IFSCC 2025 full paper (IFSCC2025-498)

"An upcycled green synthetic personal care ingredient with high antioxidant and antimicrobial properties"

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1. Introduction

The cosmetic industry has witnessed a growing trend towards natural, sustainable, and multifunctional ingredients derived from plant sources. In particular, botanical extracts rich in polyphenols and flavonoids are increasingly favored for their skin-beneficial properties, including antioxidant, anti-inflammatory, and antimicrobial activities [1, 2]. Among these, guava (Psidium guajava L.) leaf extract has emerged as a promising candidate due to its high content of bioactive phytochemicals and long-standing use in traditional medicine [3].

Guava leaves are a major agricultural by-product in tropical and subtropical regions. In Taiwan, guava is cultivated across over 7,900 hectares, producing thousands of tons of guava leaves annually [4]. These leaves are often treated as waste and disposed of through burning or burial, leading to not only environmental pollution and greenhouse gas emissions but also the loss of valuable plant-based resources [5]. The valorization of such agro-waste into functional ingredients for cosmetics aligns with global efforts to promote sustainable raw materials and circular economy principles within the personal care industry [6].

Phytochemical analyses of guava leaves have identified several bioactive constituents, such as ellagic acid, gallic acid, and quercetin, known for their strong antioxidant properties that help protect skin cells against oxidative damage caused by UV radiation and pollution [7, 8]. These compounds also demonstrate antimicrobial effects, particularly against Cutibacterium acnes and Staphylococcus aureus, two key pathogens involved in acne and skin irritation [9, 10]. Therefore, guava leaf extract offers dual-functionality as a natural preservative and as an active ingredient for anti-acne and anti-aging formulations.

Traditional extraction methods using heat or organic solvents may degrade thermolabile compounds or raise environmental concerns. In contrast, enzyme-assisted extraction (EAE) has gained attention as a green technology that enhances the recovery and stability of phenolic compounds while maintaining their bioactivity [11]. By breaking down plant cell walls through enzymatic hydrolysis, EAE facilitates the release of bound phenolics under mild conditions, making it ideal for cosmetic applications that require clean-label and eco-friendly processing [12].

In this study, we applied an optimized enzyme-assisted depolymerization process to extract functional compounds from guava leaves. The resulting extracts were analyzed for their phenolic content, antioxidant capacity, and antimicrobial activity against acne-related bacteria. Our goal is to develop a high-performance, natural ingredient suitable for use in cosmetic formulations, offering both efficacy and sustainability.

2. Materials and Methods

2.1. Chemicals and reagents

Guava leaves were obtained from organic guava farms in central and southern Taiwan. ABTS (99%), potassium persulfate (99%), Folin-Ciocalteu reagent (99%), sodium carbonate (Na_2CO_3, 99%), and enzymes were purchased from Sigma-Aldrich and Merck. Standards for HPLC analysis (ellagic acid, gallic acid, and quercetin) were of analytical grade and purchased from Thermo Fisher Scientific.

2.2 Preparation of guava leaf extract

Guava leaves were ground into powder with an average particle size of 0.25 mm using a rotor mill. The guava leaf powder (1 g) was then dissolved in deionized (DI) water to form a suspension, and the pH was adjusted to pH 5 by using citric acid. The suspension was incubated for 1 hour at 45°C in an oven, followed by the addition of 0.1 g of a complex enzyme mixture (CE) containing arabinose, cellulase, β-glucosidase, hemicellulose, and xylanase, or 0.1 g of tannase, or a combination of 0.1 g CE and 0.1 g tannase. Subsequently, 15 mL of ethanol was added, and the mixture was heated at 60°C and sonicated for 1 hour. After the reaction, the solution was filtered using 6 μm and 1 μm filter paper (Whatman) sequentially. The supernatant was then further filtered using a 0.22 μm filter and stored at 4°C for further studies. The extract without enzyme assistance was also processed for comparison.

