

Ameliorative Effect of *Bouea macrophylla* Griffth Seed Extract Against Bacteria-Induced Acne Inflammation: *in vitro* study

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Abstract: Currently, bioactive compounds derived from nature have been thought to be promising anti-acne substances owing to the variety of potential biological effects. This study aimed to evaluate the ameliorative effect of *Bouea macrophylla* Griffth seed extract against bacteria-induced acne inflammation for the first time in terms of antibacterial effects against acne-inducing bacteria, anti-inflammatory, and antioxidant properties. Initially, extracting procedures were optimized and five different extracts were obtained. Considering their antibacterial activities against *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, ethanolic and ethyl acetate fractions exerted a notable effect which were highly superior above those of polyphenol standards. Additionally, these two extracts presented outstanding antioxidant capacities in terms of DPPH and ABTS radicals scavenging effects, reducing power, and inhibitory effect on lipid peroxidation which also play a role in the exacerbation of acne inflammation. Besides, inhibition on lipid peroxidation and reducing power of ethanolic fraction were significantly ($p < 0.05$) better than those of ethyl acetate fraction which was corresponding to their phenolic and ellagic acid contents. However, flavonoids found in ethyl acetate fraction might play an important role in its potentials. After that, the anti-inflammatory effects of the extracts were elucidated by means of inhibition on nitric oxide production from LPS-induced RAW 264.7 cell lines at which the effects of both extracts were dose-dependency. Taken together, our findings have apparently proven that *B. macrophylla* seed extracts exerted a variety of potential properties including antioxidation, anti-acne-inducing bacteria, and anti-inflammatory effects which could serve as a promising anti-acne agent for cosmeceutical applications.

Key words: acne inflammation, antibacterial effect, antioxidation, anti-inflammation, maprang

1 Introduction

Acne inflammation is undoubtedly considered as a primary concern to the patient resulting in painfulness, post-inflammatory hyperpigmentation, and scarring^{1,2)}. At the initial stage of acne inflammation, exacerbation of comedogenesis is mainly attributed to *Cutibacterium acnes*-induced interleukin (IL)-1 α secretion provoking hyperkeratinization within the follicular epithelium in which an enormous number of *C. acnes* reside¹⁾. In consequence, *C. acnes* colonization chiefly contributes acne inflammation

owing to the reaction between *C. acnes*-produced enzymes including lipase, proteases, hydrolases and follicular tissue³⁾. Furthermore, the overexpression of pro-inflammatory cytokines and mediators are mainly attributed to the stimulation of Toll-like receptors (TLRs) presenting on immune cells by *C. acnes* heat shock proteins (HSPs)¹⁾. Indeed, IL-8 as one of the *C. acnes*-resulted cytokines plays a crucial role in neutrophil recruitment at the acne lesion at which pustule formation and excessive formation of free radicals and oxidative stress occur resulting in

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tissue inflammation^{1, 4)}. Admittedly, *C. acnes* has been recognized as one of the most important targets for ameliorating acne inflammation^{1, 5)}. Aside from *C. acnes*, the presences of *Staphylococcus aureus* and *Staphylococcus epidermidis* within the lesion additionally contribute exacerbation of acne inflammation⁶⁾. Until now, it has been proven that therapeutic failure of acne inflammation is chiefly resulted from bacterial resistance to antibacterial agents as well as low tolerance to adverse effects of synthetic compounds. As a result, an increase in demand for effective natural substitution exerting a variety of therapeutic potentials has been ubiquitous^{3, 7)}.

Bouea macrophylla Griffth, so-called as plum mango or maprang (Thailand), is categorized in the plant family of Anacardiaceae which is widely cultivated throughout South East Asia, particularly Thailand, Malaysia, Indonesia, and Philippines^{8, 9)}. The varieties of *B. macrophylla* grown in Thailand are typically classified by taste which include sour maprang, sweet maprang, and mayong chid of which taste presents sour sweetness. Amongst all varieties, the sweet variety is the most famous variety thanks to its sweetness and juicy texture⁸⁾. As a result, sweet maprang is regarded as one of the most widely consumed fruits in Thailand. Generally, *B. macrophylla* contains seeds presenting purple cotyledons which are wastes from processing and consumption. Noticeably, previous studies widely reported that *B. macrophylla* seed is a rich source of antioxidants⁹⁾. Furthermore, the seed extracts derived from ethanolic extraction and its aqueous formations exerted a notable antioxidant, antimicrobial, anti-aging, and anticancer effects owing to their polyphenol components^{8, 9)}. On the other hand, there has been no evidence regarding the anti-acne effect of *B. macrophylla* seed extract so far.

Therefore, the aims of the present study were to evaluate the ameliorative effect of *B. macrophylla* seed extract against bacteria-induced acne inflammation for the first time in terms of antibacterial effects against acne-inducing bacteria, anti-inflammatory, and antioxidant properties. In addition, extracting solvents were optimized and phytochemical content of the extract was elucidated. The knowledge from this study potentially enhances the value of agricultural waste and possibly provides a promising bioactive substance for ameliorating acne inflammation.

