pneumoniae,* and *Escherichia coli* (34-36). HC might have great potential to be used as an antimicrobial, anti-inflammatory, and antiallergic agent.

HMB-321 is a combination that has a bitter taste as its main ingredient. Our study demonstrated that the ethanolic extracts of HMB-321 inhibited the production of NO whereas the single herb extract of the bitter-tasting herb showed no activity in inhibiting NO production. HMB-321 showed no significant difference in inhibiting β-hexosaminidase release compared to chlorpheniramine, a standard antihistamine, and the single herb extract. The combination HMB-321 also exhibited antibacterial activity against *S. aureus* and *S. epidermidis*.

HMB-231 is a combination that has a sweet-astringent taste as its main ingredient. Our study showed that the ethanolic extracts of HMB-231 had antibacterial activity against all tested bacteria and fungi (*S. aureus, S. epidermidis, P. Aeruginosa,* and *C. albicans*). Furthermore, HMB-231 inhibited β-hexosaminidase release to a greater extent than chlorpheniramine and demonstrated inhibition of NO production.

HMB-123 is a combination that has a pungent taste as its main ingredient. Our study determined that HMB-123 inhibited β-hexosaminidase release to a greater extent than chlorpheniramine, a conventional antihistamine. HMB-123 and PB showed comparable inhibition of NO production. The combination HMB-123 also exhibited antibacterial activity against all tested bacteria and anti-fungal activity.

In addition, the toxicity can also be demonstrated with the effect of MTT on normal cells. It was determined that cytotoxicity against normal cells (HaCaT) of combination HMB-123 was greater than a single PB extract. While HMB-123 showed strongest biological activities across the anti-allergic, anti-inflammatory, and anti-microbial activities. AD patients' skin may be affected by high PB doses. It was revealed that steamed betel leaf makeup caused leukomelanosis (37). The Combination HMB-123 reduces PB. HMB-123 may reduce PB effects when its actions are not statistically different from a single PB.

Pharmaceutical antibiotics such as ampicillin and amphotericin B are isolated chemical constituents that contain a single compound which makes them easier for bacteria to adapt to and counteract and lead to antibiotic-resistant infections (38). Conversely, herbs are much more complicated with many natural compounds and biological activities. The different compounds work together to resist bacterial infections and produce better than expected results. In combination HMB-123, each ingredient is fundamentally different and may be used as one of the treatments to achieve comprehensive effectiveness and reduce drug overdose.

In future investigations, fingerprint data generated by the HPLC technique can be applied to confirm whether the remedy contains these compounds, and will have related biological activities with each other, including HC, as there is a relationship with anti-inflammatory and anti-allergic effects. The combination HMB-123, in which HC is the highest amount of compound, appeared to be the most effective combination for AD due to its strongest biological activities across the anti-allergic and anti-inflammatory effects.

A combination of medications can improve therapeutic efficacy and reduce side effects and toxicity. The combination HMB-123 was most beneficial for AD due to its significant anti-allergic, anti-inflammatory, and anti-microbial biological activity. *P. betle* has similar anti-allergy, anti-inflammatory, and anti-microbial properties as HMB-123, especially against *C. albicans*. The antibacterial activity of *G. mangostana* extract was superior against all tested strains. Although HMB-123 had weaker anti-inflammatory activity than prednisolone, it had substantial antimicrobial and anti-allergic actions related to AD symptoms. Thus, HMB-123 may be the best for co-infected AD.

CONCLUSION

Based on our research, we have developed a promising combination for the treatment of AD by incorporating the principles of Dhatu, taste of drug, and adjusting the proportions of traditional drugs. The combination includes HR, betel leaves, and mangosteen peels in a ratio of 1:2:3 and has demonstrated potent biological activities against AD symptoms. Further preclinical testing is needed to validate its efficacy and safety, and if results are promising, the combination can be advanced to human studies.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

A. Itharat conceived and supervised the project. U. Saesiw performed the experiments, analyzed the data, and wrote the manuscript in consultation with A. Itharat, S. Ruangnoo, and W. Ketjinda. N.M. Davies provided scientific insight and analysis, structure, syntax, and grammatical flow to the manuscript. K. Phumlek and P. Sriumpai performed the experiments. All authors read and approved the finalized article.

