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Assessment of *in vitro* Anti-inflammatory and Anti-acne-inducing Bacterial Activities of Kameng (*Eclipta prostrata* Linn.) Cultivated in Suphan Buri Province, Thailand

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Abstract

This research investigates extracts from Kameng (*Eclipta prostrata* Linn.), originally employed in traditional medicinal recipes, with the aim of developing an extract that can serve as an alternative to antibiotics. For this purpose, the anti-inflammatory and anti-acne-inducing bacterial activities of Kameng crude ethanol extract, grown in Suphan Buri Province were investigated, with anti-inflammatory properties assessed by the inhibition of protein degradation and proteinase inhibitory assays. Findings indicated the potential of Kameng extracts as anti-inflammatory drugs that reduce protein degradation and proteinase activities. Kameng extract concentrations ranging from 0.4 to 4.0 x 10^4 ppm inhibited protein degradation and proteinase activity by 14.66%-50.75% and 28.57%-72.08%, respectively. The agar disc diffusion method revealed that Kameng extracts had strong inhibitory effects against *S. aureus* and *C. acnes*, with inhibition zone diameters of 31.15±0.47 and 37.28±0.47 mm, respectively. The minimal inhibitory concentration (MIC) of Kameng extract against *S. aureus* and *C. acnes* was 5.0 mg/mL and 2.5 mg/mL while minimal bactericidal concentration (MBC) of Kameng extract against *S. aureus* and *C. acnes* was 20.0 mg/mL and 10.0 mg/mL, respectively. Results suggested the potential of Kameng extract to cure acne and skin inflammation as a herbal cosmetic product.

Keywords**:** *Kameng extract; Anti-acne inducing bacterial activities; In vitro anti-inflammatory*; *Protein degradation; Proteinase inhibitory assays*

1. Introduction

Kameng, called Kameng taw-meea in Thai with the scientific name *Eclipta prostrata* Linn. The plant grows well in humid areas such as rivers, riverbanks, and rice fields. It is common in tropical countries and subtropical regions of Thailand, China, India, Nepal, Brazil and other parts of the world (Timalsina, & Devkota, 2021). Kameng is used to treat skin diseases, heal wounds, reduce inflammation, and alleviate swelling in both traditional Thai medicine and traditional folk medicine in many countries (Gautam, 2011; Jahan et al., 2016). The leaves and stems are used for hair and scalp care, with crushed stems placed directly on wounds to promote healing (Adhikari et al., 2019; Ambu et al., 2020). Laxative medications are made from the roots and leaves, while the roots is also used to replenish the blood and treat abdominal pain, bronchitis, asthma, and chest tightness. The whole plant is used for cancer patients with chronic wounds that are difficult to heal. A decoction of the plant is also used to treat dandruff (Plant Genetic Conservation Project Under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn, 2019).

In addition to these traditional medicinal uses, Kameng is also utilized in formulations for treating constipation, muscle pain, flatulence, and

indigestion as well as in remedies for neurological disorders,while also providing relief from symptoms of abdominal pain and bloating. The whole Kameng plant including roots, stems, leaves, flowers, and seeds, is used as herbal medicine (Department of Thai Traditional and Alternative Medicine, Ministry of Public Health, 2019). Many previous research reports have addressed the potential abilities of Kameng extracts to alleviate pain, combat cancer, and reduce inflammation (Boonyaprapat, & Chokchaicharernporn, 1999; Arunachalam et al., 2009; Prachayasittikul et al., 2010). Previous studies have also investigated the antimicrobial properties of Kameng extracts using different solvents such as hexane, acetone, ethanol, and methanol. The hexane extract exhibited the highest inhibitory effect against various microorganisms, including *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* (MIC < 100 µg/mL), surpassing the standard antimicrobial drug ciprofloxacin with a concentration of 25 µg/mL (Pandey et al., 2011). The petroleum ether extract demonstrated inhibitory effects against dermatophyte fungi belonging to the Microsporum and Trichophyton groups (Raveesha, & Shrisha, 2013).