2 Materials and Methods

2.1 Chemicals

Tryptic soy broth (TSB; Soybean – Casein Digest Medium), tryptic soy agar (TSA), and agar were bought from Difco™ (Bangkok, Thailand). Thioglycollate broth was purchased from Merck co., ltd. (Bangkok, Thailand). Dulbecco modified eagle medium (DMEM), sterile phosphate-buffered saline (PBS) pH 7.2, L-glutamine (4 mM), penicillin

(100 U/mL), and streptomycin (100 U/mL) were purchased from Gibco® (Grand Island, NY, USA). Acetonitrile serving as high performance liquid chromatography (HPLC) eluent was HPLC grade. All chemicals were analytical grade. De-ionized water was generated by Millipore Milli-Q Advantage (Merck, Darmstadt, Germany) for extraction.

2.2 Plant materials and extraction

B. macrophylla seeds separated from the ripe fruits (sweet cultivar) and seed shells were supplied by Thai fruit processing industry in Nakorn Nayok province, Thailand during April to May 2021. The seeds were dried using hot-air oven at 45°C overnight and then grinded into fine powder before extraction.

Hexane served as a solvent for wax extraction. The ground seed was macerated with hexane for 48 h in triplicates. After that, hexane filtrate was separated and the plant residue was then macerated with 95% (v/v) ethanol in DI water or 50% (v/v) ethanol in DI water by the identical procedure as hexane extraction. In addition, ethyl acetate and 95% (v/v) ethanol were used to fractionally macerate the plant residue, respectively. The filtrates from hexane, ethanol, and ethyl acetate extraction were concentrated by a rotary evaporator (Buchi® Rotavapor R-300, Thailand), whereas DI water remaining in the filtrate from 50% (v/v) ethanol in DI water was then dried through using spray-dryer (Buchi® Mini Spray dryer B-290, Thailand). Each extracts were stored at –4°C in an amber glass bottle until use. All extracts were quantified for ellagic acid contents and analyzed fingerprint using HPLC for quality control (Unpublished data).

2.3 Determination of antimicrobial properties against acne-inducing bacteria

2.3.1 Bacterial strains

The protocol for determining the antimicrobial effects of the extracts was approved from Institutional Biosafety Committee (IBC), Chiang Mai University. Standard bacterial strains of *C. acnes* DMST 14916, *S. aureus* DMST 8840, and *S. epidermidis* DMST 15505 were purchased from the Department of Medical Sciences, Ministry of Public Health, Thailand. *C. acnes* was inoculated in modified thioglycollate broth pH 7.4 whereas *S. aureus* and *S. epidermidis* were inoculated in TSB. Anaerobic growth environment for the *C. acnes* was generated using anaerobic gas pack (Oxoid™ AnaeroGen™, Thermo Fisher Scientific™, Thailand) in anaerobic jar.

2.3.2 Inhibition zone

The antimicrobial properties of the extracts in a term of inhibition zone were initially determined by agar well diffusion assay following the method of Poomanee et al.⁵⁾. The inoculated *C. acnes* was cultured in modified thioglycollate broth and the final density was adjusted at McFarland standard turbidity No. 0.5 (1×10^8 CFU/mL). The diluted

bacterial suspension was streaked onto thioglycollate agar. The inoculated *S. aureus* and *S. epidermidis* with an identical McFarland standard turbidity of the *C. acnes* (1×10^6 CFU/mL) were streaked onto TSA. The agar was pierced to create 6 mm-diameter well and each sample solution (50 μ L) was added into each well. After 72 h of anaerobic incubation at 37°C for *C. acnes* and 24 h of aerobic incubation at 37°C for *S. aureus* and *S. epidermidis*, the inhibition zone of each sample was measured in millimeters. Clindamycin (CM; 10 mg/L), gallic acid (GA; 50 mg/mL), and ellagic acid (EA; 50 mg/mL) served as positive controls while culture broth served as a negative control and 25% (v/v) ethanol in culture broth was a solvent control.

2.3.3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC values of the extracts against *C. acnes*, *S. aureus*, and *S. epidermidis*, regarded as a minimum concentration of the compound that inhibit microorganism growth, were determined using the broth microdilution method following the procedure of Poomanee *et al.*⁵⁾. Each sample solution was two-fold serially diluted with culture broth in sterile 96-well plate. The bacterial suspension at McFarland standard turbidity No. 0.5 was then added into each well. After incubation at the aforementioned conditions, the well with no visible growth of tested organisms was observed and imposed as MIC. In regard to MBC determination, 5 μ L of each sample concentration with no visible growth was cultured onto the specific agar of each strain. MBC was then identified as the lowest concentration that totally kill tested microorganisms. Negative controls included culture broth and 25% (v/v) ethanol in culture broth while CM, GA, and EA were used as the positive controls.

