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Research Article

Antioxidant, tyrosinase inhibitory, and anti-acne activities and phytochemical screening of hydroethanolic seed extract from *Bouea oppositifolia* (Roxb.) Meisn

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ABSTRACT

Bioactive compounds from plants are widely utilized as promising raw materials in cosmetics and pharmaceuticals to prevent and treat a variety of diseases. This study aimed to optimize extraction conditions for bioactive compounds from the seeds of *Bouea oppositifolia* (Roxb.) Meisn. using the EtOH:H_2O mixtures (100:0, 50:50, and 0:100, % v/v) with extraction times of 1, 2, and 3 h. The extracts were evaluated for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity to identify the most potent extract for further investigation of tyrosinase inhibitory, antibacterial, and phytochemical properties relevant to cosmeceutical applications. The results demonstrated that extraction with 50:50 (% v/v) EtOH:H_2O for 2 h (EW2 extract) yielded the highest extract recovery (34.82%) and exhibited the highest TPC (103.42 ± 4.42 mg GAE/g DW) and TFC (139.87 ± 8.42 mg QE/g DW), along with antioxidant activity (DPPH; IC_{50} = 0.17 ± 0.04 µg/mL). Pearson's correlation analysis demonstrated that total phenolic and flavonoid contents significantly contributed to antioxidant activity. The extract also exhibited tyrosinase inhibition (IC_{50} = 183.00 ± 9.51 µg/mL) and antibacterial activity against *Staphylococcus aureus* (MIC = 0.62 mg/mL, MBC = 10.00 mg/mL), *Staphylococcus epidermidis* (MIC = 1.25 mg/mL, MBC = 5 mg/mL), and *Cutibacterium acnes* (MIC = 0.0064 mg/mL, MBC = 0.1024 mg/mL). These findings highlight that the EW2 extract of *B. oppositifolia* seed extract provided effective bioactive phytochemicals and could be further developed as a natural anti-acne agent for cosmetics and pharmaceutical products.

Keywords:
Bouea oppositifolia (Roxb.) Meisn.; Antioxidant activity; Tyrosinase inhibitory activity; Antibacterial activity; Anti-acne

1. INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the skin, which commonly occurs in teenagers and young adults. Approximately 9.4% of the population is affected by acne, which typically occurs due to the inflammation of the sebaceous gland. According to the Global Burden of Disease (GBD) study, more than 80% of teenagers are affected by chronic inflammation caused by acne, which can continue into adulthood, occurring in 3% of males and 12% of females over the age of 25 [1-2]. Several factors have been proven to lead to the evolution of acne, including hormonal, genetic, dietary, stress, and environmental factors [3]. Among these factors, the hormonal fluctuations, which are derived from the steroids, are significantly produced in excess due to the hormonal imbalance, which activates the sebaceous gland to produce excess sebum. This process activates the growth of *Cutibacterium acnes*, formerly known as *Propionibacterium acnes*, resulting in the formation of acne by disrupting the normal skin flora, which in turn activates the inflammatory reaction in the skin [4].

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The composition of *C. acnes*, which is responsible for the inflammation of the skin, may change due to the imbalance in the diversity of the normal skin flora. In addition to this, *C. acnes* is also known to coexist with other microorganisms, especially *Staphylococcus* bacteria, including *Staphylococcus aureus* and *Staphylococcus epidermidis*, which activate the innate immune response and lead to inflammation due to bacterial multiplication [5-6]. Nevertheless, it is important to note that apart from pain due to inflammation of the skin, acne also causes problems for people affected by it, such as scars, lack of self-confidence, anxiety, panic, and depression [7]. Over an extended period, different therapeutic regimens have been utilized to treat acne. These regimens include antibiotics, both topical and systemic, chemical treatments, and other treatment regimens depending on the diagnosis of the patient. On the other hand, long-term use of antibiotics and chemical treatments has been shown to lead to bacterial resistance, toxicity, and skin side effects [8]. Hence, the use of natural products containing bioactive compounds such as phenolics, flavonoids, xanthones, carotenoids, tannins, terpenoids, alkaloids, and anthocyanins is gaining popularity. These bioactive compounds are known to play significant roles in antioxidant, anti-inflammatory, antimicrobial, anti-aging, acne-reduction, skin-whitening, and wound-healing effects, thus being effective, safe, and affordable natural ingredients used in the cosmetic and nutraceutical industries [9-10].

Bouea oppositifolia (Roxb.) Meisn., (also known as its homotypic synonym of *Bouea burmanica* Griff.) is a member of the Anacardiaceae family, and is closely related to *Bouea macrophylla* Griffth., *Mangifera indica* L., *Anacardium occidentale* L., and *Spondias pinnata* (L.f.) Kurz. This plant is typically grown in Asian regions such as India, China, Sri Lanka, Myanmar, Malaysia, Laos, Vietnam, and especially Thailand. It is a dicotyledonous tree with a height of 8–15 m. This plant produces small pale yellowish inflorescences similar to those of *M. indica*, which develop into green fruits with a sour taste when unripe. When ripe, the fruits turn deep yellow-orange and have a sweet-sour flavor depending on the cultivar. Each fruit contains a single purple seed covered with a fibrous husk (Figure 1) similar to that of *B. macrophylla* [11, 12]. In Thailand, this fruit is commonly known as Ma-Yong-Chid. The ripe fruit of *B. oppositifolia* is one of the most popular fruits and generates high income for farmers. However, the plant produces fruit only once per year, resulting in seasonal production and limiting farmers to annual harvests [13]. The waste products of this plant, particularly the seeds, have not yet been extensively investigated for their biological activities and potential applications. Similarly, several studies on a closely related species, especially *B. macrophylla*, have reported that its seed extracts possess various biological properties, including antioxidant, anti-inflammatory, antibacterial, anti-acne, wound-healing, tyrosinase inhibitory, and anticancer activities [14]. The primary bioactive chemical components of the seed extract from *B. macrophylla* have been identified, including gallic acid, ellagic acid, 1,2,3,4,6-pentyl-O-galloyl-β-D-glucose, and other gallotannins [15, 16]. Furthermore, the seed extract of *B. macrophylla* has been developed as a potential cosmeceutical product, particularly for anti-acne applications using transethosome encapsulation. The results showed that the transethosome-encapsulated *B. macrophylla* seed extract exhibited spherical bilayer particles with a size of 87.17 ± 0.83 nm, a polydispersity index (PDI) of 0.271 ± 0.007, and an entrapment efficiency (EE) of 93.98 ± 0.17%. In addition, it showed lower cytotoxicity toward HaCaT cells, with an IC_{50} value of 18.07 mg/mL. The formulation also exhibited potential anti-acne activity against *C. acnes*, as evaluated by the disc diffusion method, with an inhibition zone of 15.14 ± 0.68 mm [17]. Therefore, this study aimed to determine the optimal extraction conditions, as well as the total phenolic and flavonoid contents and antioxidant activities,



