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# Phytochemical profiling, antioxidant potential, and anti-*Acinetobacter baumannii* activity of *Macadamia integrifolia* Maiden & Betche shell extracts

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**ABSTRACT**

**Introduction:** *Macadamia integrifolia* Maiden & Betche shells, which comprise approximately 80% of the total fruit weight and are often regarded as a by-product of macadamia nut production, contain bioactive compounds with potential health benefits. However, few studies have investigated the bioactive properties of *M. integrifolia* shells for pharmaceutical, cosmetic, and food applications. Therefore, this study aimed to evaluate the phytochemical contents, antioxidant activity, and inhibitory effects against *Acinetobacter baumannii* of *M. integrifolia* shell extracts.

**Methods:** Shell powder of *M. integrifolia* was extracted using 95% ethanol to yield a crude extract (ME), which was subsequently fractionated into dichloromethane (MD), ethyl acetate (MA), and water (MW) fractions. Total phenolic and flavonoid contents were determined using colorimetric assays. Antioxidant and antimicrobial activities were assessed via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and disc diffusion method, respectively.

**Results:** The *M. integrifolia* shell extracts (ME, MD, MA, and MW) contained total phenolic contents ranging from 4.27 to 26.76 mg gallic acid equivalent/g and flavonoid contents from 7.93 to 38.01 mg rutin equivalent/g. The MA fraction demonstrated the most potent antioxidant activity ($IC_{50}$ = 17.60 µg/mL). All extracts showed inhibitory effects against *A. baumannii*, with inhibition zones between 7.0 and 12.0 mm; the ME extract exhibited the largest inhibition zone (12.0 mm).

**Conclusion:** This is the first report to demonstrate the antioxidant and antimicrobial activities of *M. integrifolia* shell extracts against *A. baumannii*. Their high phenolic and flavonoid contents support potential use as natural bioactive agents. Further studies on biomarkers purification and the mechanism of action are recommended.

*Implication for health policy/practice/research/medical education:*
*Macadamia integrifolia* shell extracts exhibit antioxidant potential and antimicrobial activity against *Acinetobacter baumannii*. These findings highlight their pharmaceutical, cosmetic, and food application research promise. Further studies on biomarkers purification and the mechanism of action are recommended.

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## Introduction

Macadamia (*Macadamia integrifolia* Maiden & Betche) is a perennial plant belonging to the Proteaceae family (1). Its fruit consists of a hard brown shell encasing an edible kernel, with the kernel comprising only 20% of the total fruit weight, while the husk and shell account for approximately 42%–45% and 35%–38%, respectively (2). Despite the large volume of by-products generated, these materials are often discarded in landfills, posing significant disposal challenges and raising environmental concerns (3).

Previous research suggested that macadamia by-products could serve as valuable sources of bioactive phytochemicals. Leaves contain gallic acid, protocatechuic acid, flavonoids, and tannins (4,5), while flowers are rich in rutin, catechin, quercetin, and kaempferol (6,7). Similarly, husks and shells possess a diverse range of phenolic compounds, including gallic acid, caffeic acid, catechin, and proanthocyanidins (8,9). These by-products may have potential health and antimicrobial research applications due to their abundance in phenolic and flavonoid compounds. Phenolic compounds are well known for their antioxidant and free radical-scavenging properties, contributing to various health benefits. Macadamia husks, for example, are particularly rich in catechin and epicatechin, which exhibit potent antioxidant activity (10). Additionally, flavonoids and tannins from macadamia leaves and flowers demonstrate antimicrobial effects (11), with husk-derived phenolics being incorporated into edible films to enhance food preservation (12).

*Acinetobacter baumannii*, a multidrug-resistant (MDR) Gram-negative bacterium, is a primary global health concern, particularly in healthcare settings. *A. baumannii* exhibits high antibiotic resistance, leading the World Health Organization (WHO) to classify it as a top-priority pathogen for antibiotic research (13). Its ability to persist in hospital environments and cause infections, particularly in immunocompromised patients (14,15), highlights the urgent need for alternative antimicrobial agents.

Despite the potential of macadamia-derived bioactive compounds, research on the shell's phytochemical composition, antioxidant properties, and antimicrobial activity remains limited. This study aimed to investigate the chemical profile, antioxidant activity, and antibacterial potential of macadamia shell extracts against *A. baumannii*, providing insight into their potential therapeutic applications.