2.3.4 Time-kill kinetic

Time-kill kinetic of the extracts exerting the best antimicrobial effect against *C. acnes* were elucidated¹⁰⁾. The incubated mixture composed of *C. acnes* suspension and sample solution with a ratio of 1:1 was collected at 0, 1, 3, and 6 h and mixed with 7% w/v polysorbate 80 in TSB which was a neutralizing solution for polyphenols¹¹⁾ in a ratio of 1:10. Colony-forming units (CFU) per milliliter of each time was subsequently counted by means of pour plate method. The time-kill kinetic was expressed as a regression of $\log_{10}$ survivors on time. The time – kill curve of each extract was calculated and expressed as time v.s. – $\log_{10}$ survivors' curve. Negative control was 25% (v/v) ethanol in culture broth.

2.4 Determination of antioxidant property

2.4.1 Free radical scavenging property

Free radical scavenging properties of the extracts were determined by means of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays⁵⁾. Regarding DPPH assay, ethanolic so-

lution of each extract was mixed with 120 mM of DPPH solution in a ratio of 1:10 and left in the dark for 30 min. Subsequently, the absorbance at 520 nm of each sample was determined using microplate reader (SPECTROstar Nano; BMG Labtech).

In regard to ABTS assay, the method of Poomanee *et al.*⁵⁾ was performed. In brief, ethanolic solution of the sample was incorporated into ABTS reactive solution which shown with a final 734 nm-absorbance of 0.70 ± 0.20 in a ratio of 1:100. The mixture was left at room temperature for 6 min. Then, the absorbance at 734 nm of each sample was measured using microplate reader. Ascorbic acid (AC), GA, EA, and Trolox served as positive controls for both assays. Percent inhibition on DPPH and ABTS radicals was then calculated using the following equation;

$$\text{Percent inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{sample}}} \times 100$$

Where A_{control} implies absorbance of control radical solution whereas A_{sample} implies absorbance of sample solution. In addition, the free radical scavenging effects were expressed as IC_{50} value that implies the lowest concentration inhibiting 50% of the radicals. In case of ABTS assay, Trolox equivalent antioxidant capacity (TEAC) of each sample indicating mg Trolox per g of sample was also calculated according to Trolox calibration curve as a following equation;

$Y = -0.1705X + 0.5615$, $R^2 = 0.9995$; Y is an absorbance and X is a concentration.

2.4.2 Reducing power

Ferric reducing antioxidant power (FRAP) assay was employed for evaluating the reducing powers of the extracts¹²⁾. FRAP testing solution was composed of an acidic 10 mM 2,4,6-Tris (2-pyridyl) -s-triazine (TPTZ) solution, 20 mM Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), and 300 mM acetate buffer while an ethanolic solution of the extract was mixed with DI water in a ratio of 1:10. The FRAP testing solution (2 mL) was incorporated with the premix extract solution (1 mL) and incubated in the dark for 30 min. Ferrous sulfate ($FeSO_4$) was used as a reference standard while ascorbic acid was used as a positive control. The absorbance of each concentration at 593 nm was then evaluated using microplate reader. The regression of absorbance on concentration of $FeSO_4$ was constructed to calculate FRAP value of each sample indicating mg $FeSO_4$ that is equivalent to 1 mg of sample.

2.4.3 Inhibitory effect on lipid peroxidation

Linoleic acid thiocyanate method was employed for investigating inhibitory effects of the extracts on lipid peroxidation⁵⁾. In brief, methanolic solution of each extract was incubated with a lipid test solution composed of 1.3% w/v linoleic acid, PBS pH 7.0, DI water, and 46.35 mM AAPS and left in the dark at 45°C for 4 h. The inhibition on linoleic acid peroxidation of the extracts were subsequently determined using Ferric thiocyanate method and ex-

pressed as IC₅₀ comparing to those of gallic acid and Trolox which served as positive controls.

2.5 Determination of phytochemical contents

2.5.1 Total phenolic content

Total phenolic content of the extracts expressed as mg GAE/g extract equivalent to gallic acid was evaluated through using Folin-Ciocalteu's procedure⁵⁾. In brief, ethanolic solution of each sample was incorporated with ten-fold DI water-diluted Folin-ciocalteu's reagent, and 7.5% w/v sodium carbonate (Na₂CO₃). The mixture was then left for 30 min in the dark. After that, an absorbance at 765 nm of each sample was determined by UV-VIS spectrophotometer (Shimadzu, UV-2450, Japan). A regression of absorbances of gallic acid (Y) on concentrations (X) was established as gallic acid calibration curve with an equation;

