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BIOACTIVITY OF ETHANOL EXTRACTS AND LIPOSOMAL FORMULATIONS FROM *PSIDIUM GUAJAVA* LEAVES: ANTIOXIDANT, ANTIBACTERIAL, AND α-GLUCOSIDASE INHIBITION EFFECTS

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Abstract: This study investigated polyphenol extraction from guava (*Psidium guajava*) leaves using ethanol and various organic solvents. The resulting extracts exhibited significant antioxidant, antibacterial, and α-glucosidase inhibitory activities. The EtOAc fraction demonstrated the strongest antioxidant activity (IC50 55.61 µg/mL), comparable to vitamin C (IC_{50} 65.09 µg/mL). The hexane fraction exhibited the most potent antibacterial activity (MIC 1000 µg/mL against *P. aeruginosa* and 1400 µg/mL against S. aureus). The EtOAc fraction extract showed potent α-glucosidase inhibition (IC_{50} 2.98 µg/mL), 28.16 times lower than acarbose (IC_{50} 83.91 µg/mL). Notably, nanoliposome system encapsulating the ethanol extract was successfully prepared (average particle size 159 nm, PDI 0.384, zeta potential -11.5 mV), displaying significantly enhanced antioxidant activity compared to the unencapsulated extract. These findings suggest considerable promising food and pharmaceutical applications of guava leaf extract, particularly the EtOAc fraction and its nanoliposomal formulation.

Keywords: guava leaves, polyphenols, α-glucosidase, flavonoids, quercetin, nano liposome

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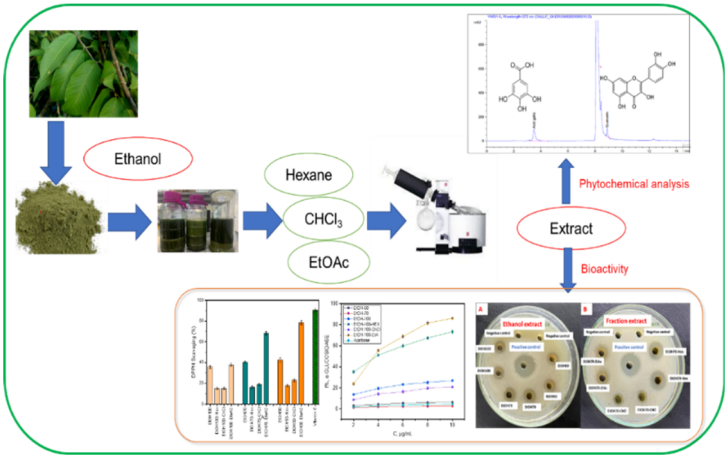
Graphical Abstract



Introduction

The guava tree (*Psidium guajava*) originates from Central and South America and is widely distributed across tropical and subtropical regions worldwide. In Vietnam, guava trees are cultivated throughout the country, from coastal plains to mountainous regions with elevations below 1500 meters. Nowadays, besides the utilization of guava fruit as a food source, numerous scientific studies have been published regarding the chemical composition of guava leaves. The leaves of *P. guajava* contain many bioactive compounds, boasting significant quantities of soluble polyphenolics.[1] Among these are gallic acid, catechin, epicatechin, rutin, and quercetin.[2,3] Additionally, the leaf oil of *P. guajava* presents a complex blend of sesquiterpene hydrocarbons and oxygenated sesquiterpenes, with notable constituents including β-caryophyllene, selin-11-en-4α-ol, and α-cadinol.[3–5] Furthermore, upon examining the guava leaf hexane fraction, researchers identified an array of compounds, including β-eudesmol, α-copaene, and phytol, among others.[6] Besides, there are many essential oil components such as β-sitosterol, guajavalic acid, cosalpha-limonene, and maslinic acid.[3–5] The

isolation process yielded flavonoidal compounds from *P. guajava* leaves, among which quercetin and its derivatives were prominent.[3] Additionally, the discovery of four new triterpenoids alongside thirteen known ones, in the leaves further emphasizes their pharmacological potential. Noteworthy among these are compounds like 2α, 3β-dihydroxy-taraxer-20-en-28-oic acid.[7]

