



Comparative study of the antibacterial and anti-inflammatory activities of the seed coat vs. seed kernel extracts from the plant *Mangifera indica* L. in inflammatory acne treatment

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ABSTRACT

Introduction: The present study was developed to investigate and compare the extraction yield, the contents of polyphenols and mangiferin, the activities against *Propionibacterium acnes*, *Staphylococcus aureus*, and *Escherichia coli* bacteria, and the anti-inflammatory activities of ethanol extracts of mango seed kernels vs. seed coats.

Methods: Mango seed kernels and seed coats were extracted using ethanol as the solvent and tested against microorganisms using the disc diffusion method. The minimum inhibitory concentration (MIC) levels of extracts were determined by the agar dilution method. The anti-inflammatory activities were assessed both *in vitro* and *in vivo* by albumin denaturation method and carrageenan-induced paw edema test, respectively.

Results: Both extracts yielded high contents of mangiferin and phenolic compounds. The antibacterial activities of both extracts showed inhibition of the tested microorganisms *Propionibacterium acnes* and *Staphylococcus aureus* but not *Escherichia coli*. Seed kernel extract (0.2 g/kg) reduced paw edema by 44.8% at 3 hours after λ -carrageenan administration. Meanwhile, 0.5 g/kg seed coat extract reduced paw edema less than the seed kernel extract (23.1% vs. 44.8%). Mango seed kernel extract, mango seed coat extract, and diclofenac sodium displayed concentration-dependent inhibition of heat-induced protein denaturation with IC₅₀ values of 137.23 μ g/mL, 292.12 μ g/mL, and 6.64 μ g/mL, respectively.

Conclusion: The obtained results confirmed the antibacterial and anti-inflammatory potential of mango seed kernel and seed coat extracts. The mango seed kernel extract was proven to be more effective than the mango seed coat extract and thus can be used in cosmetics as an anti-inflammatory and antibacterial agent.

Implication for health policy/practice/research/medical education:

The extracts from mango seed possessed antibacterial and anti-inflammatory activities. However, further clinical examination should be performed to approve these effects.

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Introduction

Inflammatory acne is a severe form of acne. The mechanism of inflammation in acne is complex and still being studied but may involve *Propionibacterium acnes* (1). Various inflammatory pathways are activated. Thus, anti-inflammatory drugs are expected to exert effects against

inflammatory acne (2,3). The use of medicinal plants for healing has existed for a very long time and continues to play a significant role in health care for people. Many plants are reputed to be useful in the treatment of infections and inflammation (4,5).

Mango (*Mangifera indica* L.), a member of the family

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Anacardiaceae, is produced worldwide. In Vietnam, mango has been cultivated in approximately 87000 ha of the whole land area, and total mango production is approximately 969 000 tons per year. One of the enormous mango wastes is mango seed, which contains a considerable amount of important nutrients and bioactive compounds and could provide an inexpensive, available, and important source for tackling an environmental problem as an eco-friendly alternative. Mango seed has many active constituents, such as antioxidant and polyphenolic compounds (6-8). Mango seed has antioxidant and polyphenolic compounds higher than the pulp and peel (9). There have been some reports on the pharmacological effects of mango seed, such as antioxidant (10,11), antimicrobial (12), anticancer (13), antidiarrheal (14) and antifungal activities (15). Mango seed powder can be utilized to address the challenge of food poisoning and infections caused by pathogenic microorganisms such as *Candida albicans* and *Escherichia coli* in the food industry (16). Choke-Anan mango seed kernel extracts from a portion of the seeds exhibited considerably strong antioxidant activity and inhibitory activity for the enzymes 5-lipoxygenase, hyaluronidase, and α -glucosidase (17). A comparative study of the antimicrobial effects of the seed, leaf, and stem bark of the plant *M. indica* showed that the seed extract had the highest antimicrobial activity against both gram-positive and gram-negative bacteria. The ethanolic extract of mango seed has more potent antibacterial activity than extracts of the stem bark and leaf at the same concentration (18).

The mango seed comprises the kernel (68%) and coat (32%) (9). Mango seed kernels possess carotenoids, tocopherol, polyphenols (mangiferin, hesperidin, rutin, quercetin, kaempferol, etc), and phenolic acids (gallic acid, caffeic acid, ellagic acid, etc). These phytochemicals are known for their high antioxidant, anticancer, antibacterial, and antidiabetic potentials (19,20). Seed oil extract had stronger antibacterial effect against *Staphylococcus aureus*, with a maximal zone of inhibition of 16.50 mm, minimum inhibitory concentration (MIC) of 0.10 μ L/mL, and minimum bactericidal concentration of 0.20 μ L/mL. Mango seed oil extract was found to have more pronounced antioxidant and antimicrobial effects than peel oil extract (21). In another study, the ethanolic fractions showed the strongest antimicrobial activity against *P. acnes* with a MIC and minimum bactericidal concentration of 1.56 mg/mL and 12.50 mg/mL, respectively (22). The inhibitory actions of the extracts on interleukin 8 secretion from lipopolysaccharide-induced RAW 264.7 cells were also mentioned. Raw *M. indica* fruit kernel extracts were efficient against aerobic and anaerobic bacteria that cause acne, particularly *P. acnes*, and expressed anti-inflammatory properties (22).