$$Y = 0.00746X + 0.00309, R^2 = 0.9995$$

2.5.2 Total flavonoid content

Total flavonoid contents of the extracts expressed as mg RU/g extract equivalent to rutin were investigated based on the standard aluminum chloride assay following the method of Poomanee *et al.*⁵⁾. The extract dissolved in absolute ethanol was diluted with DI water in a ratio of 1:10. The extract mixture was incorporated with 5% w/v sodium nitrite (NaNO₂) and 10% aluminum chloride (AlCl₃), respectively. After 5 min of incubation, 1 M sodium hydroxide (NaOH) was added. Then, an absorbance of the tested mixture was measured at 510 nm and calculated in a term of mg RE/g extract equivalent to rutin. Rutin calibration curve was constructed as a regression of absorbances (Y) on rutin concentrations (X), showing an equation;

$$Y = 0.0022X + 0.0166, R^2 = 0.9971$$

2.6 Determination of nitric oxide inhibition of the selected extract

Inhibitory effects of nitric oxide (NO) produced from lipopolysaccharides (LPS)-induced RAW 264.7 cells of the selected extracts were used to imply an anti-inflammatory effect herein following the method of Poomanee *et al.*¹³⁾. The cells, which were seeded into a sterile 96 well-plate at a density of 5×10^4 cells/well, were cultured with 5% CO₂/95% relative humidity at 37°C for 24 h and then washed twice with PBS, pH 7.4. After that, extract sample or triamcinolone acetonide (TA) as a positive control in a concentration range of 0.001 – 1 mg/mL were incorporated into the cells wells in LPS-loaded DMEM (100 ng/mL). DMEM served as a negative control. After 24 h of incubation, the supernatant solution (100 µL) was collected and incorporated with a tested mixture consisted of 100 µL Griess reagent containing 1% w/w sulfanilamide and 0.1% w/w N-1-[naphthyl]ethylenediamine dihydrochloride in 2.5% w/v phosphoric acid. The mixture was left for 10 min

and an absorbance of the solution was examined by a microplate reader at 550 nm. Percent NO reduction was then calculated using a following equation;

$$\text{Percent inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{sample}}} \times 100$$

Where A_{control} implies absorbance of negative control whereas A_{sample} implies absorbance of sample-treated well.

2.7 Quantification of ellagic acid content using HPLC analysis

In the present study, ellagic acid was identified by HPLC as a marker of ethanolic fraction of *B. macrophylla* at a retention time of 32.50 min. The ellagic acid content of the extract was thus quantified using following a modified condition of Dechsupa *et al.*⁸⁾. Mobile phase was consisted of solvent A which was 0.01% phosphoric acid in DI water and solvent B which was acetonitrile. Gradient elution was serially performed as follows: 0–5 min, 5% of B; 5–7 min, 5–10% of B; 7–12 min, 10% of B; 12–14 min, 10–15% of B; 14–19 min, 15% of B; 19–21 min, 15–20% of B; 21–26 min, 20% of B; 26–30 min, 20–25% of B; 30–35 min, 25% of B; 35–37 min, 25–30% of B; 37–45 min, 30% of B; 45–50 min, 30–100% of B; 50–55 min, 100% of B, and 55–65 min, 5% of B with a wavelength detection of 270 nm. Inertsil ODS–3 reverse phase C–18 column (5 µm, 4.6 × 250 mm, GL Science Inc. USA) was employed as a stationary phase using a column temperature of 35°C. The extract was dissolved in absolute methanol to give a final concentration of 1000 mg/L. Ellagic acid curve which shown area under the curve (mAU: Y) as a function of ellagic acid concentrations (X) was constructed for interpreting ellagic acid content of the extracts in term of percentage of ellagic acid as the following equation:

$$Y = 62.021X + 163.67, R^2 = 0.995$$

2.8 Statistical analysis

All experiments were performed in triplicates. The differences between antimicrobial, antioxidant, anti-inflammatory, and phytochemical contents were statistically analyzed by One-Way ANOVA with multiple comparison using Tukey through SPSS statistics software Version 17.7 (IBM co. Ltd., NY, USA). Statistical significance was identified as $p < 0.05$.

3 Results

3.1 *B. macrophylla* seed extracts and their characteristics

Previous studied reported that gallotannin compounds including gallic acid, ethyl gallate, and pentagalloyl glucose were found as bioactive constituents of *B. macrophylla* seed⁸⁾. Accordingly, the selection of extracting solvents for *B. macrophylla* seed was performed based on the poten-

Table 1 Percent yields and physical characteristics of *B. macrophylla* seed extracts.

Abbreviations of the extracts	Types of Extract	Percent yield (%)	Characteristics
BSH	Crude hexane extract	1.07 ± 0.20	Brownish waxy paste
BSEA	Ethyl acetate fraction	2.25 ± 0.51	Orangy pink wax
BSEE	Ethanol fraction	14.10 ± 0.77	Orangy pink powder
BSE	Crude ethanolic extract	15.14 ± 1.14	Orangy pink powder
BSHE	Crude hydroethanolic extract	20.40 ± 2.28	Orangy pink powder

tial of polyphenol extraction¹⁴⁾ of which five different polarities including hexane, ethyl acetate, ethanol, and 50% ethanol in DI water were carried out through a conventional maceration. Percent yields and characteristics of all extracts are shown in **Table 1**. The results demonstrated that ethanol and 50% ethanol were suitable solvents rendering the relative high yields compared to those of hexane and ethyl acetate. By virtue of ethyl acetate fractionation, lipophilic compounds were additionally excluded rendering a partially purified ethanolic fraction as BSEE.