Figure 1. Botanical characteristics of the fibrous husk and seed of *Bouea oppositifolia* (Roxb.) Meisn.

using EtOH:H_2O mixtures at ratios of 100:0, 50:50, and 0:100 (% v/v) for the extraction of *B. oppositifolia* seed. The extract exhibiting the highest antioxidant activity and phenolic and flavonoid contents was selected for further evaluation of tyrosinase inhibitory and antibacterial activities, as well as phytochemical profiling, with the aim of developing a potential raw material for cosmeceutical and anti-acne products.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Chemicals and reagents utilized in this study included 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3,4-dihydroxyphenylalanine (L-DOPA), gallic acid (98%), quercetin (95%), and tyrosinase enzyme from mushroom which were purchased from Sigma-Aldrich (USA). Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), ferric chloride ($FeCl_3$), Folin–Ciocalteu's phenol reagent, and L-ascorbic acid (99.5%) were supplied by Loba Chemie (India). Additionally, hydrochloric acid (HCl), dimethyl sulfoxide (DMSO), and ethanol (C_2H_5OH) were obtained from RCI Labscan (Thailand). Bacteriological culture media, including Mueller–Hinton agar/broth and Brain Heart Infusion agar/broth, as well as the vancomycin reference standard, were obtained from Oxoid Ltd. (Basingstoke, UK). All chemicals and reagents used in this study were of analytical grade and were utilized without further purification.

2.2. Plant material collection

Fresh mature fruits of *Bouea oppositifolia* (Roxb.) Meisn. were collected from a commercial orchard in Krai Nok Sub-district, Kong Krailat District, Sukhothai Province, Thailand, in March 2024. Botanical authentication was carried out by Asst. Prof. Dr. Kanjana Thananoppakun (Department of Biology, Faculty of Science and Technology, Pibulsongkram Rajabhat University). A voucher specimen (PSRU 1010) was deposited at the Herbarium, Faculty of Science and Technology, Pibulsongkram Rajabhat University (PSRU), Phitsanulok Province, Thailand.

2.3. Preparation of crude extracts

The fresh seeds of *B. oppositifolia* were separated from the fruits and cut into small pieces. The samples were then dried in a hot-air oven at 45 °C for 48 h and subsequently ground into a fine powder using a mechanical grinder. Each 10 g portion of *B. oppositifolia* seed powder was extracted with 100 mL of EtOH:H_2O solvent at volumetric ratios of 100:0, 50:50, and 0:100 (% v/v). The mixtures were subjected to continuous rotary shaking at 150 rpm at room temperature for 1, 2, and 3 h. After extraction, the mixtures were filtered through cotton followed by filter paper (Whatman® No. 1). The filtrates were concentrated by removing the solvent using a rotary evaporator (Buchi Rotavapor® R-300, Buchi Labortechnik, Switzerland) at 45 °C. The obtained crude extracts of *B. oppositifolia* seed were collected, and the extraction yield was calculated using the following equation:

$$\%\text{Yield} = \frac{\text{Mass of crude extract}}{\text{Mass of dried sample}} \times 100$$

The extracts were then stored in opaque containers at 4 °C until further analysis.

2.4. Determination of total phenolic content (TPC)

Determination of total phenolic content was performed using the Folin–Ciocalteu reagent with minor modifications [18]. Briefly, the extract was dissolved in ethanol to obtain a concentration of 1 mg/mL. A 12.5 µL aliquot of the extract was added to a 96-well microplate, followed by the addition of 12.5 µL of distilled water and 12.5 µL of Folin–Ciocalteu reagent. The mixture was allowed to stand for 6 min in the dark at room temperature. Subsequently, 100 µL of distilled water and 125 µL of 7.5% (w/v) Na_2CO_3 were added. The reaction mixture was then incubated in the dark at room temperature for 90 min. The absorbance was measured at 760 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany). Gallic acid standard solutions (0.001–0.1 mg/mL) were prepared and employed to construct the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dried sample weight (mg GAE/g DW).

2.5. Determination of total flavonoid content (TFC)

The total flavonoid content was determined using the aluminium chloride colorimetric method with slight modifications [19]. Briefly, a stock solution of the extract (1 mg/mL) was prepared, and 125 µL of the solution was added to a 96-well microplate. Subsequently, 12.5 µL of 5% (w/v) $NaNO_2$ was added, and the mixture was then incubated in the dark at room temperature for 5 min. After incubation, a 37.5 µL aliquot of 10% (w/v) $AlCl_3$ was added to the mixture. The absorbance was then measured at 510 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany). Quercetin standard solutions (0.001–0.1 mg/mL) were prepared and employed to construct the calibration curve. The total flavonoid content was expressed as

milligrams of quercetin equivalents per gram of dried sample weight (mg QE/g DW).