## Materials and Methods

### Chemicals and reagents

The analytical reagent (AR) grade of absolute ethanol, dichloromethane, ethyl acetate, deionized water, methanol, acetic acid, sodium hydrogen carbonate, and sulfuric acid, and the high-performance liquid chromatography (HPLC) grade of acetonitrile were purchased from RCI Labscan Ltd., Bangkok, Thailand. Dimethyl sulfoxide (DMSO, AR grade) was bought from AMRESCO, LLC., USA. Aluminum chloride (AR grade) was obtained from Ajax Finechem, Australia. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), vanillin, Folin–Ciocalteu reagent, and aluminum thin-layer chromatography (TLC) plate, silica gel 60 $F_{254}$, were purchased from Merck, Germany. Mueller-Hinton Broth was acquired from HiMedia Laboratories LLC, India. Stigmasterol (98.0% purity), rutin (98.0% purity), and gallic acid (97.5% purity) were bought from Sigma-Aldrich, USA. Quercetin (95.0% purity) was obtained from Tokyo Chemical Industry Co., Ltd., Japan. Colistin (98.0% purity) was purchased from Sigma-Aldrich, China. The 95% v/v ethanol (Pharmaceutical grade) was obtained from Liquor Distillery Organization, Thailand. Phloroglucinol TS (Sigma-Aldrich, USA). The 96-well plate was purchased from Greiner Bio-One (Germany). Sterile paper discs (Whatman AA, diameter 6 mm) were purchased from Cytiva (China). More details about the instruments used in this study are provided in Supplementary file 1.

### Plant authentication, collection, and microscopic analysis

Macadamia shells were collected from Phop Phra District, Tak province, Thailand (latitude: 16.490571, longitude: 98.8120993) in December 2019. *M. integrifolia* was identified and authenticated in our previous report (16) with voucher specimen number 005184, stored at the PNU Herbarium, Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand. The shells were baked at 50 °C for 4 days to remove moisture, then ground into fine powder using an electric grinder. The powder was stored in sealed plastic bags at room temperature (30±5 °C) until extraction.

The dried macadamia shell powder was analyzed for quality control using a light microscope, following WHO guidelines Geneva, 1998. The powder was sieved to obtain particle sizes (40-80 μm). Phloroglucinol TS was used as a staining reagent to examine the lignified cells, prepared by dissolving one gram of phloroglucinol in 100 mL of ethanol. Lignified cell walls were identified by their pink to cherry-red coloration.

### Plant extraction

Macadamia shell powder was extracted with 95% ethanol (1:5 ratio of powder to ethanol) by ultrasonic-assistance at room temperature (30±10 °C). The ethanolic supernatant was evaporated at 50 °C under reduced pressure to obtain an ethanolic crude extract (ME). Then 100 g of ME was subjected to liquid-liquid partition to obtain the dichloromethane, ethyl acetate, and water layers. The dichloromethane and ethyl acetate layers were evaporated at 50 °C under reduced pressure using a rotary evaporator to eliminate solvent residues, to obtain the dichloromethane fraction (MD) and ethyl acetate fraction

(MA). The remaining water layer was evaporated at 50 °C under reduced pressure, then freeze-dried to obtain the water fraction (MW). All fractions (ME, MD, MA, and MW) were stored at 4 ± 3 °C until analysis. The details of plant extraction are provided in Supplementary file 1.

### Chromatographic fingerprinting by TLC and HPLC

The chemical composition of *M. integrifolia* shell extracts (ME, MD, MA, and MW) was analyzed using TLC and HPLC analysis. For TLC, the extracts (10 mg/mL) and standards (quercetin, stigmasterol, and gallic acid, 1 mg/mL) were dissolved in methanol and spotted onto a silica gel 60 F254 Aluminum TLC plate. The mobile phase consisted of dichloromethane and methanol (90:10). Developed plates were visualized under UV light (254 and 366 nm) after spraying with 10% sulfuric acid in ethanol and heating at 110 °C. Retardation factor (Rf) values were calculated. For HPLC, the extracts (5 mg/mL) were dissolved in methanol, sonicated for 30 minutes, and filtered. A Welch® $C_{18}$ column (250 × 4.6 mm, 5 µm) was used with a gradient elution of acetonitrile (Phase A) and water (Phase B). The gradient increased Phase A from 5% to 95% over 40 minutes: 5%–20% Phase A in 5 minutes, 20%–30% in 5 minutes, 30%–45% in 5 minutes, 45%–70% in 5 minutes, and 70%–95% in 10 minutes, followed by isocratic elution at 95% Phase A for an additional 10 minutes. Detection was performed using a Photo-Diode Array Detector (PDA) 200–800 nm with a 20 µL injection volume, a flow rate of 0.8 mL/min, and a column temperature of 25 °C.