These results proposed the use of mango seed as an ideal candidate for treating inflammatory acne and as a valuable ingredient instead of an undervalued waste.

The latest studies in Vietnam have suggested that plant-derived drugs are valuable sources of antioxidants, antibiotics, and anti-inflammatory agents (23,24). However, there is no study about the antibiotics and anti-inflammatory activities of seeds from mango cultivars grown in Vietnam. In addition, earlier studies on mango seed proved the screening of bioactive compounds and activities in mango seed in general or mango seed kernel. The effect of ethanol extracts of mango seed kernel vs. seed coat on the extraction yield of bioactive components from these extracts and their pharmacological properties have not been previously announced. Therefore, this research was carried out to investigate and compare the extraction yield, the content of mangiferin and phenolic compounds, the *in vitro* antibacterial abilities of ethanol extracts of mango seed kernel vs. seed coat against *P. acnes*, *S. aureus*, and *E. coli*, and the anti-inflammatory activities of these extracts.

Materials and Methods

Materials

Cat Hoa Loc mango cultivar grown in southern Vietnam was used in this study. Fresh seeds and leaves of *M. indica* L. were collected from the O Mon ward, Can Tho province, Vietnam, from March to April 2021 and were identified in the Department of Botany-Traditional Medicine-Pharmacognosy, Can Tho University of Medicine and Pharmacy. A specimen has been reserved in the Department of Botany-Traditional Medicine-Pharmacognosy (PBTMD/2021/001). The collected seeds were washed, dried, and kept in plastic bags at room temperature until use. Three reference strains (American Type Culture Collection [ATCC]), *S. aureus* (ATCC 29213), *P. acnes* (ATCC 6919), and *E. coli* (ATCC 25922), were used to monitor the quality control of the procedures. Levofloxacin, gallic acid, and λ -carrageenan were obtained from Sigma-Aldrich Chemical Co. (United States), trimethoprim-sulfamethoxazole from Nkbiotek (Vietnam), bovine serum albumin from HiMedia (India), and diclofenac and mangiferin standards from the National Institute of Drug Quality Control (Vietnam). All of the other reagents, chemicals, and solvents were of reagent grade or higher.

Animals

Male Swiss albino mice (25–35 g) were acquired from Can Tho University's Animal Laboratory in Vietnam. The number of animals per group was 6, and there were 4 groups in the anti-inflammatory activity evaluation test. They were basal fed, given free access to water, and the animals were kept at 22 ± 3 °C with a 12-hour dark and light cycle at the pharmacology laboratory at Can Tho University of Medicine and Pharmacy for at least one week prior to the experiment. The Can Tho University of Medicine and Pharmacy Committee accepted the

experimental procedure (approval number 176/DHYDCT, January 27, 2021).

Extract preparation and quantitative phytochemical assessment

The extraction of 100 g dried powder of mango seed kernels and 100 g dried powder of mango seed coats was carried out in 0.8 L of commercial ethanol at room temperature for 24 hours. To obtain the viscous substance, the filtrates were dried under vacuum in a rotating evaporator at 40 °C. Then, these were vaporized to obtain a constant mass in a vacuum drying cabinet and kept at 4 °C to be used in experiments.

Mangiferin content determination

The samples were analyzed with a UV-vis visible spectrophotometer (Shimadzu Corp, Japan). *Standard solution*: Ten milligrams of mangiferin standard was accurately weighed and then dissolved in ethanol-water (1:1) solution to obtain a 100 µg/mL stock standard solution. After that, the stock standard was diluted with ethanol-water (1:1) solution to produce solutions with various concentrations (10, 15, 20, 25, 30 µg/mL). The samples were measured at 367 nm. *Blank sample*: Ethanol-water (1:1). *Test solution*: First, 0.5 g of mango seed extract was weighed and then ethanol-water (1:1) was added to obtain 100 mL. For 20 minutes, the test sample was extracted in a bath of ultrasound. The sample was then centrifuged, and its supernatant was taken. The resulting solution was diluted with ethanol-water (1:1) to appropriate absorbance, and photometrically measured at 367 nm.

Total phenolic content determination

The extract's total phenolic content was measured using the Folin-Ciocalteu technique (25). In brief, 60 mg of the extract was sonicated with 40 mL of distilled water for 15 minutes at 40 °C, then water was added to make 50 mL. The mixture was then filtered, and 1 mL of the filtrate was thoroughly mixed with 5 mL of Folin-Ciocalteu reagent for 2 minutes before adding 4 mL of 7.5% (w/v) sodium carbonate. After standing in the dark for a further 60 minutes, the absorbance at 750 nm was measured. The calibration curve was applied to calculate the total phenolic content, which was expressed in milligrams of gallic acid equivalent per gram of dry weight.