2.6. Determination of antioxidant activity

The antioxidant activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [20]. Briefly, a stock solution of the extract (1 mg/mL) was diluted with ethanol to obtain various concentrations using a serial dilution method. A 100 µL aliquot of each extract solution was added to a 96-well microplate, followed by the addition of 100 µL of 0.2 mM DPPH solution prepared in ethanol. The mixture was incubated in the dark at room temperature for 30 min. After incubation, the absorbance was measured at 517 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany). L-Ascorbic acid was used as the reference standard. The antioxidant activity was expressed as the half-maximal inhibitory concentration (IC_{50} value), which was estimated by plotting the percentage inhibition of DPPH against the extract concentrations. The percentage inhibition of DPPH was calculated using the following equation:

$$\%\text{DPPH radical scavenging activity} = \frac{(\text{A Control} - \text{A Sample})}{\text{A Control}} \times 100$$

2.7. Determination of tyrosinase inhibitory activity

Tyrosinase inhibitory activity was determined using the Dopachrome assay with L-DOPA as the substrate [21]. Briefly, a stock solution of the selected extract of *B. oppositifolia* seed (1 mg/mL) was prepared in DMSO and serially diluted to obtain various concentrations. The tyrosinase stock solution (46 units/mL) and 2.5 mM L-DOPA were prepared in 0.05 M phosphate buffer (pH 6.8). Subsequently, 40 µL of the extract solution was added to a 96-well microplate, followed by the addition of 80 µL of tyrosinase solution (46 units/mL). The mixture was incubated at room temperature for 10 min. After incubation, 80 µL of 2.5 mM L-DOPA was added to initiate the reaction. The reaction mixture was then allowed to stand for another 10 min. The absorbance was measured at 475 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany). Kojic acid was used as the reference standard. The enzyme inhibitory activity was expressed as the half-maximal inhibitory concentration (IC_{50} value), which was estimated by plotting the percentage inhibition of tyrosinase against the extract concentrations. The percentage inhibition of tyrosinase activity was calculated using the following equation:

$$\%\text{Tyrosinase inhibitory activity} = \frac{(\text{A Control} - \text{A Sample})}{\text{A Control}} \times 100$$

2.8. Determination of antibacterial activity against acne-associated bacteria

2.8.1. Agar disc diffusion assay

Antibacterial activity was initially evaluated using the agar disc diffusion method [22] against three reference bacterial strains (DMST) relevant to cutaneous infections and cosmeceutical product safety, including *Staphylococcus aureus* DMST 8013, *Staphylococcus epidermidis* DMST 15505, and *Cutibacterium acnes* DMST 14916. Each bacterial suspension was adjusted to a density of 1.5×10^8 CFU/mL, equivalent to McFarland standard No. 0.5, using 0.85% (w/v) NaCl. Each sterile paper disc (6 mm in diameter) was impregnated with 10 µL of the extract solution of *B. oppositifolia* seed (at a concentration of 1 mg/mL dissolved in 1% DMSO) and was allowed to dry before being placed onto the surface of Mueller–Hinton agar plates previously swabbed with the bacterial suspension (*S. aureus* and *S. epidermidis*). The plates were incubated at 37 °C for 24 h. For *C. acnes*, the impregnated discs were placed on Brain Heart Infusion agar that had been inoculated with the *C. acnes* strain. The plates were then incubated under aerotolerant anaerobic conditions at 37 °C for 48 h. After incubation, the diameters of the inhibition zones were measured to evaluate antibacterial activity and compared with those of the standard antibiotic control (vancomycin, 10 µg/disc). In addition, 1% DMSO (10 µL/disc) was utilized as the negative control.

2.8.2. Determination of MIC and MBC

The broth microdilution method was employed to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). This was performed using Mueller–Hinton broth (MHB) for *S. aureus* and *S. epidermidis*, and Brain Heart Infusion (BHI) broth for *C. acnes* [23]. Specifically, two-fold serial dilutions of the extract (0.0039–10 mg/mL) were prepared in 96-well microplates and incubated at 37 °C for 24 h for *S. aureus* and *S. epidermidis*. For *C. acnes*, the 96-well microplates were incubated under aerotolerant anaerobic conditions at 37 °C for 48 h. After incubation, the lowest concentration that showed no visible bacterial growth was recorded as the MIC. The MBC was determined by transferring 10 µL from the wells corresponding to the MIC and higher concentrations onto drug-free agar plates, followed by incubation at 37 °C for an additional 24 h. For *C. acnes*, the plates were maintained under the same conditions as those used for the MIC assay. The MBC was defined as the lowest concentration that resulted in a $\geq$ 99.9% reduction in colony count compared with the initial inoculum.

2.9. Preliminary phytochemical screening

The selected extract of *B. oppositifolia* was subjected to standard phytochemical screening tests based on precipitation and color-change reactions [24]. A total of ten phytoconstituents were qualitatively analyzed, including phenolics ($FeCl_3$ test: positive; black or blue solution), saponins (foam test: positive; persistent foam for at least 10 min), flavonoids (Shinoda's test: positive; pink or red solution), phytosterols (Salkowski's test: positive; red color appeared in the chloroform layer), alkaloids (Wagner's reagent test: positive; brown precipitate), cardiac glycosides (Keller–Killiani test: positive; brown or purple ring), anthraquinones (Bornträger's test: positive; orange, pink, or red color in the ammonia layer), diterpenes (copper acetate test: positive; emerald green solution), triterpenoids (concentrated H_2SO_4 test: positive; reddish-brown or golden-brown color in the chloroform layer), and tannins (gelatin test: positive; cloudy white precipitate).

2.10. Statistical analysis

All biological experiments were performed in triplicate (n = 3), and the results are presented as mean ± standard deviation (SD). Statistical significance for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test for multiple comparisons. Pearson's correlation coefficient was used to assess the relationships between total phenolic and flavonoid contents and antioxidant activity. Additionally, Student's *t*-test was employed to evaluate differences in tyrosinase inhibitory and anti-acne bacterial activities compared with the positive control at a 95% confidence level. A value of $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using JASP (version 0.96.0.0; University of Amsterdam, The Netherlands).

3. RESULTS

3.1. Optimization of extraction conditions

The optimal extraction conditions for *B. oppositifolia* seed were evaluated at extraction times of 1, 2, and 3 h using solvent combinations of EtOH:H_2O at ratios of 100:0, 50:50, and 0:100 (% v/v). The results showed that the EW2 extract yielded the highest crude extract yield (34.82%), whereas the W1 extract exhibited the lowest yield (15.21%), as shown in Table 1. Additionally, the physical characteristics of the crude extracts obtained from different solvent combinations (EtOH:H_2O at ratios of 100:0, 50:50, and 0:100, % v/v) were observed. The results indicated that the solvent system influenced the physical appearance of the extracts. Extraction using EtOH:H_2O at a ratio of 100:0 (% v/v) produced a light purple solid at all extraction durations. In contrast, the solvent combinations of EtOH:H_2O at ratios of 50:50 and 0:100 (% v/v) yielded brown semi-solid extracts at all extraction durations (Figure 2).