### Quantitative analysis of total phenolic and flavonoid contents

The extracts' phytochemicals were determined using the standard colorimetric assay protocols previously described (17-19). Briefly, the total phenolic content was determined using the Folin-Ciocalteu reagent, gallic acid as a standard, absorbance at 765 nm, and then reported as total phenolic content in mg gallic acid equivalent (GAE)/ g extract. The total flavonoid content was measured using the Aluminum chloride solution, rutin as a standard, absorbance at 415 nm, and finally reported total flavonoid content as mg rutin equivalent (RTE)/ g extract. All quantitative analyses were conducted in triplicate, using a microplate reader. The detailed protocols are available in Supplementary file 1.

### Evaluation of *in vitro* antioxidant activity

The free radical scavenging activity was evaluated according to Sembiring et al and Zahratunnisa et al's protocols (17,20) with minor modifications. The assay was performed in a 96-well microplate. Briefly, 125 µL of macadamia shell extract at various concentrations (1, 10, 100, 1000, 2500, and 5000 µg/mL) or ascorbic acid standard (0–50 µg/mL) prepared in methanol was mixed with 75 µL of 0.5 mM DPPH solution in methanol. The mixture was incubated at room temperature (25 ± 2 °C) for 30 minutes. The absorbance was recorded at 517 nm using a microplate reader. All experiments were conducted in triplicate. The half-maximal inhibitory concentration of DPPH activity ($IC_{50}$) was determined for each sample.

### Determination of inhibitory effect against *Acinetobacter baumannii*

#### *Acinetobacter baumannii culture*

The Gram-negative bacterium *A. baumannii* ATCC 19606 was obtained from the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand. The bacterium was cultured in Mueller-Hinton Broth and incubated at 37°C for 24 hours. The bacterial suspension was then adjusted to a turbidity of 0.5 McFarland standard, which corresponds to approximately $10^8$ colony-forming units (CFU) per mL. The microbiological experiments were conducted following approval from the Biosafety Committee of Naresuan University (NUIBC MI 66-04-014).

#### *Paper disc diffusion assay*

The inhibitory effect against *A. baumannii* growth of macadamia shell extracts was evaluated using the paper disc diffusion method (11). The suspension of the test microbe was swabbed on Mueller-Hinton agar plates. Then, twenty microliters of the four extracts (10.0 mg/mL in DMSO) were loaded onto sterile 6 mm paper discs (200 µg/disc). Colistin (10.0 mg/mL) was used as a positive control, while DMSO was a negative control. The plates were incubated at 37 ± 2 °C for 24 hours. After incubation, the diameter of the inhibition zone was measured in millimeters using a vernier caliper.

### Statistical analysis

The percentage yield of macadamia shell extracts and their phytochemical contents were presented as means (n = 3) ± standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA) with post hoc Bonferroni test ($P < 0.05$) in MS Excel 2019. The relationship between phytochemical contents (phenolics and flavonoids) and antioxidant properties was assessed using the correlation coefficient method (21), with values correlation coefficients ranging from -1 to +1. A coefficient of 0 indicates no association, while values closer to +1 or -1 represent stronger positive or negative correlations.

## Results

### Microscopic analysis

The macadamia shell had a hard brown exterior, 26-30 mm in diameter, and 2.7-3.0 mm-thick brown skin. Inside, a thin creamy-dark brown tissue covered the kernel (Figure 1).


**Figure 1.** *Macadamia integrifolia* shell macroscopic analysis.

Microscopic analysis showed thick-walled parenchyma in the seed coat (Figure 2A) and reticulate vessels with a net-like pattern (Figure 2B). Spiral vessels exhibited a helical structure (Figure 2C1-C2), and the endocarp contained lignified sclereids in various shapes (Figure 2D1-D6).

## Physical appearance and extraction yield

The physical appearance and percentage yield of each fraction from the extraction and fractionation of *M. integrifolia* shells are presented in Table 1. ME and MA had similar physical appearances, with dark brown viscous liquid characteristics, while MD was lighter, with dark yellow viscous liquid. MW was a brown powder. Compared to the weight of the dry shell of macadamia, the ethanolic crude extract (ME) yielded 1.47 %. After fractionation, MD had the highest yield at 0.78 %, followed by MW at 0.42% and MA at 0.25 %.