Antibacterial activity evaluation

Bacterial susceptibility determination with an agar well diffusion assay

Reference strains were sub-cultured on agar and underwent incubation for 24 hours at 37 °C. After 24 hours, 3-4 colonies were selected and blended in 5 mL of saline. The bacterial turbidity was then adjusted to 0.5 McFarland (10^8 CFU/mL equivalent). To determine their

antibacterial properties of the diluted extracts, they were dissolved in dimethyl sulfoxide at varied concentrations of 25 mg/mL, 50 mg/mL, 100 mg/mL, and 200 mg/mL. Levofloxacin and trimethoprim/sulfamethoxazole were used as positive controls. One hundred microliters of bacterial suspension were added to Muller Hinton agar plates (for *E. coli*-ATCC 25922 and *S. aureus*-ATCC 29213) and tryptic soy agar plus 5% blood plates (for *P. acnes*-ATCC 6919). A sterile cork borer with an 8 mm diameter was used to cut wells into the agar. Four wells were filled with 60 µL of diluted extracts. Two remaining wells were filled with positive (levofloxacin, 5 µg or trimethoprim/sulfamethoxazole 1:19, 5 µg) and negative controls (dimethyl sulfoxide, DMSO). Agar plates were kept for 5 minutes, allowing the extracts to diffuse. The plates of cells were then incubated at 37 °C for 48 hours. The diameter of the clear zone in millimeters was used to calculate the zone of inhibition.

Minimum inhibitory concentration determination

The agar dilution technique was used to determine the MIC of extracts. Before solidification, extracts at various strengths (5, 2.5, 1.25, 0.625, and 0.312 mg/mL in DMSO with 0.05% Tween 80) were added to the agar media. The suspension of bacteria was adjusted to reach the McFarland criteria of 0.5. Diluted extracts were mixed with autoclaved agar (121 °C for 15 minutes) at 50 °C and given into plates. The filled plates were left to dry for approximately 10-15 minutes. In parallel, a control plate with agar without extract inoculated with strains was set up to validate the viability of the cultures (positive control). 10 µL of the bacterial suspension was used to inoculate the plates. After standing for 5 minutes, the plates were incubated at 37 °C for 18 hours. The MIC was the lowest concentration of the extract at which all bacterial strains were inhibited.

Anti-inflammatory activity evaluation

Acute toxicity study

Six animals (three animals for each step) can be used in a limit test at a single dose level of 5000 mg/kg body weight. Further testing at the next lower limit of 2000 mg/kg may be required if test substance-related fatality is found (26). Mice were orally administered mango seed kernel and seed coat extracts suspended in water at an amount of 1 mL/100 g body weight to each mouse for 14 days in the morning with fasting conditions. The mortality and unusual behavior of the animals were noticed after 30 minutes for 6 hours and then after 24 hours for 14 days. The oral acute toxicity experiments were carried out in accordance with the OECD/OCDE 423 guidelines (26).

In vitro anti-inflammatory activity evaluation

The anti-inflammatory properties of the extracts of seed kernels and seed coats were studied using a method

modified slightly from a study by Shah et al (27). A reaction mixture containing 150 µL of a sample of varied concentrations and 150 µL of bovine serum albumin (BSA) was prepared. These mixtures were left to incubate for 20 minutes at 27 °C and then for 20 minutes at 80 °C. At 660 nm, the absorbance of the reaction mixtures was measured. Diclofenac was used as a standard drug. The test was performed in triplicate, and the percent of protein denaturation inhibition was calculated using the following formula:

$$\text{Denaturation inhibition \%} = \frac{\text{Absorbance control} \times \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

In vivo anti-inflammatory activity evaluation

The anti-inflammatory properties of the extracts were tested using the experimental carrageenan-induced mouse paw edema model. In brief, mice were randomly assigned to different groups, and the volume of their paws up to the ankle joint was recorded with a plethysmometer (Ugo Basile 37140, Italia). Subsequently, mice were orally administered 1% DMSO in water, diclofenac (10 mg/kg), mango seed coat extract (500 mg/kg), and mango seed kernel extract (200 mg/kg) 1 time/day for 3 days. Test samples were dissolved and dispersed in DMSO 1% solution. The samples were inserted directly into the mice's stomach with a flexible plastic tubing oral gavage needle. These mice were injected in the right hind paw with 50 µL of 1% carrageenan (w/v) one hour after the last dose. The paw volume of the mice was recorded at 1, 3, 5, and 7 hours after carrageenan injection. The following formula was used to calculate edema:

$$\text{Paw edema volume} = \text{paw volume at the time} - \text{paw volume base}$$

Statistical analysis

The antibacterial activity results are presented as the mean ± standard deviation. All anti-inflammatory data were given as the mean ± standard error for 6 animals per group. The statistical analyses were calculated using one-way ANOVA, and a *t* test was utilized to assess the significant differences between the results. With a 95% confidence level, we used a level of significance of 0.05.