2.3 Total phenolic content analysis

The total phenolic content was determined using the Folin-Ciocalteu reagent. Standard gallic acid solutions with concentrations of 20 μg/mL, 40 μg/mL, 60 μg/mL, 80 μg/mL, 100 μg/mL, 120 μg/mL, 160 μg/mL, and 200 μg/mL were prepared and mixed with 0.5 N Folin-Ciocalteu reagent and 7.5% Na_2CO_3. The mixtures were transferred to a 96-well plate and measured at 760 nm using an ELISA reader to construct the standard curve [13]. A 0.25 mL aliquot of guava leaf extract was diluted with DI water and mixed with 0.5 N Folin-Ciocalteu reagent and Na_2CO_3 solution. The mixture was incubated for 1 hour in the dark and analyzed using the ELISA reader. The actual total phenolic content was calculated based on the dilution factor.

2.4 Total polysaccharide content analysis

The total polysaccharide content was determined using the 3,5-dinitrosalicylic acid (DNS) method. First, 1 mL of the sample solution was mixed with 1 mL of DNS reagent, forming the reaction mixture. The reaction mixture was then heated at 100°C for 10 minutes to promote the reaction. After the reaction, the mixture was allowed to cool to room temperature and further chilled in an ice bath. Next, 3 mL of distilled water was added to dilute the cooled reaction mixture, and the absorbance was measured at 540 nm using a spectrophotometer. The total polysaccharide content in the sample was calculated by comparing the absorbance values to the corresponding standard curve equation.

2.5 HPLC Analysis

Phenolic compounds in guava leaf extracts were analyzed using high-performance liquid chromatography (HPLC) with UV detection. Separation was performed on a C18 reversed-phase column using a gradient elution of methanol and 0.1% formic acid in water. The flow rate was set at 1.0 mL/min, and detection wavelengths were 270 nm for gallic acid, 254 nm for

ellagic acid, and 370 nm for quercetin. Identification and quantification were carried out by comparing retention times and UV spectra with those of standard compounds.

2.6 Antioxidation activity.

The antioxidant capacity of the sample was evaluated using the ABTS free radical scavenging assay[14]. In this method, ABTS and potassium persulfate react to form a stable ABTS•$^{+}$ radical ion, which has an absorption peak at 734 nm. The concentration of ABTS•$^{+}$ is measured by the absorbance at 734 nm. When an antioxidant is added, it neutralizes the radicals, causing the solution to fade and the absorption value to decrease, which indicates the scavenging ability of the antioxidant.

2.7 Antimicrobial susceptibility test

The antibiotic activity against Cutibacterium acnes (formerly Propionibacterium acnes) and Staphylococcus aureus strains was tested using the minimum inhibitory concentration (MIC) method [15, 16] with minor modifications. The two-fold dilution method was employed to determine the MIC of guava leaf extract against these two bacterial strains. The guava leaf extract was serially diluted in 96-well plates, and the bacterial suspension was added to achieve final concentrations of the extract at 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, and 0.98 μg/mL. The final inoculum concentration of the bacterial suspension was 1.0×10^5 CFU/mL. Erythromycin, diluted in 0.85% NaCl, was used as a positive control. The plates were incubated anaerobically for 20 hours at 37°C in a permanent incubator. The lowest concentration showing no visible growth was recorded as the MIC.

3. Results

3.1 Total Phenolic Cotent and total polysaccharide content

The total phenolic content of guava leaf extract in different enzyme-assisted systems is shown in Table 1. Sample OGU1C-A was extracted without any enzyme assistance. Samples OGU1C-B and OGU1C-C were extracted using a complex enzyme (CE) and tannase, respectively. OGU1C-D was extracted using a mixture of CE and tannase. The total phenolic content of the extract was higher when CE or the CE and tannase mixture was used for extraction. Extracts processed without enzyme assistance or with only tannase showed a lower phenolic content. The total polysaccharide content of all extracts was also analyzed. enzyme-assisted extracts exhibited higher total polysaccharide content, particularly the CE and tannase mixture, which had a total polysaccharide content as high as 11.84 mg/mL, more than twice the amount found in the extract without enzyme assistance.