## Chromatographic fingerprinting by TLC and HPLC

The TLC spots are shown in Figure 3. MD, MA, and MW had spots with Rf values between 0.10 and 0.90. Major spots of MD were observed at Rf values 0.55-0.85. MA presented various compounds from 0.10 to 0.90, and MW showed the polar compounds with Rf values ranging from 0.05 to 0.1. The MD and MA bands matched those of quercetin (Rf 0.50), stigmasterol (Rf 0.85), and gallic acid (Rf 0.05). However, unclear spots of ME (1 mg/mL, 5 µL) were noted under UV 254 nm, 366 nm, and after spraying sulfuric acid, then heating at 110 °C.

Reverse-phase HPLC chromatograms of blank (methanol, Figure 4A), quercetin (standard, Figure 4B), and *M. integrifolia* shell extracts (ME, MD, MA, MW; Figures 4C-4F, respectively) were used to analyze their chemical composition as shown in Figure 4. The ME chromatogram showed small peaks at retention times around 12.72–15.40 minutes, while MW had two peaks at 3.54 and 4.79 minutes. MD and MA illustrated multiple peaks at retention times of 9.73–20.01 minutes and 6.96–20.02 minutes, respectively. Nineteen peaks across the four extracts were selected. Table 1 presents detailed reverse-phase HPLC chromatogram data—retention time, peak area, height, and purity index, offering more insight into Figure 4. Although some peaks share similar retention times (e.g., peak 13 at ~14.66 minutes), differences in area and height reflect varying concentrations across fractions. The purity index helps distinguish well-resolved peaks (purity >0.95) from potential co-eluted compounds or impurities (purity <0.5), aiding in identifying bioactive markers and optimizing fraction selection for further purification.

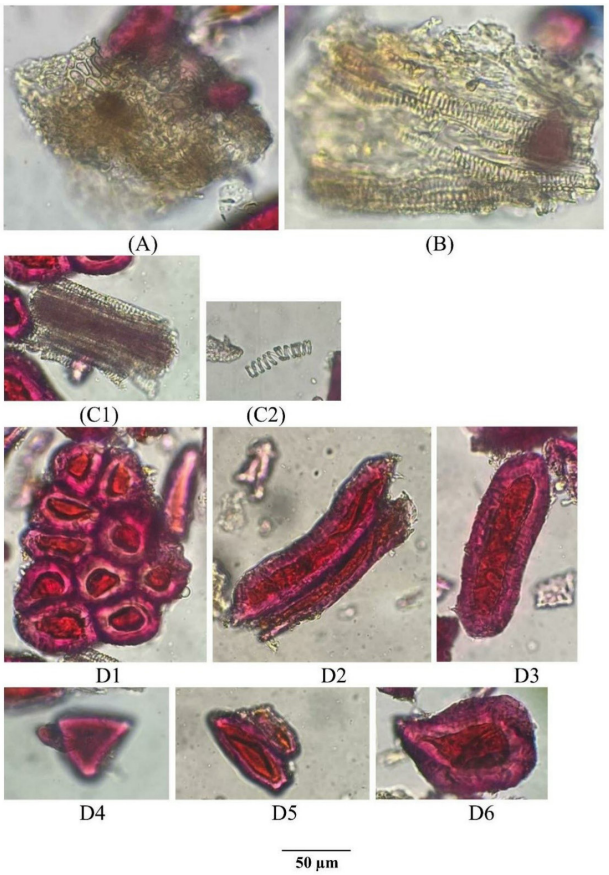


**Figure 2.** Microscopical analysis of the powder of the shell of *Macadamia integrifolia*. (A) Thick-walled parenchyma of the seed coat; (B) Parenchyma of the seed coat and underlying reticulate vessels; (C1), (C2). Spiral vessels; (D1)-(D6) Sclereids of endocarp.

## Phytochemical contents

The total phenolic contents (mg GAE/g extract) of the macadamia shell extracts were 15.15 ± 0.50 for ME, 4.27 ± 0.30 for MD, 26.76 ± 0.63 for MA, and 18.54 ± 0.74 for MW. Statistical analysis revealed significant differences in phenolic content across the fractions, with the MA fraction exhibiting the highest content, followed by MW and ME. In contrast, the MD fraction had the lowest phenolic content. The flavonoid contents (mg RTE/g extract) of the macadamia shell extracts were 21.15 ± 2.31 for ME, 7.93 ± 1.04 for MD, 29.15 ± 2.70 for MA, and 38.01 ± 0.94 for MW. Significant differences were observed among the fractions, with the MW fraction having the highest flavonoid content, followed by MA and ME. In contrast, the MD fraction had the lowest flavonoid content, as shown in Table 2.