3.2. Total phenolic and flavonoid contents

Based on the linear calibration curves, the total phenolic content (TPC) and total flavonoid content (TFC) were estimated using gallic acid ($y = 5.0423x + 0.0959$, $R^2 = 0.9983$) and quercetin ($y = 1.7312x + 0.0636$, $R^2 = 0.9997$) as standards, respectively. The results demonstrated that the EW2 extract yielded the highest significant levels ($P < 0.05$) of TPC and TFC, with values of 103.42 ± 4.42 mg GAE/g DW and 139.87 ± 8.42 mg QE/g DW, respectively. In contrast, the lowest TPC was observed in the W3 extract, with a value of 19.53 ± 0.52 mg GAE/g DW. Similarly, the lowest TFC was found in the W1 extract, with a value of 7.29 ± 0.92 mg QE/g DW, as shown in Table 2.

Table 1. Crude extract yields of *B. oppositifolia* seed under varying EtOH:H_2O ratios and extraction durations.

Duration (h)	Extraction ratios of EtOH:H_2O (%v/v)	Extract code	Physical characteristics	Weight (g)	%Yield
1	100:0	E1	Light purple solid	1.719	17.19
2	100:0	E2	Light purple solid	1.535	15.35
3	100:0	E3	Light purple solid	3.013	30.13
1	50:50	EW1	Brown semi-solid	3.017	30.17
2	50:50	EW2	Brown semi-solid	3.482	34.82
3	50:50	EW3	Brown semi-solid	3.174	31.74
1	0:100	W1	Brown semi-solid	1.521	15.21
2	0:100	W2	Brown semi-solid	2.160	21.60
3	0:100	W3	Brown semi-solid	1.531	15.31

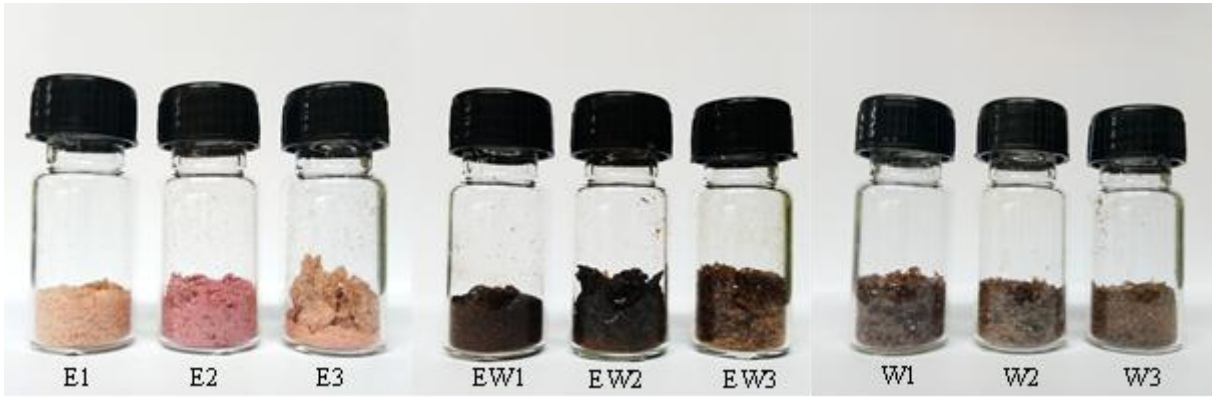


Figure 2. Physical characteristics of the crude extracts of *B. oppositifolia* seed under different extraction conditions: (E1) EtOH:H_2O (100:0, % v/v), 1 h, (E2) EtOH:H_2O (100:0, % v/v) 2 h, (E3) EtOH:H_2O (100:0, % v/v) 3 h, (EW1) EtOH:H_2O (50:50, % v/v) 1 h, (EW2) EtOH:H_2O (50:50, % v/v) 2 h, (EW3) EtOH:H_2O (50:50, % v/v) 3 h, (W1) EtOH:H_2O (0:100, % v/v) 1 h, (W2) EtOH:H_2O (0:100, % v/v) 2 h, (W3) EtOH:H_2O (0:100, % v/v) 3 h.

3.3. Antioxidant activity using the DPPH assay

Relating to hydrogen donation, the antioxidant activity of *B. oppositifolia* seed extracts was estimated using the DPPH assay. Based on the IC_{50} value of DPPH radical inhibition, a lower IC_{50} value represents greater antioxidant capacity. The results showed that the EW2 extract exhibited the highest antioxidant capacity in the DPPH assay, with an IC_{50} value of 0.17 ± 0.04 µg/mL. Furthermore, this extract demonstrated significantly higher antioxidant activity than the other extracts ($P < 0.05$). However, no significant difference ($P < 0.05$) was observed between the EW2 extract and the reference standard, L-ascorbic acid, which showed an IC_{50} value of 0.18 ± 0.05 µg/mL (Table 2).

3.4. Correlation analysis of TPC and TFC with antioxidant activity

The relationship between total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity as determined by the DPPH assay is presented in Table 3 and Figure 3. The results demonstrated that TPC was significantly correlated with antioxidant activity, as indicated by lower IC_{50} values of the DPPH assay. Based on Pearson's correlation analysis, a moderate negative correlation ($r = -0.496$, $P < 0.01$) was found between TPC and IC_{50} values of DPPH, indicating that higher phenolic content contributes to the antioxidant effect. In contrast, TFC exhibited a weak and non-significant correlation with antioxidant activity ($r = -0.105$, $P = 0.602$), indicating that flavonoids may

Table 2. Total phenolic and flavonoid contents, and antioxidant activity of the *B. oppositifolia* seed extracts under varying EtOH:H_2O ratios and extraction durations.