Moreover, recent studies have focused on exploring the biological activity of guava leaf extracts. Szu et al., investigated the effects of aqueous and ethanol extracts of guava leaves on blood sugar and glucose metabolism in type 2 diabetic rats. Both extracts significantly reduced blood sugar levels and increased plasma insulin levels. Additionally, the aqueous extract was found to enhance the activity of hepatic enzymes related to glucose metabolism more effectively than the ethanol extract.[8] Some authors have also demonstrated that guava extract exhibits antimicrobial activity against various pathogenic microorganisms in experimental trials, such as *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus* and *Bacillus cereus.*[4,9] Multiple studies have shown that both quercetin and vitamin C in guava leaves are potent antioxidants, inhibiting the development of cancer-induced tumors. Antioxidants can regulate body weight and have biological value in treating conditions such as diabetes, dyslipidemia, hypertension, and other cardiovascular risks.[10–14]

Research into the composition and effects of guava leaf extracts has been undertaken by various research groups. However, to the best of our knowledge, up to now, the publication of studies assessing the biological activity of individual organic fractions during the liquid-liquid extraction process has been limited. Therefore, this study aims to comprehensively evaluate the qualitative and quantitative chemical constituents found in both

total ethanol extract and extracts derived from different organic fractions, as well as their antibacterial and antioxidant properties and their ability to inhibit the α-glucosidase enzyme. Through this work, we seek to gain a comprehensive understanding of the potential applications of each segmented extract in the treatment of bacterial infections and diabetes. Additionally, this research also provides reliable scientific data to offer readers valuable insights into the utilization of guava leaf products originating from Vietnam.

Materials and methods

Chemicals

All reagents utilized in the experiment were of analytical grade. Dimethyl sulfoxide (DMSO), gallic acid standard (1000 µg/mL), 1,1-diphenyl-2-picrylhydrazine (DPPH), and α-glucosidase solution (0.13 UI/mL), as well as the Folin-Ciocalteau reagent, TSA plates, TSB medium, resazurin (0.01 M), and p-nitrophenyl-α-D-glucopyranoside (p-NPG) solution (2.5 mM), were purchased from Sigma-Aldrich (Germany). Solvents ethanol 96% (EtOH100), hexane (Hex), chloroform (ChCl), ethyl acetate (EtOAc), and methanol (MeOH) were purchased from Xilong (China).

Sample extraction

The guava leaves were harvested in Binh Chanh district, Ho Chi Minh City, Vietnam. Next, they were dried under natural sunlight until the moisture content reached about 10%. After that, they were finely ground to obtain a sample with a uniform size, which was then sealed in PE bags before proceeding to the next steps. The extraction process is described in Figure 1.

For the ethanol extraction process, 10.0 grams of guava leaf powder was placed into a 250-mL heat-resistant glass beaker. Then, 100 mL of 96% ethanol (EtOH100) solvent was added to the beaker. The mixture was soaked

for 72 hours, then centrifuged, and the supernatant was decanted. The extract was evaporated using a Yamato RE301 rotary evaporator (Japan) at 60 °C, a vacuum pressure of 1000 Pa for 2 hours under vacuum to obtain the ethanol dried extract. The experiment was conducted sequentially with ethanol: water (7:3) (EtOH70) and ethanol: water (5:5) (EtOH50).

For the fractionation extraction process, the ethanol extract was diluted with methanol at a ratio of 1:10 by weight. Subsequently, liquid-liquid extraction was performed with solvents of increasing polarity: n-hexane, chloroform, and ethyl acetate. The fractions obtained were evaporated to remove the solvents at 47 ºC under low pressure using a rotary evaporator. The extracts were then dried at 47 ºC, allowed to cool, and weighed to determine their masses. The corresponding sample identifiers were EtOH100-Hex, EtOH100-ChCl, EtOH100-EtOA, EtOH70-Hex, EtOH70-ChCl, EtOH70-EtOA, EtOH50-Hex, EtOH50-ChCl, and EtOH50-EtOA. All experiments were repeated three times to calculate the average value and the standard deviation (SD).

Preparation of nano liposomes loaded with extract

Nano liposomes containing EtOH70 extract were prepared using the solvent injection method, as described in a previous report with minor modifications.[15] The preparation process was as follows: 5.0 g of lecithin was dissolved in 20 mL of dichloromethane to create a solution. Subsequently, 5.0 mL of this solution was gradually injected dropwise into 20 mL of solution content 1.0 g EtOH70 dried extract, which was combined with 2.5 mL of Tween 80, while stirring at 500 rpm with a magnetic stirrer. The liposome suspension was then heated on the stirring device to evaporate the dichloromethane at 50 °C for 30 minutes. Following this, the system was subjected to ultrasound for 10 minutes at 60% amplitude using an ultrasonic

probe (VCX750, Sonic, USA) to produce the nano liposomes containing the extract. The average droplet diameter (Z-average) and the Zeta potential of the nano liposome system were measured using the Dynamic Light Scattering method (HORIBA SZ-100, Japan).