**Table 1.** The physical appearance, yield percentage, phytochemical contents, antioxidant activity, and inhibitory effects of *Macadamia integrifolia* shell extracts against *Acinetobacter baumannii*

| Sample | Physical appearance | (%Yield) of dry plant | Phytochemical contents | | Antioxidant activities | Inhibitory effect against *Acinetobacter baumannii* |
|---|---|---|---|---|---|---|
| | | | Phenolics (mg GAE/g extract) | Flavonoids (mg RTE/g extract) | $IC_{50}$ values (µg/mL) | Growth inhibition zones diameters (mm.) |
| ME | Dark brown viscous liquid | 1.47 | 15.15±0.50[a] | 21.15±2.3[a] | 124.27±13.14[a] | 12.0±1.0 |
| MD | Dark yellow viscous liquid | 0.78 | 4.27±0.30[b] | 7.93±1.04[b] | 1,035.20±166.55[b] | 10.0±1.0 |
| MA | Dark brown viscous liquid | 0.25 | 26.76±0.63[c] | 29.15 ± 2.70[c] | 54.27±5.40[c] | 7.5±1.0 |
| MW | Brown powder | 0.42 | 18.54±0.74[d] | 38.01±0.94[d] | 168.20±8.27[d] | 7.0±1.0 |
| Ascorbic acid | - | - | - | - | 12.75±0.24[e] | |
| Colistin | | | | | | 18.0±0.0 |
| Dimethyl sulfoxide | | | | | | 0.0±0.0 |

ME: ethanolic crude extract; MD, MA, and MW: dichloromethane, ethyl acetate, and water fractions of *Macadamia integrifolia* shell, respectively. Ascorbic acid was used as a positive control in the DPPH assay, while colistin and dimethyl sulfoxide (DMSO) served as positive and negative controls in the disc diffusion assay. GAE: gallic acid equivalents; RTE: rutin equivalents; $IC_{50}$: half-maximal inhibitory concentration of the extracts. All data are presented as mean ± SD from triplicate determinations, with different superscript letters (a–e) within a column indicating a significant difference ($P < 0.05$).

These findings demonstrate distinct phenolic and flavonoid profiles across the macadamia shell fractions, highlighting their potential as valuable sources of bioactive compounds that could be optimized through the liquid-liquid partitioning technique. Specifically, the ethyl acetate fraction (MA) was identified as the richest source of phenolic compounds, while the water fraction (MW) exhibited the highest flavonoid content.

## Antioxidant activity

The antioxidant activities of *M. integrifolia* shell extracts were expressed as the $IC_{50}$ (µg/mL). Their $IC_{50}$ values ranged from 54.27 µg/mL to 1035.20 µg/mL, as shown in Table 1. The MA extract demonstrated the highest antioxidant activity among the tested extracts, with an $IC_{50}$ value of 54.27 µg/mL, significantly higher than those of ME, MW, and MD ($P < 0.05$). These findings highlight the potent antioxidant activity of the MA fraction and strengthen the importance of phenolic compounds in contributing to the overall antioxidant activity of *M. integrifolia* shell extracts. Moreover, the correlation coefficient values between total phenolic content and total flavonoid content with antioxidant properties were 0.889 and 0.815, respectively, indicating a strong correlation (21) between the phenolic and flavonoid contents of the extracts and their antioxidant potential.

## Inhibitory effect against *Acinetobacter baumannii*

The antimicrobial activity of the fractions of *M. integrifolia* shell extracts was evaluated against *A. baumannii*. *M. integrifolia* extracts exhibited anti-*Acinetobacter baumannii* activity, with inhibition zones ranging from