REFERENCES

1. Institute of Dermatology of Thailand. Dermatology disease statistics reports for Thailand. 2018. Available from: http://inderm.go.th/inderm_eng/cpgeng.php/.
2. Hall JC, Hall BJ. Sauer's manual of skin diseases. 11th ed. Lippincott Williams & Wilkins (LWW); 2017. pp.110-124. Available at https://shop.lww.com/Sauer-s-Manual-of-Skin-Diseases/p/9781496383235.
3 Abbas AK, Lichtman A, Pillai S. Cellular and molecular immunology. USA, Philadelphia: Elsevier; 2010. pp. 325-327.
4. Cheng XL, Ding F, Li H, Tan XQ, Liu X, Cao JM, *et al*, Activation of AMPA receptor promotes TNF-alpha release *via* the ROS-cSrc-NFkappaB signaling cascade in RAW264.7 macrophages. Biochem Biophys Res Commun. 2015;461(2):275-280. DOI: 10.1016/j.bbrc.2015.04.015.
5. National Eczema Association. Eczema causes and triggers. 2022. Available from: https://nationaleczema.org/eczema/causes-and-triggers-of-eczema/.
6. American College of Allergy, Asthma & Immunology. Eczema (Atopic Dermatitis) Eczema, also called atopic dermatitis, is a skin disease that causes an itchy rash. 2014. Available from: https://acaai.org/allergies/types-allergies/skin-allergy/eczema-atopic-dermatitis.
7. Byrd AL, Deming C, Cassidy SKB, Harrison OJ, Ng WI, Conlan S, *et al*. *Staphylococcus aureus* and *Staphylococcus epidermidis* strain diversity underlying pediatric atopic dermatitis. Sci Transl Med. 2017;9:397,1-22. DOI: 10.1126/scitranslmed.aal4651.
8. Inoue H, Someno T, Kawada M., Ikeda D. Citric acid inhibits a bacterial ceramidase and alleviates atopic dermatitis in an animal model. J Antibiot(Tokyo). 2010;63(10) 611-613. DOI: 10.1038/ja.2010.91.
9. Mulholland J. Thai traditional medicine: ancient thought and practice in a Thai context. J Siam Soc. 1979;67(2):80-115. PMID: 11617470.
10. Ketpet S, Taoprasert Y, Taoprasert K, Tansuwanwong S. A study of the cause of disease by the elements in Thai traditional medicine scriptures according to the five aggregates of Buddhism. Journal of MCU Nakhondhat. 2022;9(12):30-41.
11. Salguero P, White N. Thai herbal medicine: traditional recipes for health and harmony. Findhorn Press; 2016. pp. 53-59 Available at: uwobookstore.uwosh.edu/thai-herbal-medicine-traditional-recipes-health-and-harmony
12. Tewtrakul S, Subhadhirasakul S, Kummee S. Anti-allergic activity of compounds from *Kaempferia parviflora*. J Ethnopharmacol. 2008;116(1):191-193. DOI: 10.1016/j.jep.2007.10.042.
13. Tewtrakul S, Itharat A. Nitric oxide inhibitory substances from the rhizomes of *Dioscorea membranacea*. J Ethnopharmacol. 2007;109(3): 412-416. DOI: 10.1016/j.jep.2006.08.009.
14. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 2007;42(4):321-324. DOI: 10.1016/j.ymeth.2007.01.006.