Table 1. The total phenolic and total polysaccharide contents of guava leaf extracts.

Guava Leaf Extracts	Enzyme system	TPC(mg GAE /100g leaves)	Total polysaccharide content (mg/mL)
OGU1C-A	-	9686.4	4.98±0.53
OGU1C-B	CE	9728.4	6.56±0.17
OGU1C-C	Tannase	8700.4	10.65±0.04
OGU1C-D	CE +Tannase	11192.3	11.84±0.17

3.2 Phenolic Compounds analysis

The individual phenolic compound content in guava leaf extracts obtained through different extraction processes was analyzed using High Performance Liquid Chromatography (HPLC), as shown in Table 2 and Figure 1. Among the identified phenolics, the contents of ellagic acid, gallic acid, and quercetin significantly increased with enzyme-assisted extraction. Specifically, compared to the control (non-enzyme-assisted extract), the contents of ellagic

acid, gallic acid, and quercetin increased by 1.37-fold, 6.28-fold, and 5.12-fold, respectively. These results suggest that enzymatic treatment facilitates the release of bound or conjugated phenolic compounds, enhancing the functional quality of the extract.

Table 2. Phenolic compounds content of different extract process.

Guava Leaf Extracts Process	Phenolic compounds content (%)		
	Ellagic acid	Gallic acid	Quercetin
No enzyme-assisted	0.727	0.388	0.042
Enzyme-assisted	0.994	2.438	0.215

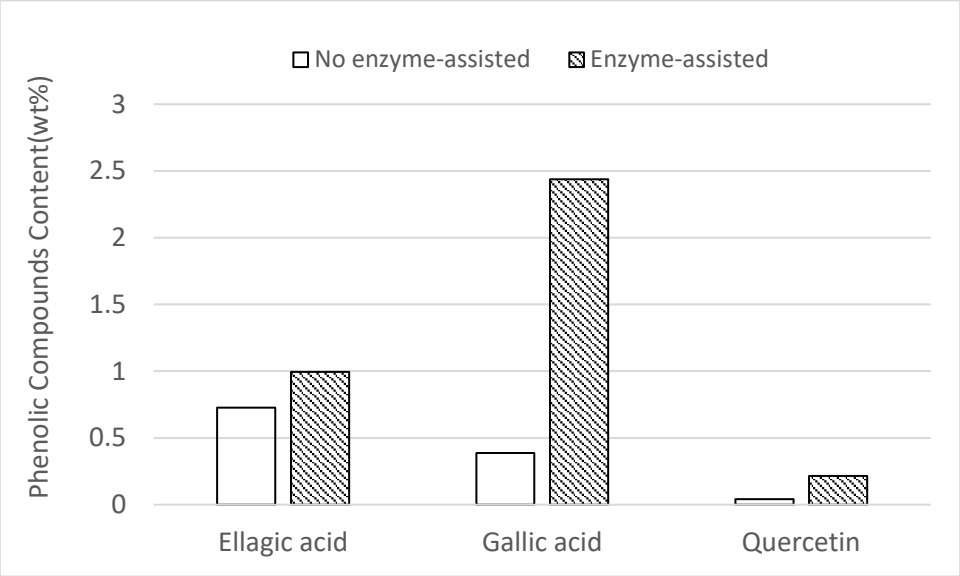

Figure 1. Individal phenolic compound contents extracted with enzyme assisted(OGU1C-D) and no enzyme assisted analysis by High Performance Liquid Chromatography(HPLC).

3.2 Antioxidant Activity

Antioxidant activities of the guava leaf extracts from different enzyme-assisted systems were analyzed by measuring ABTS•⁺ radical scavenging activities. The ABTS assay is commonly used to evaluate the total antioxidant capacity of various biological samples by measuring radical scavenging through electron donation [17]. All extracts exhibited very high antioxidant activity even at very low concentrations.