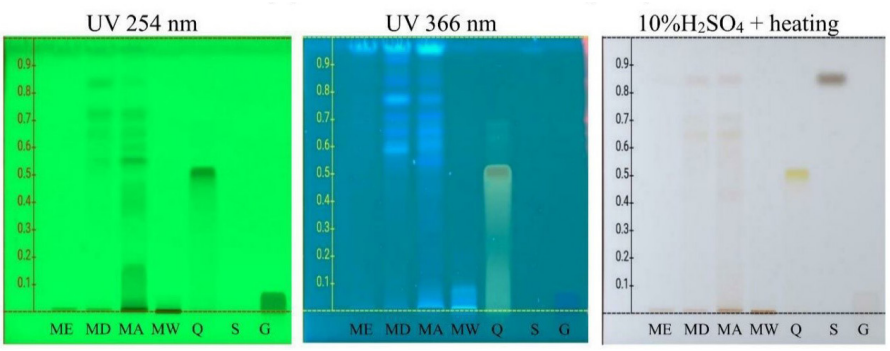


**Figure 3.** Thin-layer chromatography fingerprints of *Macadamia integrifolia* shell extracts. Dichloromethane: methanol at a 90:10 ratio was used as developing solvents. ME: ethanolic crude extract; MD, MA, and MW dichloromethane, ethyl acetate, and water fractions of *Macadamia integrifolia* shell, respectively. Q, S, and G represent quercetin, stigmasterol, and gallic acid, respectively.

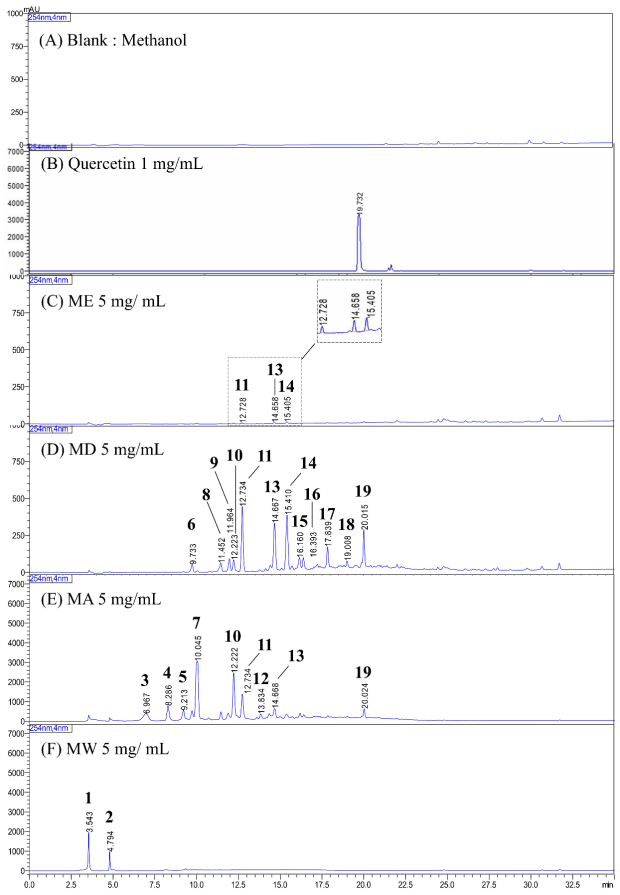


**Figure 4.** The high-performance liquid chromatography chromatograms were observed at 254 nm. ME: the ethanolic crude extract; MD, MA, and MW dichloromethane, ethyl acetate, and water fractions of *Macadamia integrifolia* shell, respectively.

7.0 ± 1.0 to 12.0 ± 1.0 mm. The average diameters of the inhibition zones are presented in Table 1, while Figure S1 displays representative images of the inhibition zones on the agar plate. The ME exhibited the highest inhibition zone among the tested extracts, measuring 12.0 ± 1.0 mm. The MD extract demonstrated slightly lower potency, exhibiting inhibition zones of 10.0 ± 1.0 mm. The MA and MW fractions showed moderate antimicrobial activity, with inhibition zones ranging from 7.0 ± 1.0 mm to 7.5 ± 1.0 mm. These findings suggest that the phytochemicals in *M. integrifolia* shell extracts may serve as key biomarkers for inhibitory activity against *A. baumannii*. Furthermore, the enhanced antimicrobial effect observed in ME indicates a possibility of the synergistic effect of the combined compounds, resulting in greater efficacy.

## Discussion

The macroscopic and microscopic analysis of the macadamia shell raw material in this study revealed the presence of a hard brown shell, a thin creamy-dark brown tissue inside the shell (Figure 1), along with parenchyma cells and underlying reticulate and spiral vessels (Figure 2A-2D), consistent with a previous report on the cross-section of macadamia shells (22).