Results

Extraction yield and quantitative phytochemical assessment

Each 100 g dried powder of mango seed kernels and 100

g dried powder of mango seed coats was soaked in 0.8 L of commercial ethanol for 24 hours and provided a 4.00 g yield of mango seed kernel extract and 1.02 g yield of mango seed coat extract. Data in Table 1 demonstrates the contents of phenolics and mangiferin in the ethanol extracts of mango seed coat and mango seed kernel.

The content of mangiferin extracted from the mango seed kernel extract was approximately equal to that in the mango seed coat extract. However, the extraction yield of phenolic components (654.56 mg GAE/g) in the mango seed kernel extract was higher. Both of these extracts had high contents of mangiferin (1-1.5%, w/w) and phenolics (55-65%, w/w).

Results of antibacterial activity

Agar well diffusion assay

The antibacterial properties of mango seed coat and mango seed kernel extracts against bacteria were measured as a zone of inhibition in mm. Mango seed kernel extract showed a smaller zone of inhibition (17.7 ± 1.4 mm) against *P. acnes* at the lowest concentration of the extract (25 mg/mL). Mango seed kernel extract showed a zone of inhibition >10 mm at all concentrations of the extracts (Table 2). The positive control (trimethoprim/sulfamethoxazole) showed a zone of 34 ± 1 mm against *P. acnes*. The positive control (levofloxacin) showed zones of 39.0 ± 1.4 mm and 28.0 ± 1.4 mm against *E. coli* and *S. aureus*, respectively. Trimethoprim/sulfamethoxazole (control) and plant extracts had significantly different inhibitory zones (*P* < 0.05) and between seed coat extracts and seed kernel extracts (*P* < 0.05). There was no zone of inhibition around the wells that were merely submerged in solvents (negative control); all extracts were effective against *E. coli*. The extracts were tested for antibacterial activity using agar dilution methods for inhibitory concentration assays (Figure 1).

Determination of minimal inhibitory concentration

Subsequent experiments were conducted to identify the inhibitory concentrations of all extracts. Figure 2 and Table 3 show the results of the extracts' MIC. The control showed that the culture medium without the seed extracts had no inhibiting impact on bacterial growth. The seed kernel extract of *M. indica* showed a greater antibacterial effect. The MIC values of mango seed kernel and seed coat extracts against *P. acnes* were 1.25 and 5 mg/mL, respectively (Table 3).

Table 1. Phenolics and mangiferin content of *Mangifera indica* seed coat extract and seed kernel extract

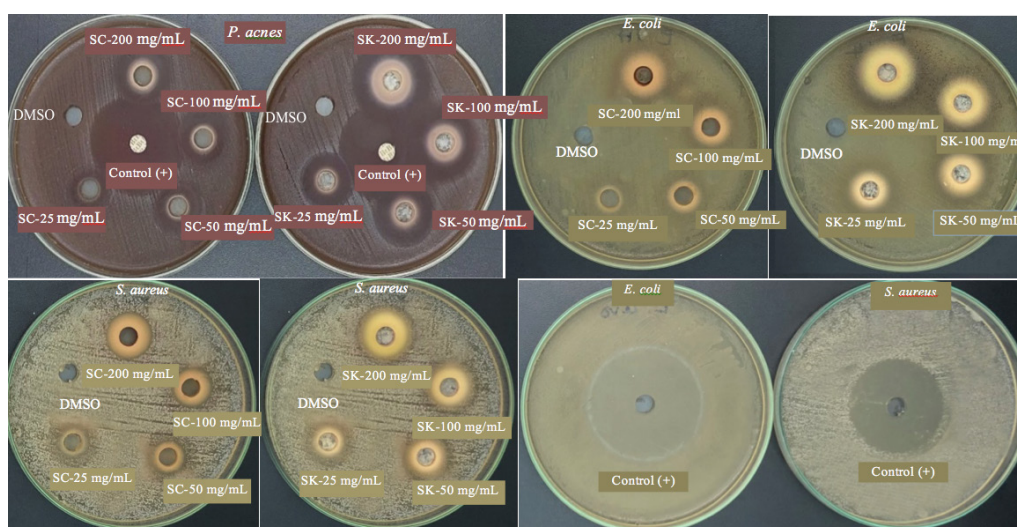
Extraction	Phenolics (mg GAE/g extract)	Mangiferin (mg/g extract)
Mango seed kernel	654.56 ± 2.99	13.72 ± 0.11
Mango seed coat	548.47 ± 1.95	14.78 ± 0.22

Note: GAE: Gallic acid equivalent; Values are mean ± SD (n = 3).