Extract code	Total phenolic content; TPC (mg GAE/g DW)	Total flavonoid content; TFC (mg QE/g DW)	Antioxidant activity; IC_{50} of DPPH (µg/mL)
E1	23.51 ± 1.51^{f}	83.87 ± 9.48^{d}	2.24 ± 0.08^{a}
E2	30.73 ± 0.52^{e}	111.24 ± 2.65^{c}	0.41 ± 0.06^{bc}
E3	34.44 ± 0.65^{de}	92.57 ± 3.79^{d}	0.45 ± 0.14^{bc}
EW1	30.73 ± 2.69^{e}	114.54 ± 3.84^{bc}	0.93 ± 0.07^{b}
EW2	103.42 ± 4.42^{a}	139.87 ± 8.42^{a}	0.17 ± 0.04^{c}
EW3	54.27 ± 0.99^{b}	127.82 ± 6.56^{ab}	0.46 ± 0.02^{bc}
W1	39.98 ± 2.21^{cd}	7.29 ± 0.92^{e}	0.52 ± 0.14^{bc}
W2	42.22 ± 0.15^{c}	14.00 ± 2.74^{e}	0.58 ± 0.15^{bc}
W3	19.53 ± 0.52^{f}	17.46 ± 1.11^{e}	0.86 ± 0.43^{b}
L-Ascorbic acid	-	-	0.18 ± 0.05^{c}

Note: All experiments were performed in triplicate (n = 3), and the results are expressed as mean ± SD. Different superscript letters within the same column indicate statistically significant differences ($P < 0.05$) based on Tukey's HSD test.

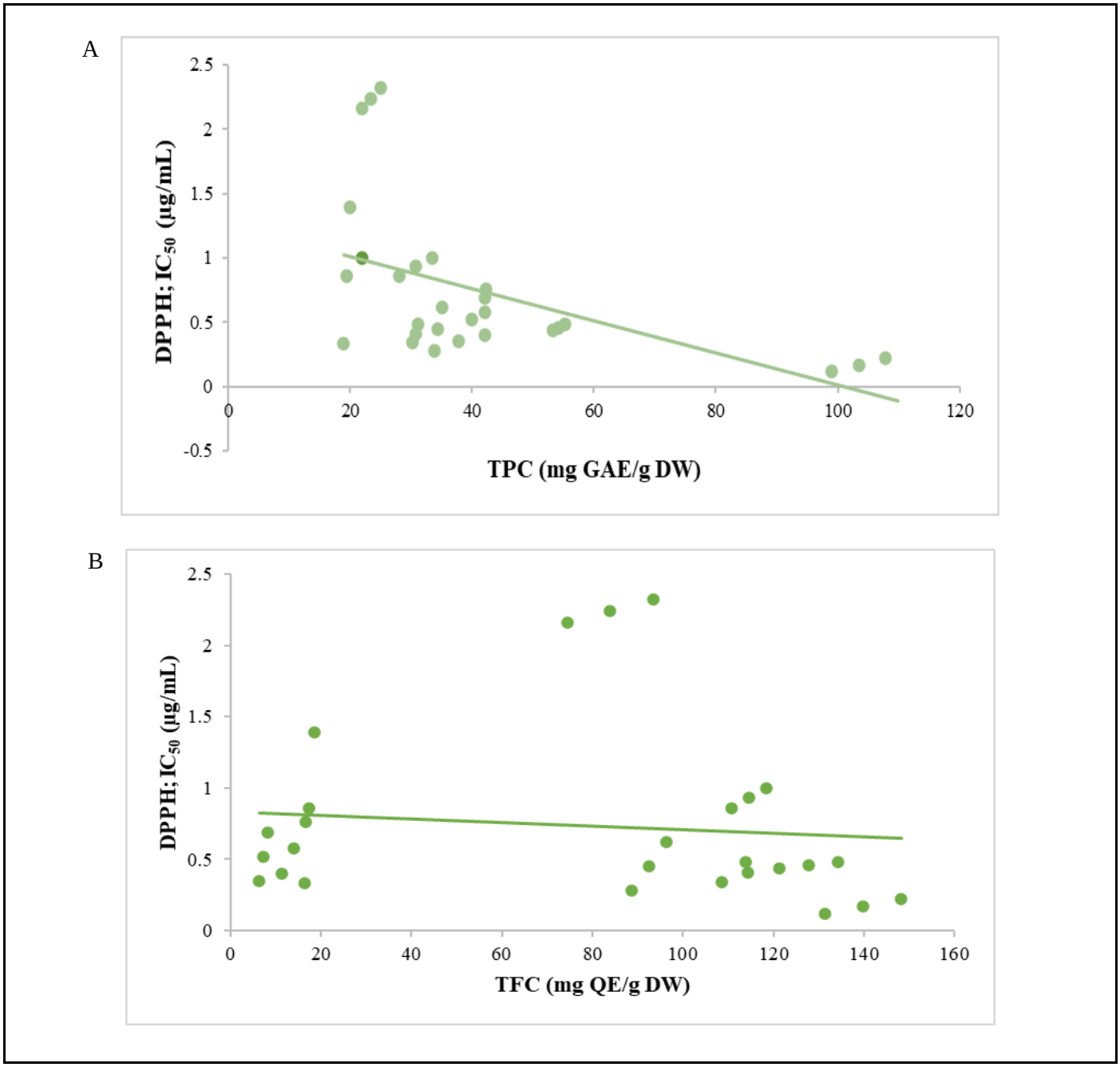


Figure 3. Scatter plots showing the correlation between TPC and DPPH (A) and TFC and DPPH (B).

play a less dominant role in contributing to the DPPH radical scavenging activity in this study. In addition, a moderate positive correlation was observed between TPC and TFC (r = 0.465, $P < 0.05$), indicating that flavonoids constitute a portion of the total phenolic compounds.

3.5. Tyrosinase inhibitory activity using the Dopachrome assay

Tyrosinase inhibitory activity was determined using the Dopachrome assay, and the IC_{50} value was used to indicate the potential enzyme inhibitory effect

Table 3. Pearson's correlations between TPC and TFC with antioxidant activity.

	TPC	TFC	DPPH
TPC	1.000		
TFC	0.465*	1.000	
DPPH	-0.496**	-0.105	1.000

Note: *$P < 0.05$ (two-tailed), **$P < 0.01$ (two-tailed).