Ethanol was used as a universal solvent to extract a broad range of phytochemicals from *M. integrifolia* shells. This was followed by liquid-liquid fractionation to separate compounds based on polarity into dichloromethane, ethyl acetate, and aqueous fractions, expecting to obtain nonpolar, moderately polar, and polar compounds, respectively. Several previous studies (23-26) have indicated that polarity-guided fractionation is crucial in bioassay-guided isolation for identifying bioactive constituents that may contribute to antimicrobial activity in this study. Interestingly, the TLC (Figure 3) and HPLC (Figure 4) fingerprints of macadamia shell extracts showed that liquid-liquid partitioning effectively enriched compounds in the ME. Initially, unclear spots in ME were observed due to low compound concentration. However, fractionation resulted in clearer MD, MA, and MW spots. The Rf values of these spots (0.50 for MD, 0.85 for MA, and 0.05 for MW) matched those of quercetin, stigmasterol, and gallic acid, confirming the possibility of the presence of these compounds in the macadamia shell as previously reported (8).

The HPLC chromatogram, Figure 4C, showed that the ME chromatogram had only three peaks. In contrast, multiple peaks with high purity indexes (0.72–0.99, Table 2) were seen in the MD (Figure 4D) and MA (Figure 4E) fractions, with retention times ranging from 9.73–20.01 minutes and 6.96–20.02 minutes, respectively. MW illustrated two peaks at 3.54 and 4.79 minutes, suggesting the presence of more polar compounds. Nineteen peaks (1–19) were analyzed for compound similarity in each fraction. The peaks 11, 13, and 14 in ME were abundant in MD, while 11 and 13 appeared in MA, but 14 was absent. The peaks 3–10, 12, and 15–19, lacking in ME, showed up in MD or MA. Peak 1 (3.54 minutes, purity 0.99) appeared in MW. None matched quercetin's retention time (19.73 minutes).

Although the 19 peaks found in this study's chromatograms are yet to be identified, previous studies using HPLC to analyze bioactive compounds in macadamia by-products have shown the presence of gallic acid, caffeic acid, rutin, catechin, stigmasterol, and other compounds in macadamia flowers, leaves, and husks (4,7,9,23). Previous HPLC analysis with a $C_{30}$ column has also identified gallic acid, caffeic acid, apigenin, quercetin, and rutin in macadamia shell extracts (8). These compounds might also be present in this study's extracts. Further analysis by mass spectrometry may be required. However, TLC and HPLC results confirmed the effectiveness of liquid partitioning in enriching bioactive compounds in the ME.

The solvents used in this study are commonly used in natural product research; however, complete removal is necessary to avoid non-specific antimicrobial effects and ensure the accuracy of bioassay results. In this study, all

**Table 2.** The high-performance liquid chromatography parameters of the extracts from *Macadamia integrifolia* shells

| Fractions (5 mg/mL) | Peak (No.) | Retention time (min) | Area (mAU.s) | Height (mAU) | Purity index |
|---|---|---|---|---|---|
| ME | 11 | 12.72 | 35 036 | 5052 | 0.99 |
| | 13 | 14.65 | 68 881 | 8655 | 0.87 |
| | 14 | 15.40 | 82 437 | 9961 | 0.93 |
| MD | 6 | 9.73 | 384 050 | 50 264 | 0.99 |
| | 8 | 11.45 | 478 928 | 54 608 | 0.03 |
| | 9 | 11.96 | 548 899 | 79 656 | 0.96 |
| | 10 | 12.22 | 480 943 | 71 415 | 0.78 |
| | 11 | 12.73 | 2 925 739 | 420 823 | 0.99 |
| | 13 | 14.66 | 2 362 634 | 302 756 | 0.99 |
| | 14 | 15.41 | 2 952 843 | 362 053 | 0.99 |
| | 15 | 16.16 | 482 520 | 67 742 | 0.94 |
| | 16 | 16.39 | 353 350 | 64 593 | 0.96 |
| | 17 | 17.83 | 823 248 | 131 597 | 0.88 |
| | 18 | 19.00 | 202 934 | 37 383 | 0.97 |
| | 19 | 20.01 | 1 332 787 | 243 993 | 0.95 |
| MA | 3 | 6.96 | 7,653,264 | 347 713 | 0.99 |
| | 4 | 8.28 | 7 237 216 | 755 577 | 0.72 |
| | 5 | 9.21 | 4 009 435 | 468 113 | 0.99 |
| | 7 | 10.04 | 31 327 715 | 2 851 052 | 0.98 |
| | 10 | 12.22 | 16 710 989 | 2 215 973 | 0.85 |
| | 11 | 12.73 | 9,899,984 | 1 247 066 | 0.37 |
| | 12 | 13.83 | 1 496 742 | 224,480 | 0.90 |
| | 13 | 14.66 | 4 234 166 | 473 551 | 0.86 |
| | 19 | 20.02 | 2 015 084 | 408 105 | 0.99 |
| MW | 1 | 3.54 | 8 030 480 | 1 900 327 | 0.99 |
| | 2 | 4.79 | 3 404 643 | 961,006 | -0.23 |
| Quercetin (1 mg/mL) | | 19.73 | 37 442 775 | 3 269 913 | 0.94 |