Table 2. Antibacterial efficacy of *Mangifera indica* seed kernel and seed coat extracts against *Propionibacterium acnes*, *Staphylococcus aureus*, and *Escherichia coli*

Test concentration (mg/mL)	Diameter of inhibition zone (mm)					
	<i>Propionibacterium acnes</i>		<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
	SK	SC	SK	SC	SK	SC
200	23.7 ± 2.2	19.6 ± 1.3	20.0 ± 1.5	16.0 ± 1.2	0 ± 0	0 ± 0
100	24.0 ± 1.8	17.4 ± 0.8	16.0 ± 1.3	14.0 ± 1.3	0 ± 0	0 ± 0
50	20.7 ± 0.9	16.2 ± 1.6	13.0 ± 1.2	9.0 ± 1.2	0 ± 0	0 ± 0
25	17.7 ± 1.4	12.6 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Trimethoprim/sulfamethoxazole	34.0 ± 1.0		-		-	
Levofloxacin	-		28.0 ± 1.4		39.0 ± 1.1	
DMSO	0 ± 0		0 ± 0		0 ± 0	

SC: Mango seed coat extract; SK: Mango kernel extract. SD: Standard deviation. DMSO: Dimethyl sulfoxide. Values are represented as mean ± SD from triplicate experiments.

**Figure 1.** Formation of inhibition zones of *Mangifera indica* seed kernel extracts (SK) and seed coat extracts (SC) against *Propionibacterium acnes* (Positive control: Trimethoprim/sulfamethoxazole (Control (+)); Negative control: dimethyl sulfoxide (DMSO)); against *Staphylococcus aureus*, and *Escherichia coli* (Positive control: Levofloxacin (Control (+)); Negative control: dimethyl sulfoxide (DMSO)).

Assessment of anti-inflammatory activity

Acute toxicity studies

For seed coat extract, mice given a high dose of 5000 mg/kg survived for 14 days with no evidence of acute toxicity. However, one mouse died 4 hours after administering seed kernel extract at the same dose. Then, a next lower dose of 2000 mg/kg was used. The results showed that the animals remained alive for 14 days. Thus, a 200 mg/kg

dose of seed kernel extract and a 500 mg/kg dose of seed coat extract were chosen for *in vivo* anti-inflammatory testing.

Heat-induced protein denaturation activity inhibition

The *M. indica* seed kernel extract and seed coat extract were tested for anti-inflammatory activity *in vitro* using a heat-induced protein denaturation assay (Figure 3). Both of these extracts inhibited heat-induced protein denaturation in a dose-dependent manner. The IC₅₀ values (μg/mL) were discovered in seed kernel extract (137.23 ± 2.24 μg/mL) < seed coat extract (292.12 ± 3.22 μg/mL). The percentage inhibition ranges of seed kernel extract were 2.4% and 70.85% at concentrations ranging from 6.25 μg/mL to 200 μg/mL and seed coat extract (2.7%–68.05%) at concentrations ranging from 12.5 μg/mL to 400 μg/mL. Diclofenac was used as a standard drug and exhibited an IC₅₀ value of 6.64 ± 0.13 μg/mL, and

Table 3. Minimum inhibitory concentration values of *Mangifera indica* seed kernel extract and *Mangifera indica* seed coat extract

Extracts	Test organisms/MIC (mg/mL)		
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Propionibacterium acnes</i>
SK	>5	>5	1.25
SC	>5	>5	5

SC: Mango seed coat extract, SK: Mango kernel extract. MIC: Minimum inhibitory concentration.