Table 4. Tyrosinase inhibitory activity of the selected EW2 extract of *B. oppositifolia* seed.

Sample	Tyrosinase inhibitory activity; IC_{50} (µg/mL)
EW2 extract	183.00 ± 9.51**
Kojic acid	9.60 ± 1.65

Note: The experiment was performed in triplicate (n = 3), and the results are expressed as mean ± SD. Statistical significance compared with the reference standard was determined using Student's *t*-test (**$P < 0.01$).

in comparison with the reference standard (kojic acid). Similar to antioxidant activity, a lower IC_{50} value indicates stronger enzyme inhibitory activity. The results exhibited that the selected EW2 extract of *B. oppositifolia* seed had lower tyrosinase enzyme inhibition, with an IC_{50} value of 183.00 ± 9.51 µg/mL, which was significantly higher ($P < 0.01$) than that of kojic acid (IC_{50} value of 9.60 ± 1.65 µg/mL), as shown in Table 4.

3.6. Antibacterial activity against acne-associated bacteria

The antibacterial activity of the selected EW2 extract from *B. oppositifolia* seed was evaluated against three acne-associated bacteria, including *S. aureus*, *S. epidermidis*, and *C. acnes*. The antibacterial activity was expressed as the diameter of the clear zone of inhibition (mm) and compared with the reference antibiotic, vancomycin (10 µg/disc). The results demonstrated that the selected extract exhibited antibacterial activity against all tested microorganisms, with inhibition zone diameters of 13.00 ± 0.00 mm for *S. aureus*, 14.00 ± 0.00 mm for *S. epidermidis*, and 12.17 ± 0.29 mm for *C. acnes*. However, the antibacterial activity of the extract was significantly lower ($P < 0.01$) than that of vancomycin, which produced inhibition zones of 15.00 ± 0.00 mm, 16.17 ± 0.29 mm, and 35.00 ± 0.00 mm against *S. aureus*, *S. epidermidis*, and *C. acnes*, respectively (Figure 4). Subsequently, the extract was further evaluated to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the three tested microorganisms. The results revealed that the extract exhibited the MIC values of 0.62 mg/mL, 1.25 mg/mL, and 0.0064 mg/mL against *S. aureus*, *S. epidermidis*, and *C. acnes*, respectively. Similarly, the MBC values were estimated to be 10.00 mg/mL, 5.00 mg/mL, and 0.1024 mg/mL against *S. aureus*, *S. epidermidis*, and *C. acnes*, respectively (Table 5).

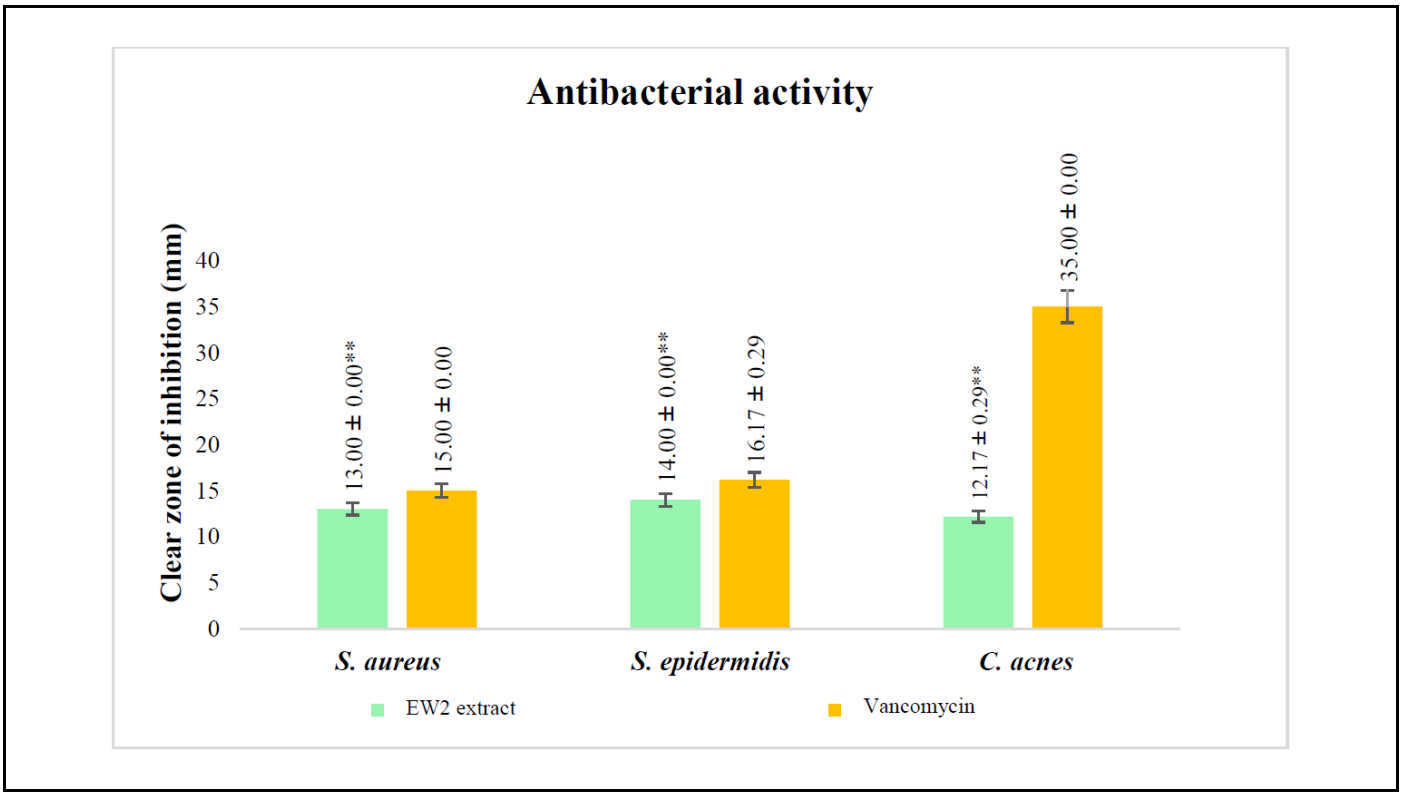


Figure 4. Anti-acne activity based on acne-associated bacteria of the selected EW2 extract of *B. oppositifolia* seed compared with vancomycin (10 µg/disc). **$P<0.01$ (Student's *t*-test).