ME: the ethanolic crude extract; MD, MA, and MW dichloromethane, ethyl acetate, and water fractions of *M. integrifolia* shell, respectively. The high-performance liquid chromatography parameters were obtained from an ultraviolet detector at 254 nm.

extract samples (ME, MD, MA, MW) were concentrated at 50 °C under reduced pressure (~37.5 mm Hg) using a rotary evaporator, eliminating solvent residues. This temperature exceeded the boiling points of the solvents under reduced pressure—ethanol (20 °C at 44.0 mm Hg), dichloromethane (−10 °C at 86.6 mm Hg), ethyl acetate (10 °C at 44.9 mm Hg), and water (40 °C at 55.2 mm Hg) (27) ensuring the reliability and safety of the antimicrobial assay results.

The macadamia shell fractions exhibited varying phytochemical contents (Table 1). The MA fraction had the highest phenolic content, whereas the MW fraction contained the highest flavonoid content. These variations may result from the effects of different solvents on compound extraction (2). This study also revealed a strong positive correlation between phenolic and flavonoid contents in *M. integrifolia* shell extracts (21), with significant antioxidant effects, supported by DPPH assay correlation coefficients of 0.889 and 0.815. This aligns with previous findings that high phenolic and flavonoid levels in macadamia flowers (7) and husks (2) contribute to enhanced antioxidant activity.

All macadamia shell extracts tested in this study exhibited antimicrobial activity against the Gram-negative bacterium *A. baumannii*, aligning with previous research on the antimicrobial properties of macadamia plant parts, including the leaf and flower (11), and kernel (28-30). Compounds like ferulic acid, catechin, phlorizin, quercetin, and kaempferol (7) may contribute to this activity. The ME extract showed comparable antimicrobial effects at the same concentration as colistin (10 mg/mL, 20 µL per disc). In this context, *M. integrifolia* shell crude extracts represented a promising and novel source of alternative bioactive compounds with the potential to combat *A. baumannii* effectively. These findings may be attributed to the disc diffusion assay, where phytochemicals' solubility and diffusion properties in the extract are critical for obtaining a clear inhibition zone (31,32). The diverse phytochemicals in the ME likely diffused more effectively through the agar compared to the non-polar components in other fractions. To address this limitation of the disc diffusion method, a minimum inhibitory concentration assay could be performed further to confirm the antimicrobial activity of *M. integrifolia*

shell extracts.

This study highlights the potential of *M. integrifolia* shells as a source of antioxidant and antimicrobial compounds. As a by-product, macadamia shells should be considered a resource for obtaining active substances for further pharmaceutical, cosmetic, or food development research.

## Conclusion

The extract and fractions (ME, MD, MA, and MW) extracted from *M. integrifolia* Maiden & Betche shells, which are by-products of macadamia nut production, contain high levels of phenolic and flavonoid compounds with antioxidant activities Among the tested extracts, ME showed the most excellent antimicrobial activity. These findings shed light on *M. integrifolia* shells as a promising new natural antioxidant and antimicrobial agent source. Further studies on purification, a minimum inhibitory concentration assay, the underlying mechanisms of antioxidant and antimicrobial activity, and the identification of bioactive markers are recommended.

## Authors' contribution

**Conceptualization:** Supawadee Parhira, Nareeluk Nakaew, and Khemmachat Pansooksan.

**Data curation:** Khemmachat Pansooksan, Supawadee Parhira, and Nareeluk Nakaew.

**Formal analysis:** Khemmachat Pansooksan, Supawadee Parhira, and Nareeluk Nakaew.

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**Project administration:** Supawadee Parhira.

**Resources:** Supawadee Parhira.

**Software:** Supawadee Parhira.

**Supervision:** Supawadee Parhira and Nareeluk Nakaew.

**Validation:** Supawadee Parhira and Nareeluk Nakaew.

**Visualization:** Supawadee Parhira and Nareeluk Nakaew

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## Conflict of interests

All authors declared that they have no conflict of interest.

## Ethical considerations

The microbiological experiments were conducted following approval from the Biosafety Committee of Naresuan University (NUIBC MI 66-04-014).

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## Supplementary file

Supplementary file 1 contains details of the instrument, plant extraction protocol, and the determination of total phenolic and flavonoid contents, as well as Figure S1.

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