3.2 Antimicrobial activities of the extracts against acne-inducing microorganisms

In the present study, the antibacterial effects of *B. macrophylla* seed extracts against *C. acnes* were reported for the first time. **Figure 1** illustrating the antimicrobial activities of the derived extracts against three acne-inducing microorganisms: *C. acnes*, *S. aureus*, and *S. epidermidis* in a term of inhibition zone, exhibited that all extracts exerted a potent antimicrobial activity since their inhibition zone were wider than 15 mm¹⁵⁾. Besides, the antimicrobial effects of BSEE, BSEA, and BSE were outstanding among all extracts. Noticeably, it is worth nothing that the effects of gallic acid and ellagic acid as positive controls were significantly lower than all extracts. The results demonstrated that *C. acnes* was more susceptible to the extracts than *S. aureus* and *S. epidermidis*.

The results from broth microdilution assay were in correspondence with those from inhibition zone as shown in **Table 2**. Considering on the bactericidal effect against *C. acnes*, BSEE exerted the greatest effect among all extracts with the lowest MBC value of 0.156 mg/mL, while BSEA showed an outstanding bactericidal effect against *Staphylococci* spp. at a concentration of 0.625 mg/mL. Noticeably, the antimicrobial activities of all extracts were superior above those of phenolic positive controls. Nonetheless, the effects of all extracts and tested phenolic compounds were significantly lower than those of CM which is considered as an antibiotic for topical acne treatment.

According to the notable antimicrobial effects of BSEE and BSEA, these two extracts were subsequently tested for their lethal effects against *C. acnes* over time expressed as time-kill kinetic curve during 6 h as shown in **Fig. 2**. The

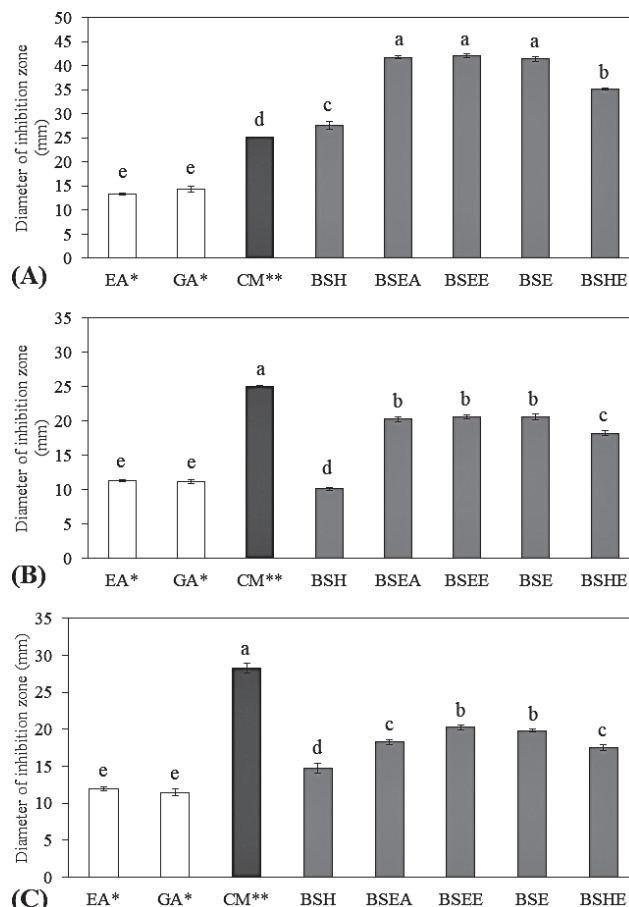


Fig. 1 Inhibition zone (inclusive of 6 mm-well diameter) of *B. macrophylla* seed extracts against (A) *C. acnes*, (B) *S. aureus*, and (C) *S. epidermidis*. Superscript letters (a, b, c, d, e) indicate significant differences between groups analyzed by One-Way ANOVA with multiple comparison using Tukey ($p < 0.05$), *a tested concentration of 50 mg/mL, **a tested concentration of 10 mg/L.

tested concentrations were varied into MBC, 2 × MBC, and 4 × MBC. The 3-log reduction in log CFU/mL is normally regarded as a bactericidal effect¹⁰⁾. The results illustrated that bactericidal effects of both extracts at MBC could be observed after 3 h of incubation. Moreover, at 2 × MBC and 4 × MBC, almost all microorganisms could be rapidly killed

Table 2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *B. macrophylla* seed extracts against *C. acnes*, *S. aureus*, and *S. epidermidis*.