Table 5. MIC and MBC of the selected EW2 extract of *B. oppositifolia* seed against acne-associated bacteria.

Microorganism	MIC (mg/mL)	MBC (mg/mL)
S. aureus	0.62	10.00
S. epidermidis	1.25	5.00
C. acnes	0.0064	0.1024

3.7. Phytochemical screening

In terms of the preliminary phytochemical screening, the selected EW2 extract from *B. oppositifolia* seed was conducted based on precipitation and color-change reactions. The results indicated that seven out of the ten tested phytochemical constituents were present in the extract, including phenolics, flavonoids, phytosterols, cardiac glycosides, anthraquinones, triterpenoids, and tannins, as shown in Table 6.

4. DISCUSSION

Differences in extraction yield, physical characteristics, and color of the extracts may be attributed to the polarity of the solvents used, which influences the types of bioactive compounds extracted. Variations in the polarity index of solvent systems enable the selective dissolution of phytochemicals with similar polarity, resulting in noticeable changes in the physical appearance of the crude extracts [25]. Notably, the utilization of polar solvents, particularly ethanol and water, can effectively extract hydrophilic phytoconstituents containing hydroxyl (–OH) groups attached to aromatic rings in their molecular structures, such as phenolic compounds, flavonoids, tannins, and other highly polar constituents. This is due to the presence of hydroxyl groups in the solvent molecules, which enhance their polarity and ability to form hydrogen bonds with polar phytochemicals. Therefore, the combination of ethanol and water under optimized conditions provides a suitable polarity index, facilitating the efficient extraction of phytochemicals with similar polarity from plant materials [26]. Furthermore, the use of green extraction solvents, such as ethanol and water, is advantageous for the development of natural ingredients in healthcare products. These solvents are considered safer and more environmentally friendly compared with conventional organic solvents used for extracting bioactive compounds, such as methanol, ethyl acetate, hexane, chloroform, and benzene, which may cause toxic effects to human health and contribute to environmental pollution [27].

Several studies have reported that solvent mixtures containing ethanol (approximately 50–80%) are effective for extracting a wide range of phenolic and flavonoid compounds. This efficiency is attributed to the ability of hydroethanolic solvents to penetrate and disrupt plant cell walls, thereby enhancing the release of intracellular phytochemicals. Consequently, the functional groups of the solvent molecules can more easily interact with the bioactive compounds produced by plants, leading to improved extraction efficiency [28-29]. However, higher levels of total phenolic content (TPC) and total flavonoid content (TFC) may vary depending on numerous factors, including the plant species, the plant parts used for extraction, the geographical area of plant collection, and the extraction techniques employed, all of which can significantly affect the extraction yield and total phenolic and flavonoid contents of crude extracts [30].

Moreover, evidence from previous studies indicates that elevated levels of TPC and TFC are significantly correlated with robust antioxidant activity. Due to the negative correlation between TPC and TFC with IC_{50} values, the high levels of phenolic and flavonoid compounds are significantly associated with lower IC_{50} of

Table 6. Preliminary phytochemical screening of the selected EW2 extract of *B. oppositifolia* seed.

Phytoconstituent	Test name	Result
Phenolics	$FeCl_3$ test	+++
Saponins	Foam test	-
Flavonoids	Shinoda's test	+++
Phytosterols	Salkowski test	+
Alkaloids	Wagner's test	-
Cardiac glycosides	Keller-Killiani test	++
Anthraquinones	Bornträger's test	++
Diterpenes	Copper acetate test	-
Triterpenoids	Conc. H_2SO_4 test	+
Tannins	Gelatin test	+++

Note: (+++) Highly positive (significantly visible color change), (++) Moderately positive, (+) Mildly positive, (–) Negative (Phytoconstituent not detected).

the DPPH radical scavenging activity [31]. However, a stronger correlation was observed for TPC compared to TFC in this study, indicating that phenolic compounds are the major contributors to antioxidant activity. These compounds play an important role in stabilizing free radicals through several mechanisms, including radical scavenging, hydrogen atom donation, neutralization of free radicals, metal ion chelation, and singlet oxygen quenching [32-33]. Thus, the EW2 extract was selected for further investigation of its tyrosinase inhibitory and antibacterial activities to determine its potential as a natural raw material for developing in the field of healthcare products.

Similarly, some studies have demonstrated that TPC and TFC are positively correlated with both antioxidant and tyrosinase inhibitory effects. However, other bioactive compounds may also contribute to these activities. This is supported by findings indicating that biological properties are often influenced by a complex mixture of phytochemicals rather than a single class of compounds. Moreover, the compounds responsible for enzyme inhibition may vary significantly depending on the plant species [34]. This variation can be attributed to differences in the composition of bioactive compounds, particularly their functional groups and molecular structures, which enable effective interaction with the active site of the tyrosinase enzyme. Such interactions may enhance binding affinity and result in stronger tyrosinase inhibitory activity [35-36].

In terms of antibacterial activity against acne-associated bacteria, a previous study has reported that seed extracts from the plants in the Anacardiaceae family possess notable antibacterial properties. In particular, *B. macrophylla* seed extracts have been demonstrated to exhibit promising anti-acne bacterial activity against *S. aureus*, *S. epidermidis*, and *C. acnes*, with inhibition zone diameters wider than 15 mm. Among the tested extracts, the ethanolic fraction (BSEE), ethyl acetate fraction (BSEA), and crude ethanolic extract (BSE) showed outstanding antibacterial activity in the disc diffusion assay. In addition, the MIC and MBC of these seed extracts were also evaluated. The results revealed that the BSEE, BSEA, and BSE of *B. macrophylla* exhibited the MIC values of 0.625 mg/mL and MBC values ranging from 0.625 to 2.5 mg/mL against *S. aureus*. For *S. epidermidis*, the MIC values ranged from 0.15625 to 0.3125 mg/mL, while the MBC values ranged from 0.625 to 1.25 mg/mL. Furthermore, the extracts also showed antibacterial activity against *C. acnes*, with the MIC values ranging from 0.00975 to 0.0195 mg/mL and MBC values ranging from 0.15625 to 0.3125 mg/mL, respectively [37].