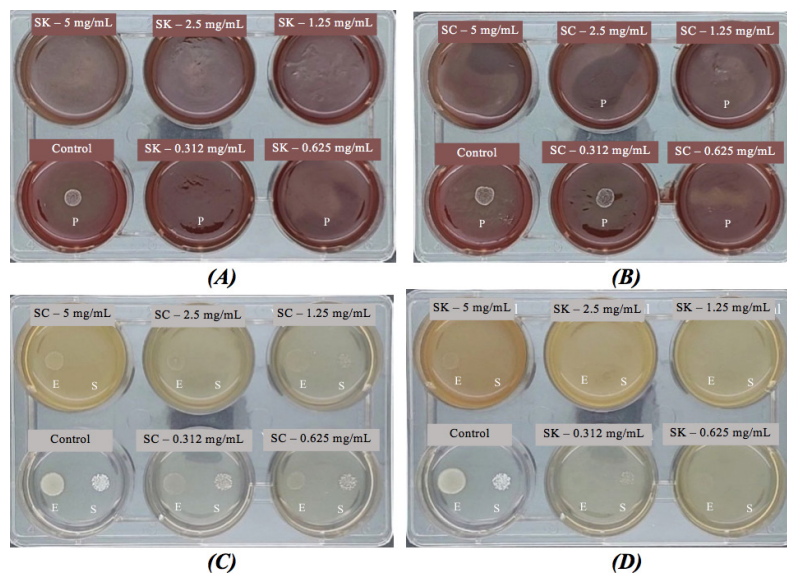


Figure 2. Results of minimum inhibitory concentration tests of *Mangifera indica* seed kernel extracts (SK) and seed coat extracts (SC) against *Propionibacterium acnes* (A and B with Control: Levofloxacin); *Staphylococcus aureus* and *Escherichia coli* (C and D with Control: Trimethoprim/sulfamethoxazole). S: *Staphylococcus aureus* ATCC 29213; E: *Escherichia coli* ATCC 25922; P: *Propionibacterium acnes* ATCC 6919.

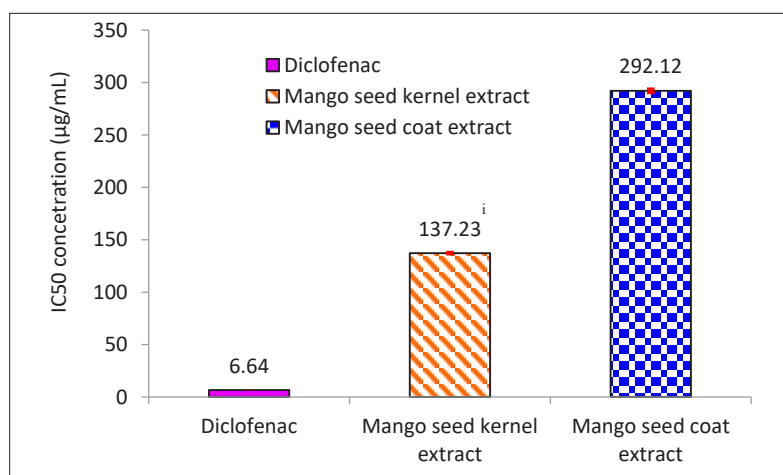


Figure 3. The IC₅₀ (µg/mL) values of *Mangifera indica* seed kernel extract, seed coat extract, and diclofenac (control). Each bar represents the mean ± SD (n = 3). At $P < 0.05$, superscript (i) indicates significance for certain *Mangifera indica* seed kernel extract to others.

the percentage inhibition of diclofenac solution varied between 2.11% and 73.08% at concentrations ranging from 0.3125 µg/mL to 10 µg/mL (Table 4).

In vivo anti-inflammatory activity

Table 5 and Figure 4 show that carrageenan injection caused local edema in the mouse's hind paw, which peaked at the third hour after carrageenan administration but gradually declined after that. When compared to the control, the administration of 200 mg/kg seed kernel extract of *M. indica* considerably reduced paw edema volume from the 3rd hour (that is, 50.9% at the 7th hour from the administration of carrageenan). Table 6 shows that 10 mg/kg of diclofenac and 200 mg/kg of mango

kernel extract significantly reduced paw edema volume from the 1st to 3rd hour when compared to the control. The highest percent inhibition of edema (50.9%) in this study was predicted at the 7th hour after administering carrageenan.

Discussion

The main purpose of the present investigation was to determine whether *M. indica* seed extracts had any potential for treating inflammatory acne due to their dual antibacterial and anti-inflammatory capabilities. Another purpose was to determine which mango seed extract was more potent at potentially treating inflammation acne, mango seed kernel extract or mango seed coat extract.

Table 4. Effect of seed kernel and seed coat extracts of *Mangifera indica* and diclofenac sodium on heat-induced protein denaturation

Test	Concentration (µg/mL)	%Inhibition	Test	Concentration (µg/mL)	%Inhibition	Test	Concentration (µg/mL)	%Inhibition
Diclofenac	0.3125	2.12	Mango seed kernel extract	6.25	2.40	Mango seed coat extract	12.5	2.70
	0.625	6.85		12.5	5.90		25	4.36
	1.25	12.33		25	11.53		50	9.11
	2.5	19.92		50	20.66		100	18.16
	5	40.58		100	38.63		200	34.53
	10	73.08		200	70.85		400	68.05

Table 5. Anti-inflammatory activity of mango seed kernel, seed coat extract, and diclofenac by the hind paw edema method in mice