Samples/ Standards	Susceptibility of bacteria (mg/mL)					
	<i>C. acnes</i>		<i>S. aureus</i>		<i>S. epidermidis</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
BSH	0.625	NA	NA	NA	NA	NA
BSEA	0.00975	0.3125	0.625	0.625	0.15625	0.625
BSEE	0.00975	0.15625	0.625	2.5	0.3125	1.25
BSE	0.0195	0.3125	0.625	1.25	0.15625	0.625
BSHE	0.0195	0.3125	0.625	1.25	0.3125	0.625
EA	3.125	6.25	2.5	5	2.5	5
GA	6.25	6.25	5	5	5	5
CM	0.08*	10.00*	3.13*	6.25*	3.13*	12.5*

NA – no activity.

*MIC and MBC values of CM were reported in mg/L.

MIC break point (mg/L) of CM against *C. acnes* and *Staphylococci* (*S. aureus* and *S. epidermidis*); *C. acnes*: susceptible ≤ 2 , and resistant ≥ 8 , *Staphylococci*: susceptible ≤ 0.25 , and resistant ≥ 0.5 (data from the Clinical and Laboratory Standards Institute (CLSI))

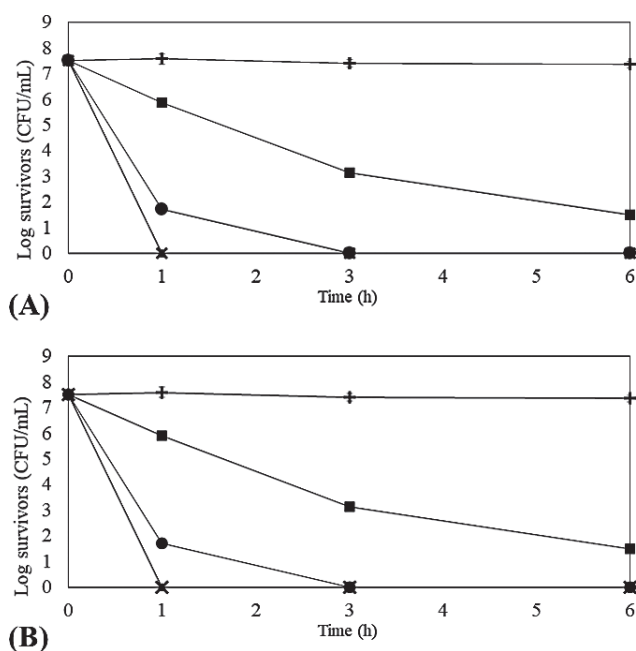


Fig. 2 Time-kill kinetic plots of *B. macrophylla* seed extracts against *C. acnes*, (A)– BSEE (ethanolic fraction) and (B) BSEA (ethyl acetate fraction); (+) Negative control, (■) 1MBC, (●) 2MBC, (×) 4MBC.

within 1 h. This finding could confirm that the BSEE and BSEA could be potential bioactive compounds for inhibiting bacteria-induced acne aggravation.

3.3 Antioxidant properties of the selected extracts

On account of the most outstanding antibacterial properties, BSEA and BSEE were selected for antioxidant determination. Table 3 showed that BSEA and BSEE exerted potent free radicals scavenging properties and strong inhibitory effect on linoleic acid peroxidation which were comparable to the antioxidant standards. In case of DPPH radical scavenging properties, the effects of BSEE and BSEA were significantly higher than those of Trolox and ascorbic acid which are regarded as strong antioxidant compounds. Likewise, under a hydrophilic condition of ABTS assay¹⁷⁾, these two extracts additionally exerted an equivalent effect to Trolox. Besides, BSEE significantly exhibited stronger inhibitory effect against ABTS radicals than BSEA.

The attenuating properties on lipid peroxidation of the BSEE and BSEA are also shown in Table 3 in a term of IC_{50} value. It could be clearly demonstrated that the effects of the extracts were equivalent to that of Trolox. Although, there is non-significant difference between BSEA and BSEE, yet the IC_{50} value of BSEE was lower than that of BSEA indicating a stronger potential which was in correspondence with the ABTS radical scavenging property. Additionally, reducing power of the extracts was reported in a term of FRAP value as shown in Table 3. The results were coincided with other antioxidant assays of which BSEE exerted better reducing power than BSEA.

3.4 Phytochemical contents of the selected extracts

The amounts of phenolic acids and flavonoid compounds

Table 3 Antioxidant properties of the selected extracts in terms of DPPH, ABTS radicals scavenging effects and attenuating effect on lipid peroxidation (Mean $\pm$ SD).