The main bacterium that causes acne is *Propionibacterium acnes*, currently reclassified as *Cutibacterium acnes* (Corvec et al., 2019). This bacterium thrives in anaerobic conditions and triggers allergic reactions and inflammation. In most cases, the formation of pus-filled acne starts with the clogging of hair follicles due to various factors such as imbalanced skin bacteria, pollution, medication, cosmetics, and hormonal changes. This obstruction leads to an anaerobic environment in the hair follicles, promoting the growth of *C. acnes*, which ultimately results in inflamed acne. Other bacterial groups such as *S. aureus* and *S. epidermidis* are also found on the skin and contribute to acne formation by producing enzymes that trigger acne development (Lertcanawanichakul et al., 2018).

The treatment for acne includes skin cell turnover enhancement and antibiotics such as clindamycin and tetracycline which inhibit bacterial growth. Prolonged antibiotic use may lead to side effects and antibiotic resistance, and these drugs are not suitable for patients with kidney disease, allergies to antibiotics or similar drugs, photosensitive individuals, and pregnant or lactating women (Ramathibodi Poison Center, 2019; Lee et al., 2013). Isotretinoin, a vitamin A derivative, is considered for severe cases but has significant side effects and is contraindicated for patients with liver disease, pregnant women, and breastfeeding mothers (Zesch, 1988). Therefore, many herbal extracts have been studied for their use in acne treatment as potential alternatives to antibiotics; Buachard et al. (2015) who reported on the antimicrobial activity against acne-inducing bacteria of 15 herbal plants extracts. The results showed that the MIC of water extracts of *Zingiber zerumbet* (L.) Smith and *Alpinia conchigera* Griff was 15.63 – 31.25 mg/ml, and the MBC was 31.25 and 31.25 – 62.5 mg/ml against *S. epidermidis*, respectively. The MIC and MBC for P. acne of *Eleutherine palmifolia* (L.) Merr. extracts were 7.81 and 15.63 mg/ml, respectively. Somkhow et al. (2022) who tested the efficacy of acne gel containing *Allium ascalonicum* L. The results showed that the shallot extract had *P. acnes* inhibitory activity with the MIC at 25.00 mg/mL and the MBC at 50.00 mg/mL. It was then developed into acne gel at concentrations of 0.25, 0.50 and 1.00%. Moreover, studies have been conducted on the use of Kameng in anti-inflammatory and antibacterial resistance. Subramanian, Pazhani, & Ravichandran (2009) reported the Anti-inflammatory activity of methanolic extract of leaves of *Eclipta prostrata* L. by using Carrageenin-induced rat paw oedema and Egg white induced hind paw oedema methods. The results revealed that the methanolic extract of leaves of *E. prostrata* at 100 and 200 mg/kg exhibited 34.02 and 38.80% inhibition respectively in carrageenin induced hind paw oedema, while idomethacin showed 48.47%. In 2015, Cherdtrakulkiat et al. (2015) reported the bioactive triterpenoids, antimicrobial, antioxidant and cytotoxic activities of the aerial parts of *Eclipta prostrata* Linn. A mixture of crude was isolated by column chromatography. The results demonstrate beneficial effects of E. prostrata as antimicrobials and bioactive compounds for medicinal usages. However, limited research exists on the ability of Kameng extracted against *S. aureus* and *C. acnes*. Most previous studies focused on individual parts of Kameng and often used dried powdered Kameng available in herbal stores, which may have reduced antimicrobial potential due to storage and other factors such as the drying and extraction processes.

2. Objectives

Therefore, this study investigated the anti-inflammatory and anti-acne-inducing bacterial activities of fresh Kameng whole plant crude extract including the stem, leaves, flowers, and seeds, using the Soxhlet extraction method with 95% (w/w) ethanol as the solvent. Results significantly contribute to further development of Kameng extract herbal cosmetic products as a cure for acne and skin inflammation.

3. Materials and methods

3.1 Plant preparation

Kameng (*Eclipta prostrata* Linn) grows in Suphan Buri Province, Thailand and no insecticides are used. The collcted plant samples were classified by classification based on botanical classification, such as the characteristics of roots, leaves, and flowers by using BGO Plant Database (BGO Plant Database, The Botanical Garden Organization, 2019). Samples of the whole plant of fresh Kameng were soaked in a vegetable wash solution (KING STELLA VEGGA brand) at a ratio of 15 mL of solution per 1 L of water for 10 min before rinsing with clean water and then washing with distilled water. The samples were then cut into small pieces and dried in a hot air oven at 60^{o}C for 24 h.