Phytochemical qualification

The qualitative chemical composition of guava leaf extracts, including polyphenols, flavonoids, alkaloids, terpenoids, and tannins, was determined through methods for screening compound groups, following the protocol outlined by Hazmi et al.[16] Specifically, the $FeCl_3$ screening analysis was conducted to detect phenolic in the guava leaf extracts. This involved placing 2 mL of extracts in test tubes and boiling for 2 minutes. Subsequently, after cooling to 25 °C, 5 drops of 0.5 M $FeCl_3$ were added. The appearance of a dark green coloration confirmed the presence of phenolic compounds.

A 10% sodium hydroxide (NaOH) test was conducted to indicate the presence of flavonoid compounds, as described in a previous work.[17] In this test, 3 mL of the extract were added to test tubes containing 10 mL of distilled water. Then, 1 mL of NaOH solution (10% w/v) was added to the resulting solution and vortexed. The presence of flavonoid compounds was confirmed by the yellow color.

Terpenoids were assessed using Salkowski's test.[18] The extract was combined with 2 mL of chloroform, followed by the careful addition of 2 mL of concentrated sulfuric acid, and gently shaken. The formation of a reddish-brown coloration in the interphase indicated the presence of terpenoids.

To detect tannins in the sample, a qualitative gelatin-salt test was performed. A 1.0 mL of 1.0% gelatin solution containing 10% NaCl was added to 2 mL of the sample solution. The formation of a white or cloudy

precipitate indicated the presence of tannins. To test for alkaloids, 2 mL of extract solution were treated with 1 mL of Wagner's reagent. A reddish-brown precipitate indicated the presence of alkaloids[19]

To test for glycosides, the extract was mixed with 2 mL of glacial acetic acid containing 2 drops of 2% $FeCl_3$. The resulting mixture was then poured into another tube containing 2 mL of concentrated sulfuric acid. A brown ring observed at the interphase indicates the presence of glycosides.

HPLC-UV analysis

Furthermore, the HPLC-UV method was also applied to analyze the total content of gallic acid and quercetin. The extracted sample (0.5 mL) was centrifuged (3 minutes, 15000 rpm) to remove solid particles. Subsequently, the sample was diluted five times before analysis. Quercetin and gallic acid were determined by liquid chromatography (HPLC) coupled with a UV detector (1260 Infinity, Agilent, USA), at a wavelength of 272 nm. The stationary phase was a Zorbaz Eclipse C18 analysis column (4.6 × 100 mm, 3.5 μm, Agilent, USA). The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), each containing 0.1% formic acid. The flow rate was set at 1 mL/min, and the gradient mode was programmed as follows: 0 - 4 min, 5 - 15% B; 4 - 7 min, 15 - 55% B; 7 - 9 min, 55 - 90% B; 9 - 12 min, 90% B; 13 - 18 min, 5% B.

Total phenolic compound quantitation

For the quantification of total phenols, we used 10% (v/v) Folin-Ciocalteu reagent, which was used as follows: take 0.5 ml of extract solution and mix well with 2.5 ml of the Folin-Ciocalteu reagent in distilled water. This mixture is allowed to stabilize for 5 minutes. Subsequently, add 1.25 ml of a 10% (w/v) Na_2CO_3 solution in distilled water to the mixture and incubate it in the dark at room temperature for 1 hour. The optical absorbance of the

reaction mixture is measured at a wavelength of 765 nm using a UV-Vis spectrophotometer. Gallic acid is utilized as the standard compound to construct the calibration curve for calculating the total phenolic content. The final results are expressed as mg of gallic acid equivalents per gram of dry sample (mg GAE/g dry sample) as shown in Equation (1):

$$\mathrm{TPC(mgGAE/g\ dry\ sample)} = C \times k \times \frac{V}{m} \quad (1)$$

Where, C is the concentration of gallic acid equivalents (GAE) from the calibration curve (mg/L); k is the dilution factor applied to the sample; V is the total volume of the extract in which the sample is dissolved (L) and m is the weight of the sample used for extraction (g).