Samples/ Standards	Free radical scavenging effects			IC ₅₀ [†] on lipid peroxidation	FRAP value ^{†††}
	IC ₅₀ on DPPH [†]	IC ₅₀ on ABTS [†]	TEAC ^{††}		
BSEA	4.23 $\pm$ 0.34 ^c	1.16 $\pm$ 0.27 ^c	0.64 $\pm$ 0.05 ^d	13.48 $\pm$ 1.01 ^a	103.42 $\pm$ 0.02 ^b
BSEE	3.73 $\pm$ 0.54 ^c	1.26 $\pm$ 0.20 ^c	1.05 $\pm$ 0.06 ^b	8.20 $\pm$ 0.80 ^a	117.75 $\pm$ 0.06 ^a
Trolox	8.32 $\pm$ 0.10 ^d	1.16 $\pm$ 0.15 ^c	—	16.78 $\pm$ 3.00 ^a	—
EA	2.48 $\pm$ 0.28 ^b	0.57 $\pm$ 0.07 ^{a,b}	0.86 $\pm$ 0.10 ^c	82.56 $\pm$ 9.48 ^b	—
GA	0.95 $\pm$ 0.03 ^a	0.85 $\pm$ 0.06 ^{b,c}	1.32 $\pm$ 0.01 ^a	25.43 $\pm$ 3.04 ^a	—
AC	7.21 $\pm$ 0.12 ^c	0.35 $\pm$ 0.04 ^a	1.32 $\pm$ 0.02 ^a	139.46 $\pm$ 11.46 ^c	34.29 $\pm$ 0.01 ^c

EA – Ellagic acid; GA – Gallic acid; AC – ascorbic acid

[†]IC₅₀ values are reported in μ g/mL; ^{††}TEAC values are reported in mg Trolox/mg extract or standard;

^{†††}FRAP values are reported in mM FeSO₄/mg extract or standard.

Superscript letters (^a, ^b, ^c, ^d, ^e) indicate significant differences between groups analyzed by One-Way ANOVA with multiple comparison using Tukey ($p < 0.05$).

in the selected extracts were expressed in terms of total phenolic and total flavonoid contents which were equivalent values to those of gallic acid and rutin, respectively. The results showed that BSEA and BSEE contained 136.94 ± 3.03 and 160.27 ± 0.50 mg GAE/g extract, respectively, while the total flavonoid contents of BSEA and BSEE were 92.84 ± 0.07 and 62.70 ± 1.20 mg RU/g extract, respectively. It is worth noting that ethyl acetate as an extracting solvent proportionately extracted flavonoids which generally show hydrophobicity whereas phenolic compounds that present rather hydrophilicity could be suitably extracted by ethanol.

3.5 Anti-inflammatory activities of the selected extracts

Figure 3 illustrated that BSEA and BSEE exerted inhibitory effect on NO production in a dose-dependent manner. Nonetheless, there was no significant difference between the effects of BSEA and BSEE in similar concentrations. In comparison with TA, BSEA and BSEE showed approximately 2-fold lower inhibitory effect.

3.6 Ellagic acid contents of the selected extracts

From our preliminary results, ellagic acid was found as a marker of BSEA and BSEE in HPLC analysis. The ellagic acid contents of BSEA and BSEE were 18.240 ± 1.334 and 25.310 ± 1.165 mg EA/g extract, respectively.

4 Discussion

B. macrophylla seed, a by-product from fruit consumption and processing, has been reported for its several biological properties and secondary metabolites⁹. However, there is no information regarding the ameliorative effect of *B. macrophylla* seed extract against bacteria-induced

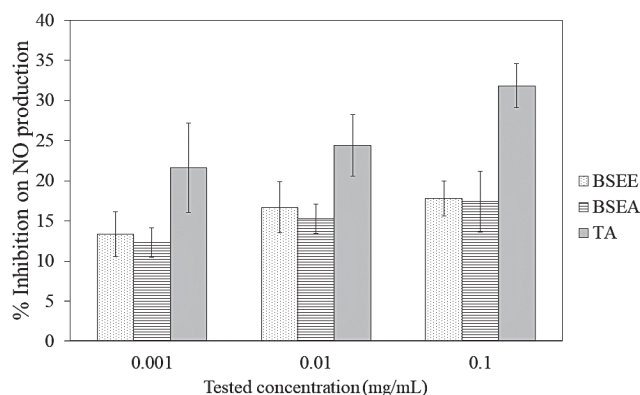


Fig. 3 Percent inhibition of *B. macrophylla* seed extracts and triamcinolone acetonide (TA) on nitric oxide production from RAW 264.7 cell line.

acne inflammation. Our study thus elucidated the anti-acne potential of the seed extracts for the first time by means of evaluating antimicrobial effect against acne-inducing microorganisms, antioxidation, and anti-inflammation which chiefly involve in the progression of acne inflammation⁵.