3.2 Sample preparation

Kameng samples (100 g) from 3.1 were wrapped in thin white cloth and subjected to Soxhlet extraction using 95% ethanol as the solvent. The extraction process was carried out for 2 h. The resulting extract was filtered using Whatman filter paper No.1, then filtered again for two more rounds. The filtered extract was collected in a known-weight round-bottom flask and subjected to evaporation under reduced pressure using a rotary evaporator (PHLAROTA 4011). The resulting crude extract was dark green with a slight yellowish-brown tint. The weight of the extract was measured, and the percentage of the extracted material was determined. A small amount of solvent was used to elute the extract from the evaporating flask into the vial and then dried it with nitrogen gas. The vial was then sealed and stored in light-tight containers at -20^{o}C for further study.

3.3 Testing the anti-inflammatory ability by evaluating the inhibition of protein degradation

The inhibition of protein degradation was assessed following the modified method of Gunathilake et al. (2018). A 5 mL solvent solution was prepared consisting of 1% bovine serum albumin with a volume of 0.2 mL, 4.78 mL of phosphate buffer solution (pH 6.4), and 0.02 mL of Kameng extract (with varying concentrations: $0.4x10^4$, $0.8x10^4$, $1.2x10^4$, $1.6x10^4$, and $4.0x10^4$ ppm). The solvent solution was then immersed in a water bath (WNE Series, Memmert GmbH + Co. KG, Germany) at a controlled temperature of 37^{o}C for 15 min. After incubation, the solution was heated to 70^{o}C for 5 min and allowed to cool before measuring the turbidity at a wavelength of 660 nm using a UV-visible spectrophotometer (Jenway 6850), with phosphate buffer solution as the control. The percentage of protein degradation inhibition was calculated by Equation (1).

$$\% \text{ Inhibition of protein degradation} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

where $A_{control}$ is the absorbance value of the control, and A_{sample} is the absorbance value of the sample substance.

3.4 Testing anti-inflammatory ability by assessing proteinase inhibition activities

The inhibition of proteinase enzyme activity was conducted following a modified method of Gunathilake et al. (2018). A solution of 2 mL was prepared, consisting of 0.06 mg of trypsin, 20 mM Tris-HCl buffer (pH 7.4) with a volume of 1 mL, 0.02 mL of Kameng extract (with varying concentrations: 0.4×10^4, 0.8×10^4, 1.2×10^4, 1.6×10^4, and 4.0×10^4 ppm), and 0.98 mL of 95% methanol. The mixture was incubated in a water bath at a controlled temperature of 37^{o}C for 5 min. After that, 0.8% casein solution (w/v) was added at a volume of 1 mL and further incubated for 20 min. Then, 2 mL of 70% perchloric acid was added to terminate the reaction. The resulting solution was centrifuged to separate the supernatant. The absorbance of the supernatant at 210 nm was measured using a phosphate buffer solution as the control, the percentage of proteinase enzyme activity inhibition was calculated using Equation (2).

$$\%\text{Proteinase inhibition activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

Where $A_{control}$ is the absorbance value of the control, and A_{sample} is the absorbance value of the sample substance.

3.5 Ability to inhibit bacterial growth assessed using the disc diffusion method.

The bacterium *S. aureus* used as a source in this experiment is *S. aureus* TISTR746 from the department of biology, faculty of science and technology, Rajamangala university of technology suvarnabhumi and the bacterium *C. acnes* used as a source was isolated from a patient with a skin disease at the Manose Health and Beauty Research Center.

Kameng extract was assessed for the ability to inhibit bacterial growth by the paper disc diffusion method modified from Bauer et al. (1966); Supaphon (2018); Banjara et al. (2012). In this research, the two bacterial strains, *S. aureus* and *C. acnes* were cultured in liquid media. *S. aureus* was cultured in Nutrient broth (NB) at 37°C for 24 h, while *C. acnes* was cultured in Brain Heart Infusion (BHI) broth at 37°C for 48 h under anaerobic conditions. The bacterial suspensions were adjusted to approximately 0.5 McFarland standard (10^8 CFU/mL).