Groups	Average paw size (mL) ± SEM					Average rise in paw volume (mL) ± SEM			
	Pre drug	1 h	3 h	5 h	7 h	1 h	3 h	5 h	7 h
Control	0.94 ± 0.03	1.33 ± 0.05	1.57 ± 0.03	1.48 ± 0.05	1.42 ± 0.06	0.39 ± 0.01	0.63 ± 0.01	0.54 ± 0.02	0.48 ± 0.03
Diclofenac 10 mg/kg	0.87 ± 0.02	1.16 ± 0.02	1.26 ± 0.06*	1.18 ± 0.03*	1.14 ± 0.03*	0.29 ± 0.01	0.39 ± 0.04	0.31 ± 0.01	0.27 ± 0.01
SK 200 mg/kg	0.84 ± 0.01	1.04 ± 0.02	1.20 ± 0.03*	1.20 ± 0.01*	1.10 ± 0.02*	0.20 ± 0.01	0.36 ± 0.02	0.36 ± 0.01	0.26 ± 0.01
SC 500 mg/kg	0.72 ± 0.03	0.95 ± 0.04	1.16 ± 0.05*	1.17 ± 0.07*	1.15 ± 0.07*	0.23 ± 0.01	0.44 ± 0.02	0.45 ± 0.03	0.43 ± 0.04

SEM: Standard error of the mean; SC: mango seed coat extract; SK: Mango kernel extract.

Values are shown as the mean ± SEM (n=6). **P* < 0.05 significantly different in comparison to the others with the control group at the same time.

The ethanolic crude extracts of *M. indica* seed kernel had an extract yield of 4.00% w/w, while the seed coat had an extract yield of 1.02% w/w. This result was less than the extract yield in the Poomanee et al study despite different extraction processes ($18.34 \pm 1.85\%$ for mango seed kernel extract) (22). The amounts of phenolic and mangiferin compounds were found to be quite high in both kinds of *M. indica* seed extracts. These compounds are also well known for their anticancer, antidiabetic, anti-inflammatory, skin-protecting, neuron-protecting, antimicrobial, and antiaging effects (9). Each 100 g seed

kernel had 2618.24 mg phenolics, higher than that amount of the previously reported and investigated phenolics content, at approximately 23 to 838 mg/100 g phenolics in mango seed kernel (9). In a study, it was found that the amount of mangiferin in the peel of mango cultivars (7.34-7.49 mg/g dry weight) was higher than in seed kernels (1.04-2.43 mg/g dry weight) (28). The reported mangiferin content in mango seed kernels (54.88 mg/100 g) was lower than that reported in previous investigations. Compared to mango seed kernels, mango seed coats had a lower content of mangiferin (15.07 mg/100 g).

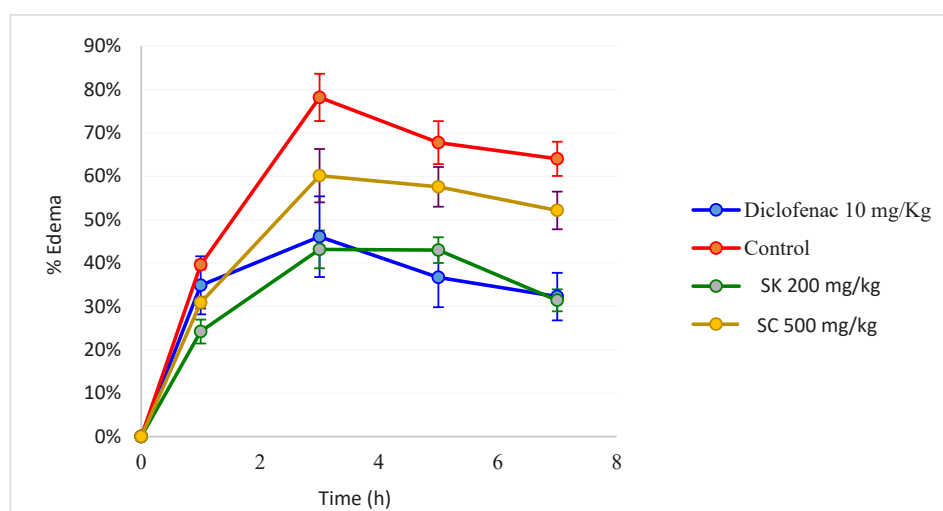
**Figure 4.** Carrageenan-induced paw edema in mice underwent treatment with 200 mg/kg mango seed kernel extract (SK), 500 mg/kg mango seed coat extract (SC), and 10 mg/kg diclofenac compared to the control (Data are mean ± standard error).

Table 6. The percent inhibition of edema in mice treated with mango seed kernel, seed coat extract, and diclofenac

Groups	% Inhibition of edema			
	1 h	3 h	5 h	7 h
Control	-	-	-	-
Standard-Diclofenac 10 mg/kg	11.9%	41.1%	45.9%	49.6%
SK 200 mg/kg	38.9%	44.8%	36.6%	50.9%
SC 500 mg/kg	22.0%	23.1%	15.0%	18.6%

SC: Mango seed coat extract; SK: Mango kernel extract.