Several studies have demonstrated that phytochemical constituents present in plant extracts play important roles in the prevention and management of chronic diseases. The compounds were identified in the selected EW2 extract of *B. oppositifolia* seed, such as phenolics, flavonoids, phytosterols, cardiac glycosides, anthraquinones, triterpenoids, and tannins, which are widely recognized for their diverse biological activities. In particular, they have been reported to exhibit antioxidant, anticancer, antitumor, antibacterial, antiviral, antimicrobial, anti-inflammatory, antidiabetic, neuroprotective, enzyme inhibitory, immunomodulatory, anti-aging, and skin-whitening effects [32, 38, 39].

The biological activities and major bioactive compounds of *B. oppositifolia* remain relatively underexplored. However, previous studies have reported the phytochemical composition and biological properties of different plant parts, particularly twig and leaf extracts. Earlier findings demonstrated that twig extracts of *B. oppositifolia* exhibit notable antioxidant activity, as evaluated by multiple assays. Among the three extracts, the methanolic extract showed the highest antioxidant potential, as evidenced by DPPH and ABTS assays, with IC_{50} values of 1.14 ± 0.16 µg/mL and 47.86 ± 1.39 µg/mL, respectively, along with a FRAP value of 27.87 ± 3.85 mg $FeSO_4$/g DW. This extract also contained the highest levels of TPC (20.24 ± 3.77 mg GAE/g DW) and TFC (5.41 ± 0.12 mg QE/g DW), which likely contributed to its strong antioxidant activity. In contrast, cytotoxicity evaluation using HaCaT cell lines revealed that the ethyl acetate extract at a concentration of 1,000 µg/mL exhibited moderate cytotoxicity (%cytotoxicity of 39.19 ± 0.04), indicating lower toxicity compared to the other extracts [40].

Additionally, leaf extracts of *B. oppositifolia* have been investigated for their phytochemical constituents, total phenolic and flavonoid contents, and biological activities. The results revealed the presence of various phytochemicals, including phenolics, saponins, flavonoids, phytosterols, glycosides, anthraquinones, diterpenes, triterpenoids, and tannins. Among these, the methanolic extract exhibited the highest TPC and TFC (74.25 ± 3.31 mg GAE/g dried leaf and 71.48 ± 1.94 mg QE/g dried leaf, respectively), along with the strongest antioxidant capacity, with IC_{50} values of 1.39 ± 0.40 µg/mL and 21.97 ± 0.31 µg/mL for DPPH and ABTS assays, respectively, and a FRAP value of 505.55 ± 24.32 mg $FeSO_4$/g dried leaf. In contrast, the ethyl acetate extract demonstrated the strongest tyrosinase inhibitory activity, with an IC_{50} value of 242.90 ± 7.48 µg/mL, and exhibited the lowest cytotoxicity toward HaCaT cells (%cell viability of 59.04 ± 0.15 at a concentration of 1,000 µg/mL) [12].

In the present study, solvent extraction conditions using a combination of ethanol and water were optimized to enhance the extraction efficiency of bioactive compounds from *B. oppositifolia* seeds, while avoiding the use of toxic organic solvents and promoting safer applications in the cosmetic industry. The results

indicated that the optimal extraction condition was an EtOH:H_2O ratio of 50:50 (% v/v) with an extraction time of 2 h. Under these conditions, the extract achieved the highest extraction efficiency and exhibited the highest levels of TPC and TFC. Moreover, this extract showed the lowest IC_{50} values in the DPPH assay compared to other extracts ($P < 0.05$), indicating superior antioxidant capacity. Furthermore, this extract demonstrated notable tyrosinase inhibitory activity and strong antibacterial effects against acne-associated bacteria, particularly *C. acnes*.

These findings suggest that the EW2 extract of *B. oppositifolia* seed represents a promising natural source of phytoconstituents, especially phenolic and flavonoid compounds, which are key contributors to its antioxidant, tyrosinase inhibitory, and antibacterial activities. Therefore, this extract shows strong potential as a natural anti-acne agent and may be developed as a raw material for cosmetic formulations. Nevertheless, further investigations, including *in vivo* studies, clinical trials, cytotoxicity evaluation, anti-inflammatory activity assessment, and structural identification of active compounds, are required to ensure its safety and efficacy for future applications.

5. CONCLUSIONS

The findings of this study demonstrate that the EW2 extract of *B. oppositifolia* seed provided the highest levels of total phenolic and total flavonoid contents, as well as the strongest antioxidant activity as determined by the DPPH assay. In addition, the extract exhibited tyrosinase inhibitory activity and showed promising antibacterial effects against acne-associated bacteria, especially *S. aureus*, *S. epidermidis*, and *C. acnes*. The extract was also found to be rich in bioactive phytochemical constituents. These results suggest that the EW2 extract of *B. oppositifolia* seed may serve as a promising natural resource for further development of anti-acne products in the cosmeceutical and pharmaceutical industries. However, further in-depth studies, including *in vivo* experiments and clinical trials, are required to evaluate its cytotoxicity and anti-inflammatory activities, as well as to isolate and identify the key bioactive compounds responsible for its biological activities, in order to ensure its safety and therapeutic potential for broader future applications.

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Author contribution

Conceptualizations: P.S. and A.J.; Methodology: N.L., P.K., P.P., P.K., and P.S.; Formal analysis: N.L. and P.S.; Investigation: N.L., P.K., P.P., P.K., and P.S.; Data curation: N.L., P.S., and A.J.; Writing—original draft: N.L. and P.S.; Writing—review and editing: N.L., A.J., and P.S.; Supervision: P.S. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

Ethical approval was not required for this study, as it involved only *in vitro* experiments using standard bacterial strains obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand.

Declaration on the Use of Generative AI in Scientific Writing

During the preparation of this manuscript, the authors used ChatGPT to improve the language and readability of the text. The authors carefully reviewed and edited the output and take full responsibility for the content of the final manuscript.

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