Each bacterial strain was then swabbed onto the surface of agar plates (NB agar for *S. aureus* and BHI agar for *C. acnes*) using sterile cotton swabs. The agar plates were allowed to dry for approximately 3-5 min. The Kameng extract was then tested by applying it to paper discs on the agar surface. The experiment followed a completely randomized design (CRD). The extract was dissolved with ethanol to obtain concentrations of 1200 mg/mL and filtered through a membrane filter with a pore size of 0.2 µm.

To test the antibacterial activity against *S. aureus*, the extracts were diluted with 95% ethanol to obtain samples with concentrations of 10, 100, 1000 and 1200 mg/mL. Two control methods were used: a negative control using 95% ethanol and a positive control using a standard solution of clindamycin with a concentration of 30 mg/mL. Then, 10 µL of each extracted sample (with varying concentrations) was applied onto 6 mm filter paper discs to achieve concentrations of 0.1, 1.0, 10, and 12 mg/disc. For the control agents, 10 µL of clindamycin 0.30 mg/disc solution and 95% ethanol were also applied to separate discs and the discs were allowed to dry. Each bacterial strain was swabbed using a sterile cotton swab onto NB agar plates. Three replicates of the antibacterial test for each concentration were performed. The filter paper discs containing the extract samples and controls were placed onto the surface of the bacterial swabbed agar plates and then incubated in a bacterial incubator at 37±1°C for 48 h.

For testing the antibacterial activity against *C. acnes*, the crude Kameng extracts were diluted with 95% ethanol to obtain samples with concentrations of 10, 100, 1000 and 1200 mg/mL. A positive control was also prepared using a standard solution of clindamycin at a concentration of 0.2 mg/mL. Then, 10 µL of each extracted sample (with varying concentrations) was applied on 6 mm filter paper discs to achieve concentrations of 0.01, 0.10, 1.0, and 1.2 mg/disc. For the clindamycin positive control, 10 µL of a 0.002 mg/disc solution and 95% ethanol were also applied to separate discs and the discs were allowed to dry. The *C. acnes* bacterial strain was swabbed using a sterile cotton swab onto BHI broth agar plates. Three replicates of the antibacterial test for each concentration were performed for the same bacterial strain. The filter paper discs containing the galangal extract samples and controls were placed on the surface of the bacterial swabbed agar plates, and the plates were then transferred to an anaerobic jar to create an oxygen-free environment. The jars were incubated at 37±1°C for 48 h. After the incubation period, the plates were taken out, and the inhibition zones, measured in mm, were recorded as the diameter of the area around the discs where bacterial growth was inhibited.

Table 1 Concentration of Kameng extract and clindamycin (CM) in test wells (1-12)

Samples	**Concentration in each test well**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Extracts (mg/mL)	20	10	5.00	2.50	1.25	0.62	0.31	0.08	0.04	0.02	0.01	0.005
CM (µg/mL)	50	25	12.50	6.25	3.12	1.56	0.78	0.39	0.20	0.10	0.05	0.025

3.6 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Kameng extract against bacterial strains using the broth microdilution and drop plate methods

Kameng extract was first diluted with 10% DMSO to a concentration of 40 mg/mL, the dilutions were filtered through a 0.2 µm filter membrane. Next, a 96-well microplate was prepared and the wells were filled with liquid culture media (Nutrient Broth for *S. aureus* and Brain Heart Infusion Broth for *C. acnes*), with each well containing 100 µL. The extract was added to the first well with a volume of 100 µL. The substances were mixed and then 100 µL was taken from the first well and added to the second well, and so on until well number 12. In the 12^{th} well, 100 µL of the mixture was discarded (2-fold serial dilution). The same experimental procedure was followed but the Kameng extract was changed with the antibiotic clindamycin at a concentration of 100 µg/mL. The concentrations of substances in each well bewere recorded, as shown in Table 1.

The MIC was determined as the lowest concentration of the extract that inhibited visible bacterial growth. The MBC was determined by transferring samples from the wells with no visible growth onto agar plates and incubating them further to check for complete bacterial killing. The concentration at which there was no bacterial growth on the agar plates was recorded as the MBC.