15. Jeong D, Yi YS, Sung GH, Yang WS, Park JG, Yoon K, *et al*. Anti-inflammatory activities and mechanisms of *Artemisia asiatica* ethanol extract. J Ethnopharmacol. 2014;152(3):487-496. DOI: 10.1016/j.jep.2014.01.030.
16. Coleman JW. Nitric oxide in immunity and inflammation. Int J Immunopharmacol. 2001;1(8):1397-1406. DOI: 10.1016/s1567-5769(01)00086-8.
17. Passante E, Ehrhardt C, Sheridan H, Frankish N. RBL-2H3 cells are an imprecise model for mast cell mediator release. J Inflamm Res. 2009;58(9): 611-618. DOI: 10.1007/s00011-009-0028-4.
18. Lomaestro BM, Piatek MA. Update on drug interactions with azole antifungal agents. Ann Pharmacother. 1998;32(9):915-928. DOI: 10.1345/aph.17271.
19. Jia W, Gao WY, Yan YQ, Wang J, Xu ZH, Zheng WJ. The rediscovery of ancient Chinese herbal formulas. Phytother. Res. 2004;18(8):681-686. DOI: 10.1002/ptr.1506.
20. Zhou X, Seto SW, Chang D, Kiat H, Razmovski-Naumovski V, Chan K, *et al*. Synergistic effects of Chinese herbal medicine: a comprehensive review of methodology and current research. Front Pharmacol. 2016;7:201,1-16. DOI: 10.3389/fphar.2016.00201.
21. Chen LG, Yang LL, Wang CC. Anti-inflammatory activity of mangostins from *Garcinia mangostana*. Food Chem Toxicol. 2008;46(2):688-693. DOI: 10.1016/j.fct.2007.09.096.
22. Chomnawang MT, Surassmo S, Nukoolkarn V, Gritsanapan W. Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium acnes.* Fitoterapia. 2007;78(6):401-408. DOI: 10.1016/j.fitote.2007.02.019.
23. Ashok PK, Upadhyaya K. Tannins are astringent. J Pharmacogn Phytochem. 2012;1(3) 45-50.
24. Maisetta G, Batoni G, Caboni P, Esin S, Rinaldi AC, Zucca P. Tannin profile, antioxidant properties, and antimicrobial activity of extracts from two Mediterranean species of parasitic plant Cytinus. BMC Complement Altern Med. 2019;19(1):82,1-11. DOI: 10.1186/s12906-019-2487-7.
25. Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials-a minireview. Materials (Basel). 2020;13(14):3224,1-13. DOI: 10.3390/ma13143224.
26. Ntie-Kang F. Mechanistic role of plant-based bitter principles and bitterness prediction for natural product studies II: prediction tools and case studies. Phys Sci Rev. 2019;4(8),1-16. DOI: 10.1515/psr-2019-0007.
27. Cheriet T, Ben-Bachir B, Thamri O, Seghiri R, Mancini I. Isolation and biological properties of the natural flavonoids pectolinarin and pectolinarigenin-a review. J Antibiotics. 2020;9(7):417,1-32. DOI: 10.3390/antibiotics9070417.
28. Juckmeta T, Thongdeeying P, Itharat A. Inhibitory effect on β-hexosaminidase release from RBL-2H3 cells of extracts and some pure constituents of Benchalokawichian, a Thai herbal remedy. Evid base Complement Alternat Med. 2014;2014:1-8. Available from: DOI: 10.1155/2014/828760.
29. Nuaeissara S, Kondo S, Itharat A. Antimicrobial activity of the extracts from Benchalokawichian remedy and its components. J Med Assoc Thai. 2011;94(7):172-177. PMID:22619925.
30. Dragoş D, Petran M, Gradinaru TC, Gilca M. Phytochemicals and inflammation: is bitter better? Plants. 2022;11(21):2991,1-13. DOI: 10.3390/plants11212991.
31. Wirotesangthong M, Inagaki N, Tanaka H, Thanakijcharoenpath W, Nagai H. Inhibitory effects of *Piper betle* on production of allergic mediators by bone marrow-derived mast cells and lung epithelial cells. Int Immunopharmacol. 2008;8:453-457. DOI: 10.1016/j.intimp.2007.11.005.
32. Biswas P, Anand U, Saha SC, Kant N, Mishra T, Masih H, *et al*. Betel vine (*Piper betle L.*): a comprehensive insight into its ethnopharmacology, phytochemistry, and pharmacological, biomedical and therapeutic attributes. J Cell Mol Med. 2022;26(11):3083-3119. DOI: 10.1111/jcmm.17323.
33. Sharma S, Ali KI, Ali I, Ali F, Kumar M, Kumar A, *et al*. Evaluation of the antimicrobial, antioxidant, and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. Antimicrob Agents Chemother. 2009;53(1):216-222. DOI: 10.1128/aac.00045-08.
34. Subri LM, Dewi W, Satari MH. The antimicrobial effect of *Piper betel* leaves extract against *Streptococcus mutans*. Padjadjaran J Dent. 2012;24(3):174-178. DOI: 10.24198/pjd.vol24no3.26835.
35. Elfrida E, Junaida E, Ariska RN, Jayanthi S. Effect of *Piper betle* linn extract on the growth of *Staphylococcus aureus* atcc 25923. Bp Int Res Critic. 2020;3(4):3028-3034. DOI: 10.33258/birci.v3i4.1325.
36. Nayaka NMDMW, Sasadara MMV, Sanjaya DA, Yuda PESK., Dewi, NLKAA, Cahyaningsih, E, *et al*. *Piper betle (L)*: recent review of antibacterial and antifungal properties, safety profiles, and commercial applications. Molecules. 2021;26(8):2321,1-21. DOI: 10.3390/molecules26082321.
37. Liao YL, Chiang YC, Tsai TF, Lee RF, Chan YC, Hsiao CH. Contact leukomelanosis induced by the leaves of *Piper betle L*. (Piperaceae): a clinical and histopathologic survey. J Am Acad Dermatol. 1999;40(4):583-589. DOI: 10.1016/s0190-9622(99)70441-x.
38. Buhner SH. Herbal antibiotics: natural alternatives for treating drug-resistant bacteria. North Adams MA: Storey Publishing; 2012. pp. 467.