At an extract concentration of 0.2%, the ABTS•⁺radical scavenging activity of enzyme-assisted extracts reached 100%. In contrast, the extract without enzyme assistance showed an ABTS•⁺radical scavenging activity of 99.8%. The results are shown in Table 3. Enzyme-assisted extracts(OGU1C-B, OGU1C-C, OGU1C-D) exhibited 100% radical scavenging activity at an extract concentration of 0.2%. The antioxidant activity at different extract concentrations is also analyzed (Figure 2). The enzyme-assisted extract OGU1C-9 scavenged ABTS•⁺radicals in a concentration-dependent manner, with 96.3% scavenging activity at an extract concentration of just 0.1%.

Table 3. ABTS radical scavenging activity.

Guava Leaf Extracts	Concentration (%)	Inhibition activity
OGU1C-A	0.2	99.8%±0.10
OGU1C-B	0.2	100%±0.10
OGU1C-C	0.2	100%±0.20
OGU1C-D	0.001	96.3%±0.17
OGU1C-D	0.2	100%± 0.10
OGU1C-D	0.5	100%±0.20
CON	0.05	100%±0.00

1)All measurements were done in triplicate; All values are mean±SD

2)Asorbic acid is uesed as positive control

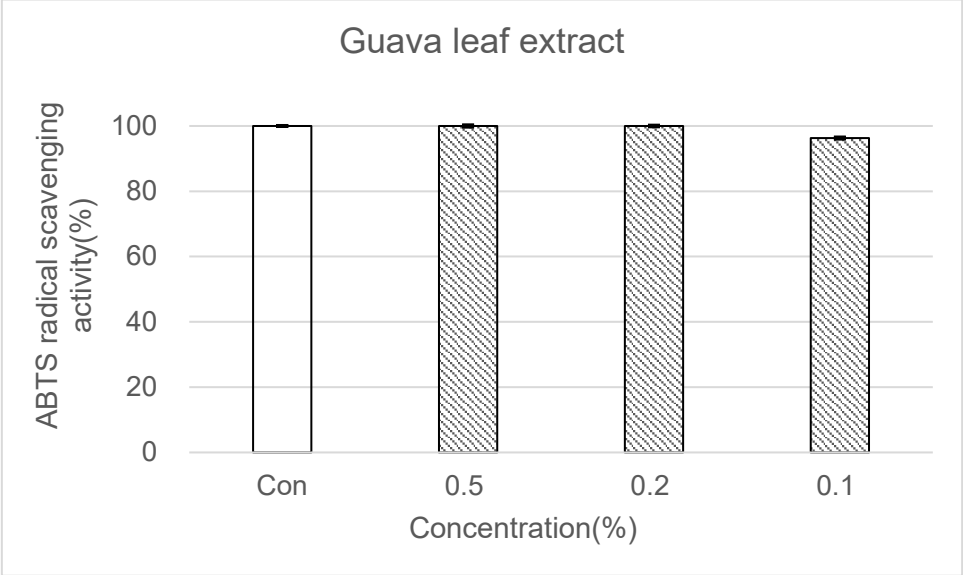

Figure 2. ABTS radical scavenging activity of guava leaf extract with enzyme assisted(OGU1C-D)

3.3 Antimicrobial susceptibility

The minimum inhibitory concentrations (MICs) of guava leaf extract against Cutibacterium acnes (C. acnes) and Staphylococcus aureus (S. aureus) are presented in Table 4 and Table 5. The results showed that various dilutions of the enzyme-assisted guava leaf extract (OGU1C-D) effectively inhibited both bacterial strains. The guava leaf extract demonstrated antimicrobial activity against both C. acnes and S. aureus, with MIC values of 31.3 mg/mL and 62.5 mg/mL, respectively.

These findings suggest that the guava leaf extract is more effective against C. acnes, as it requires a lower concentration for inhibition compared to S. aureus. This highlights the potential of guava leaf extract as an antimicrobial agent, particularly for targeting C. acnes at lower concentrations.

Table 4. The MIC of Guava leaf extract against four C. acnes strains.