To determine the MIC of Kameng extract against *C. acnes* and *S. aureus* using the Drop Plate method, the bacterial strains were adjusted to a turbidity equivalent to 0.5 McFarland standards in each well, with a volume of 100 µL per well. The microplate was then incubated at 37±1ºC for 48 h (in an anaerobic condition for *C. acnes*). After the incubation period, the last well that appeared clear with no bacterial growth at the bottom of the well was used to determine the MIC value. The turbidity was compared-using 100 µg/mL of clindamycin as a positive control and 10% DMSO as a negative control. All experiments were conducted in triplicate.

For the determination of the MBC of the extract, the concentration at which the bacteria were killed, was determined using the Drop Plate method. A 5 µL sample from wells where no visible growth was observed (last wells) in the determination of MIC was dropped onto the surface of the agar plates (NB for *S. aureus* and BHI agar for *C. acnes*) and then incubated again at 37±1 ºC for 48 h (in an anaerobic condition for *C. acnes*). After the incubation, the plates were examined for the absence of bacterial growth on the agar surface. The minimum concentration of Kameng extract that resulted in no bacterial growth on the agar plate was recorded as the MBC. The same procedure was repeated in triplicate for each bacterial strain.

Statistical Analysis

Statistical analysis was conducted using both descriptive and inferential statistics to analyze the general data and perform hypothesis testing at a significance level of 0.05. Descriptive statistics, including percentages, mean, and standard deviations, were used to summarize and describe the data, while inferential statistics as parametric tests, were employed to analyze the data using a completely randomized design at a significance level of 0.05.

4. Results and discussion

Kameng extract prepared using the Soxhlet extraction method with 95% ethanol as the solvent was a dark green with a tinge of brown, and the quantity of crude extract ranged from 2.50 to 3.75% by weight.

4.1 Anti-inflammatory test based on protein degradation inhibition

Kameng extract was tested for anti-inflammatory ability by evaluating its capacity to inhibit protein degradation. The extract was prepared at five concentrations: $0.4x10^4$, $0.8x10^4$, $1.2x10^4$, $1.6x10^4$, and $4.0x10^4$ ppm. The results of the experiment showed that the percentage of inhibition protein degradation increased with higher concentrations of Kameng extract. Phosphate buffer solution was used as the negative control. Results revealed a significant impact of increasing extract concentration on the inhibition of protein degradation ($F = 19529.003$, $p = 0.000$) (Table 2 and 3).

4.2 Anti-inflammatory test based on proteinase inhibition activities

The inhibition of proteinase activity by Kameng extract ranged from 23.97% to 69.55%. Results indicated that Kameng extract had the potential to inhibit proteinase activity, with the effect being more pronounced as the concentration of the extract increased. Phosphate buffer solution

was used as the negative control. Results revealed a significant impact of increasing inhibition of proteinase activity when the extract concentration increased (F = 135659-203, p = 0.000) (Table 2).

These findings concurred with Arunachalam et al. (2009), who reported on the anti-inflammatory properties of Moringa oleifera leaf extracts in a mouse model with induced swelling. In their experiment, four groups (each consisting of 6 mice) were studied. Groups 1 and 2 received Moringa oleifera leaf extracts orally at doses of 100 and 200 mg/kg, respectively after inducing swelling with 1% carrageenan (0.1 mL). Group 3 was administered 5 mL/kg of propylene glycol, while group 4 received the anti-inflammatory drug indomethacin at a dose of 10 mg/kg. Another experiment with similar conditions was conducted, replacing carrageenan with 0.1 mL of egg white and using cyproheptadine at a dose of 8 mg/kg as the standard drug. Results confirmed the significant anti-inflammatory effects of Kameng extracts. These findings concurred with Sangkaram et al. (2021) who tested the anti-inflammatory effects of Kameng extracts in ethanol using the inhibition of nitric oxide (NO) production. Results showed that Kameng extracts in 95% ethanol exhibited better anti-inflammatory properties than water extracts and the drug diclofenac, with NO inhibition percentages of 75.66 ± 2.66%, 9.76 ± 0.45%, and 66.79±0.97%, respectively.