Principally, polarity of extracting solvent plays an essential role in the identity, quantity, toxic effect, and biological properties of the derived extracts¹⁸. From the results of the extracting procedure, percent yields of hexane extract and ethyl acetate fraction were significantly lower than those of ethanolic and hydroethanolic extracts indicating the lower lipophilic compounds of *B. macrophylla* seed. In comparison with *Mangifera indica* Linn. which belongs to the similar plant family, 4-fold higher amount of wax compounds derived from *M. indica* seed were observed⁵. In addition, the percent yields reported herein were higher than those of the ethanolic *B. macrophylla* seed extracts in the study of Dechsupa *et al.*⁸. According to the results

from biological assays, BSEA and BSEE exerted an outstanding properties amongst all extracts. Taking phytochemical contents of these two extracts into consideration, total phenolic content of BSEE was higher than that of BSEA which was in correspondence with their ellagic acid contents while higher total flavonoid contents of BSEA than that of BSEE was observed. This finding implied that ethanol is the most appropriate solvent for extracting bioactive phenolic compounds from *B. macrophylla* seed offering cost-effectiveness extraction since larger amount of extracts exerting strong potentials were obtained. Yet, higher amount of flavonoids in BSEA extracted by ethyl acetate also determined its biological properties which was interesting for further elucidation.

Antibacterial effect against *C. acnes* along with time-kill kinetic of the *B. macrophylla* seed extracts were firstly elucidated herein. Antibacterial effects of *B. macrophylla* seed extracts were stronger than those of *M. indica* seed extracts reported in the study of Poomanee *et al.*⁵⁾. It is worth noting that ellagic acid, which was identified in *B. macrophylla* seed extracts herein, was considered as one of the bioactive phenolic compounds in the extracts. Ellagic acid, regarded as one of the ellagitannins, has been proven for its various biological properties including antimicrobial, antioxidant, and anticancer potentials^{7, 19)}. Mode of antimicrobial action against methicillin-resistant *S. aureus* of ellagic acid was apparently reported owing to its capability to precipitate bacterial proteins and attenuate gyrase activity which plays a vital role in DNA replication¹⁹⁾. Noticeably, the antibacterial effects of BSEA and BSEE against all tested microorganisms were significantly stronger than those of gallic acid and ellagic acid. These findings suggested that crude extracts possibly exerted a synergistic effect which was resulted from several bioactive substances in the extracts other than gallic acid and ellagic acid which was interesting for further investigation.

Aside from antibacterial properties, the promising anti-acne compounds is necessary to potentially attenuate free radicals and inflammatory process²⁰⁾. It has been proven that during acne inflammatory process, interleukin (IL)-8 secreted from CD4+ T cell mainly recruits neutrophils into acne lesions at which an enormous amount of free radicals are generated to not only eliminate the microorganisms but also damage follicular epithelium¹⁾. In addition, hydroxyl radical, which is one of the most dangerous radicals produced from neutrophils, mainly initiates lipid peroxidation resulting in exacerbation of inflammation²¹⁾. Our results illustrated that BSEA and BSEE exhibited notably antioxidant effects in both free radical scavenging property and inhibition against lipid peroxidation offering a possible attenuating properties on acne inflammation. Remarkably, DPPH scavenging properties of the seed extracts reported in the present study showed higher potency than those of ethanolic extracts reported in Dechsupa *et al.*⁸⁾. Consider-

ing on phytochemical contents of the extract, it might be interpreted that phenolic compounds determined inhibitory effect on lipid peroxidation and reducing power rather than flavonoids owing to the superior effect of BSEE above those of BSEA.

Several studies denoted that a number of pro-inflammatory cytokines secreted from not only adaptive immune cells but also innate immune systems potentiated acne inflammation²²⁾. *C. acnes*, a crucial bacterium with its unique immunomodulatory process, strongly urges the innate immune response by heat shock proteins (HSPs) acting as a ligand of Toll-like receptor (TLR) via NF- κ B mediated pathway resulting in the secretion of TNF- α , IL-1 α , IL-8 and IL-12²²⁾. In addition, Lyte *et al.*²³⁾ reported that NO, one of the pro-inflammatory mediators, was secreted from keratinocytes and immunological cells in consequence of *C. acnes* HSPs stimulation. Our study performed LPS as a mimic compound of *C. acnes* HSPs to induce RAW264.7 cells-produced NO owing to its potential to stimulate NF- κ B pathway via TLR2 which was in coincidence with *C. acnes* HSPs²⁴⁻²⁶⁾. From the results, both BSEA and BSEE exerted an inhibitory effect on NO production indicating the ameliorative effect on acne inflammation through this pathway.

5 Conclusion

In conclusion, our findings have apparently proven that *B. macrophylla* seed extracts exerted a variety of potential properties including antioxidation, anti-acne-inducing bacteria, and anti-inflammatory effects for effectively ameliorating the acne inflammation of which anti-*C. acnes* was elucidated for the first time. Owing to the potent competency of *B. macrophylla* seed extracts, it could be a promising anti-acne agent for cosmeceutical applications. Nonetheless, *in vivo* determination and safety evaluation are necessary to be further conducted before human use.

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Conflicts of Interest

The authors declare there are no conflicts of interest.

Author Contributions

W.P. and P.L. designed research; W.P. and I.V. performed research; I.V., K.T., P.L., and W.L. contributed analytical tools; W.P. analyzed data; W.P., W.L. and P.L. wrote manuscript.

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