C. acnes	Concentration (μg/mL)									
	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.98
Extracts	+	+	+	+	+	-	-	-	-	-
Control	+	+	+	+	+	+	+	+	-	-

[1)] +, with antibacterial activity, -, without antibacterial activity

[2)] Control, Erythromy

Table 5. The MIC of Guava leaf extract against four S. acnes strains.

S. aureus	Concentration (μg/mL)									
	500	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0.98
Extracts	+	+	+	+	-	-	-	-	-	-
Control	+	+	+	+	+	+	+	+	-	-

[1)] +, with antibacterial activity, -, without antibacterial activity

[2)] Control, Erythromy

4. Discussion

The results of this study demonstrate that enzyme-assisted extraction significantly enhances the bioactive compound yield and biological activity of guava leaf extracts. In particular, the use of a complex enzyme (CE) and tannase, either alone or in combination, was found to be effective in increasing both total phenolic content (TPC) and total polysaccharide content, consistent with previous reports on the use of enzymatic treatments in plant extraction processes [18,19].

The highest TPC was observed in the extract obtained with the CE and tannase combination (OGU1C-D), reaching 11,192.3 mg GAE/100 g leaves, which was notably higher than that of the control extract without enzymes (OGU1C-A). This suggests a synergistic effect between the enzymes in breaking down plant cell walls, allowing for the release of bound phenolic compounds [20]. Interestingly, although the extract with only tannase (OGU1C-C) had a relatively lower TPC, it exhibited a much higher polysaccharide content than the control, implying that tannase may also act on glycosidic bonds to release carbohydrate fractions [21].

In terms of antioxidant activity, all extracts exhibited strong ABTS radical scavenging capacity, with enzyme-assisted extracts showing complete (100%) inhibition at 0.2% concentration. Even at a lower concentration (0.1%), OGU1C-D maintained a high inhibition rate of 96.3%, comparable to that of vitamin C, a known antioxidant. These findings align with previous studies indicating that higher phenolic content often correlates with stronger antioxidant activity in plant-based extracts [22, 23]. The antioxidant properties of these extracts are of particular interest for their potential applications in protecting skin cells from oxidative stress, which is a major factor in skin aging and inflammatory skin conditions.

Moreover, the antimicrobial activity of guava leaf extracts was evaluated with a focus on their potential applications in cosmetic formulations, particularly for treating acne vulgaris. The enzyme-assisted extract (OGU1C-D) demonstrated stronger antimicrobial activity against Cutibacterium acnes, a bacterium strongly implicated in the development of acne, with a MIC value of 31.3 mg/mL. This is significant because C. acnes is a common target for acne treatment products [24]. Additionally, the extract exhibited moderate antibacterial activity against

Staphylococcus aureus (MIC = 62.5 mg/mL), a pathogen often associated with skin infections, although the effects were less pronounced compared to C. acnes.

Taken together, these findings highlight the advantage of enzyme-assisted extraction, particularly using a combination of CE and tannase, in improving the functional properties of guava leaf extracts. The enhancement in both antioxidant and antimicrobial activities correlates strongly with the increased levels of phenolics and polysaccharides, supporting the potential use of such extracts in natural cosmetic formulations, particularly for acne treatment. Moreover, as consumers increasingly seek natural alternatives, guava leaf extract could offer a sustainable and effective option for cosmetic companies looking to formulate anti-acne skincare products with natural antimicrobial agents.

5. Conclusion

Enzyme-assisted depolymerization and extraction techniques have demonstrated a remarkable ability to enhance the release, preservation, and bioavailability of bioactive compounds from plant materials. In this study, we successfully developed an optimized enzyme formulation capable of effectively breaking down the cellular structure of guava leaves. By targeting cell wall components and facilitating the cleavage of chemical bonds, this enzyme-assisted extraction process significantly improved extraction efficiency, reduced precipitation, and increased the concentration of key phytochemicals, including ellagic acid, gallic acid, and quercetin. This study demonstrates that enzyme-assisted extraction is a powerful tool for unlocking the therapeutic potential of guava leaves, offering a sustainable and efficient approach for producing high-value bioactive compounds with potential applications in skincare products.

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