4.3 Antibacterial activity test using the disc diffusion method

The antibacterial properties of Kameng extract were evaluated against *S. aureus* and *C. acnes* using the disc diffusion method. The extract exhibited significant antibacterial activity. The maximum inhibition zone diameter against *S. aureus* was 31.15±0.47 mm at a concentration of 12.00 mg/disc. By comparison with the standard antibiotic, clindamycin had an inhibition zone diameter of 34.14±0.08 mm (F = 2684.510, p = 0.000) as shown in Figure 1.

Table 2 Anti-inflammatory activities (inhibition of protein degradation) of crude ethanolic extract of Kameng

Crude extract of Kameng (ppm)	Percentage inhibition of protein degradation			
	1st	2nd	3rd	Mean ± SD
0.4 x 10^4	14.95	14.58	14.45	14.66 ± 0.26
0.8 x 10^4	17.39	17.73	17.56	17.56 ± 0.17
1.2 x 10^4	24.06	24.2	24.17	24.14 ± 0.07
1.6 x 10^4	35.48	35.74	35.6	35.61 ± 0.13
4.0 x 10^4	50.99	50.71	50.55	50.75 ± 0.22
Phosphate buffer	0.00	0.00	0.00	0.00

(n = 3)

Table 3 Anti-inflammatory activities (inhibition of proteinase activities) of crude ethanolic extract of Kameng

Crude extract of Kameng (ppm)	Percentage inhibition of proteinase activities			
	1st	2nd	3rd	Mean ± SD
0.4 x 10^4	24.03	23.89	24.00	23.97 ± 0.07
0.8 x 10^4	41.89	41.98	41.92	41.93 ± 0.05
1.2 x 10^4	50.49	50.47	50.58	50.51 ± 0.06
1.6 x 10^4	55.7	55.84	55.69	55.74 ± 0.08
4.0 x 10^4	69.68	69.45	69.53	69.55 ± 0.12
Phosphate buffer	0.00	0.00	0.00	0.00

(n = 3)

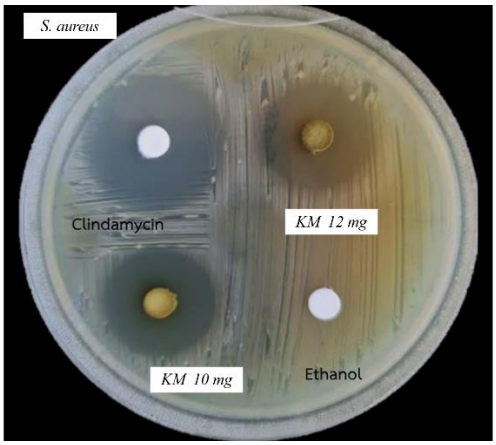

Figure 1 The inhibition zone diameter against *S. aureus* of Kameng extract (KM = Kameng extract)

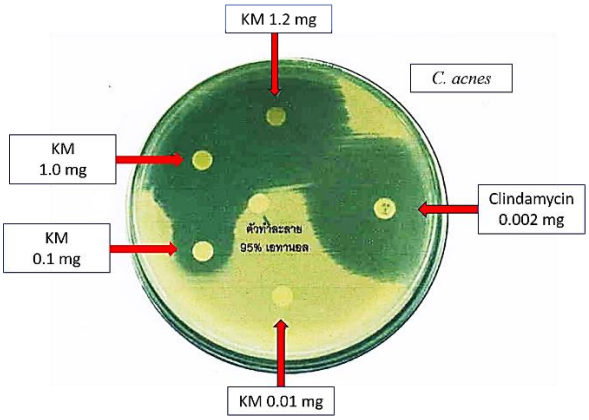

Figure 2 The inhibition zone diameter against *C. acnes* of Kameng extract (KM = Kameng extract)

Similarly, the extract showed a maximum inhibition zone diameter of 37.28±0.47 mm against *C. acnes* at a concentration of 1.20 mg/disc. By contrast, clindamycin had an inhibition zone diameter of 43.77±1.56 mm at a concentration of 0.002 mg/disc as shown in Figure 2.

Increasing the concentration of the extract significantly influenced the inhibition ability against *C. acnes* ($F = 1515.027$, $p = 0.000$) (Tables 4 and 5).