In this study, the seed kernel extracts of *M. indica* possessed significant antibacterial potency against the tested organisms. This was reported by other studies but for mango seed in general (28). The seed kernel and seed coat extracts showed inhibition zone diameters of 16 mm and 14 mm, respectively, against *S. aureus* at 100 mg/mL. It was also found that both seed kernel and seed coat extracts showed inhibition of the growth of *P. acnes* bacteria, with seed kernel extract exerting more activities on this bacterium with a zone of inhibition from 22 to 26 mm at 100 mg/mL. The test results displayed similarity in the results of trials around the world. Specifically, according to the study results of Mutua et al (16), the antibacterial zone diameters of mango seed extract (including mango seed coat and kernel collected in Kenya) varied according to concentration from 25–100 mg/mL for *E. coli* and *S. aureus* (8.7–19.3 mm and 9.0–18.7 mm, respectively). From these results, it was shown that the whole mango seed extract in Kenya had a higher ability to inhibit *E. coli* and *S. aureus* against each of the seed coat and kernel extracts in Vietnam. However, in this study, neither of the extracts showed antibacterial effect against *E. coli*. The inhibition zones produced by our mango seed extracts were less than those produced by fraction extracts in previous studies. This may be due to the low diffusion rate of mango extract in an agarose medium, a matter that needs to be further investigated.

The MIC values obtained from seed kernel extracts and seed coat extracts were higher than 5 mg/mL against *S. aureus* and *E. coli*. The major difference between the seed kernel extract and seed coat extract was seen in the ability against *P. acnes*, where MIC values from seed kernel extracts were almost three times lower than those in seed coat extracts. The same observation was found for the ethanol fractions of mango seed kernels in the study of Poomanee et al; the ethanol fractions showed the strongest antimicrobial activity against *P. acnes* with a MIC value of 1.56 mg/mL (22). This result may suggest that the antibacterial activities come from seed kernel extracts of the mango cultivar in Vietnam.

There have been no studies reporting the anti-inflammatory activities of mango seed extracts *in vivo* (20). In this study, mango seed extracts were evaluated for both *in vitro* and *in vivo* anti-inflammatory property. The results indicated that mango kernel extract with the dose

of 200 mg/kg decreased paw edema volume from the 1st to 3rd hour when compared to the control and was equivalent to 10 mg/kg diclofenac. The maximum percent inhibition of edema (50.9%) in this study was measured at the 7th hour after carrageenan administration.

According to Kuganesan et al (29), the IC₅₀ value in the human red blood cell membrane stabilization assay by the heat-induced hemolysis method of mango seed extract (without separating the kernel and seed shell) was 128–248 µg/mL, which demonstrates that the anti-inflammatory potential of mango seed extract is very strong compared to the control used in this study. The results of the current study presented that the IC₅₀ values of mango seed kernels (137.23 ± 2.24 µg/mL) were significantly lower than the reported data of mango seed coats (292.12 ± 3.22 µg/mL) in the inhibition of heat-induced protein denaturation.

Previous researches have demonstrated that mangiferin possesses anti-inflammatory activity and antibacterial effect against gram-positive and gram-negative bacteria (30,31). Our findings revealed that the effects of anti-inflammatory activity in seed kernel extract were higher than those in seed coat extract. The mangiferin content in the mango seed kernel was nearly equal to that in the mango seed coat. However, the differences may be caused by a higher content of phenolic compounds in the seed kernel extract. Hence, it was suggested that the anti-inflammatory and antibacterial activities of mango seed extracts might be related to their phenolic content.

In the present study, for the first time, mango seeds were separated from the core and coat to evaluate their biological activities. While studies in other areas of the world have identified subjects as mango seed extract, it has shown good anti-inflammatory results in *in vitro* models. The present study separated 2 research subjects, namely, the seed coat and the seed kernel, to determine the medicinal part that has stronger anti-inflammatory effects than other parts. This provides more information as well as a great reference for the development of anti-inflammatory preparations and the selection of raw materials to isolate components with anti-inflammatory activity. However, this work did not exactly determine the active components of the extracts responsible for the antibacterial and anti-inflammatory properties. In the future, different extraction techniques may be used to concentrate these specific components and identify the

main active components in mango seed kernels and seed coats for further studies.

Conclusion

The present study determined the antibacterial and anti-inflammatory effects of mango seed coat and mango seed kernel extracts. In addition, when comparing the extracts, the mango seed kernel extract was proven to be more effective than the mango seed coat extract in the antibacterial and anti-inflammatory tests. It is recommended that mango seed kernel extract be considered a choice candidate in pharmaceutical anti-inflammatory acne drug development. However, to corroborate these findings, additional preclinical and clinical testing is required.

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Authors' contributions

NNNT provided the idea and made the protocol. HCT collected samples for extracting. VDL and HTH did experiments and collected data. NNNT and DDK prepared the manuscript. NNNT revised the manuscript. DDK submitted and paid the article processing charges. All read and agreed its publication.

Conflict of interests

The authors have no conflicts of interest to declare.

Ethical considerations

The research ethics were approved by Can Tho University of Medicine and Pharmacy (approval number 176/DHYDCT, January 27, 2021).

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