4.4 Determination of MIC and MBC of Kameng extract against acne-causing bacteria (*C. acnes* and *S. aureus*) using broth microdilution and drop plate methods

The MIC and MBC values of Kameng extract against *C. acnes* and *S. aureus* were determined using the broth microdilution and drop plate methods. The MIC values of the extract against *S. aureus* and *C. acnes* were 5.00 and 2.50 mg/mL, respectively while the MBC values against *S. aureus* and *C. acnes* were 20.00 and 10.0 mg/mL, respectively. By comparison, the MIC values of the standard antibiotic, clindamycin against *S. aureus* and *C. acnes* were 1.56 and 0.78 µg/mL, respectively while the MBC values against *S. aureus* and *C. acnes* were 3.12 and 1.56 µg/mL, respectively (Table 6). Sangkaram et al. (2021) investigated the biological activities of dried Kameng extracts using ethanol as the solvent. They reported MIC and MBC values of 12.5 and 25.00 mg/mL, respectively against *S. aureus.* Kameng extracts in this study showed better efficacy, with differences possibly arising from using fresh Kameng extracts cultivated in Suphan Buri Province, while the previous study used dried Kameng from a herbal pharmacy with an unknown storage time. The processes for extraction and storage were different, leading to possible loss of potency.

Table 4 Antibacterial activity of Kameng extract against *S. aureus* by the disc diffusion method

Sample	Concentration	Zone of inhibition (mm)			
		Plate 1	Plate 2	Plate 3	Mean ±SD
Clindamycin	0.30 (mg/disc)	34.07	34.12	34.24	34.14±0.08
Ethanol	95 %	0.00	0.00	0.00	0.00±0.00
Kameng extract	0.10 (mg/disc)	0.00	0.00	0.00	0.00±0.00
	1.00 (mg/disc)	12.11	11.09	12.78	11.99 ± 0.85
	10.00 (mg/disc)	28.21	28.40	28.33	28.31±0.10
	12.00 (mg/disc)	31.05	30.74	31.66	31.15 ± 0.47

(n=3)

Table 5 Antibacterial activity of Kameng extract against *C. acne* by the disc diffusion method

Sample	Concentration	Zone of inhibition (mm)			
		Plate 1	Plate 2	Plate 3	Mean ± SD
Clindamycin	0.002 (mg/disc)	44.37	44.95	42.00	43.77±1.56
Ethanol	95 %	0.00	0.00	0.00	0.00±0.00
Kameng extract	0.01 (mg/disc)	0.00	0.00	0.00	0.00±0.00
	0.10 (mg/disc)	17.64	16.75	15.28	16.56±1.19
	1.00 (mg/disc)	34.08	32.85	34.44	33.79±0.85
	1.20 (mg/disc)	37.15	36.88	37.80	37.28±0.47

(n=3)

Table 6 MIC and MBC of Kameng extract against *S. aureus* and *C. acnes*

Sample	*S. aureus*		*C. acnes*	
	MIC	MBC	MIC	MBC
Clindamycin (µg/mL)	1.56	3.12	0.78	1.56
Kameng extract (mg/mL)	5.00	20.00	2.50	10.00

5. Conclusions

This research assessed the antiinflammatory and antibacterial properties of Kameng extract from the whole plant cultivated in Suphan Buri Province. The extracts concentrations of 0.4 - 4.0 x 10^4 ppm inhibited protein degradation and proteinase activity ranging from 14.66% - 50.75% and 28.57% - 72.08%, respectively. Findings demonstrated the anti-inflammatory effects of Kameng extracts, with activity increasing proportionally to the extract concentration.

Kameng extract also exhibited antibacterial activity against *S. aureus* and *C. acnes*, with inhibition zones of 31.15±0.47 and 37.28±0.47 mm, respectively, when tested using the agar disc diffusion method. The MIC and MBC of Kameng extracts against *S. aureus* and *C. acnes* were 5.00 and 2.50 mg/mL for MIC, and 20.00 and 10.0 mg/mL for MBC. Results suggested that Kameng extracts cultivated in Suphan Buri Province had potential as anti-acne agents, with possible further development into cosmetic products for acne treatment and skin inflammation reduction in combination with other herbal extracts. Kameng is a readily propagable plant and suitable for commercial product development, potentially reducing the reliance on antibiotics and providing an additional income source for herbal farmers. If other purification techniques are used together, such as column chromatography. It will be able to increase the efficiency of the extract even more.

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