DPPH assays

DPPH, a purple-colored free radical, exhibits maximum absorption at a wavelength of 517 nm. The DPPH free radicals are neutralized by samples possessing antioxidant properties, resulting in the formation of the 2,2-diphenyl-1-picrylhydrazine compound (DPPH-H), characterized by its pale-yellow color. This reaction induces a transition in the solution from purple to pale yellow, leading to a reduction in optical absorption at 517 nm.[20–23] Briefly, the reaction mixture was composed of 2800 µL of DPPH (OD ≈ 1) and 200 µL of high extract sample (or Vitamin C), yielding final concentrations of 20, 40, 60, 80, and 100 µg/mL for the extract (or Vitamin C) in each reaction. Vitamin C was employed as a positive control, whereas 96% ethanol was utilized as a negative control. Each experiment was conducted in triplicate. The mixture was then incubated for 30 minutes in the dark, after which the optical absorbance was measured at 517 nm using a Lambda 25 UV-visible recording spectrophotometer (USA). The antioxidant

capacity was determined based on the %I inactivated percentage by Eq. (1), and the half-maximal inhibitory concentration (IC_{50}) values.

$$\%I = \frac{A_0 - A_{sample}}{A_0}.100(\%) \quad (1)$$

where A_0 and A_{sample} are the absorbance of the DPPH standard and the sample, respectively.

Antibacterial test

The antibacterial activity of the guava leaf yield extract was assessed using the disc diffusion method to measure inhibition zones against two strains of bacteria, *Pseudomonas aeruginosa ATCC 27853 (P. aeruginosa*) and *Staphylococcus aureus ATCC 25923 (S. aureus)*. The procedure began with the sterilization of all experimental instruments at 121 °C and 1 atm pressure for 15 minutes. Cultures of both bacterial strains, *S. aureus* and *P. aeruginosa*, were prepared, grown, and diluted in a non-specific medium (Tryptic Soy Broth - TSB) to achieve a density of approximately 10^8 CFU/mL. Subsequently, 100 µL of bacterial suspension was evenly spread onto Tryptic Soy Agar (TSA) plates, and nine wells, each with a diameter of 6 mm, were created evenly spaced on the agar surface. Next, 50 µL of guava leaf extract samples at a concentration of 15,000 µg/mL in 96% DMSO solvent were added to the wells on the agar plates. Negative control samples consisted of 10 µL of pure DMSO solvent, while positive control samples contained 10 µL of chloramphenicol antibiotic at a concentration of 1000 µg/mL, both used for comparison. The plates were then incubated at 37°C for 24 hours. Each experiment was replicated three times. The antibacterial activity of the plant extract was determined by measuring the diameter of the inhibition zones (DIZ) formed around the wells on the agar plates.

The minimum inhibitory concentration (MIC) was determined through the color change of the resazurin indicator.[24] The experiment was conducted in 96-well plates, with each well containing a mixture of plant extract, Tryptic Soy Broth (TSB) medium, and bacterial suspension. Each well was loaded with a mixture consisting of 200 µL of plant extract at various concentrations: 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, and 2000 µg/mL, along with 10 µL of bacterial suspension at a density of 10^6 bacteria/mL. The negative control consisted of 100% DMSO solvent, while the positive control was chloramphenicol antibiotic at a concentration of 1000 µg/mL. All experimental wells were incubated at 37 °C. After 16 – 18 hours, 10 µL of 0.01% resazurin test solution was added to each well. The lowest concentration of plant extract at which the resazurin test solution remained blue was recorded as the minimum inhibitory concentration (MIC). Each experiment was repeated three times.

Inhibition enzyme *α*-glucosidase test

The α-glucosidase enzyme inhibition activity was conducted following a previously published procedure with minor adjustments to suit experimental conditions.[25–27] The summary is as follows: 200 µL of the sample, with final concentrations of 2, 4, 6, 8, and 10 µg/mL, respectively, were added to 40 µL of α-glucosidase enzyme solution (0.13 UI/mL). The mixture was thoroughly mixed and incubated at 30 °C for 20 minutes. Subsequently, 40 µL of the substrate p-nitrophenyl-β-D-glucopyranoside (p-NPG) was added, and thoroughly mixed, and the reaction was incubated at 30°C for 17 minutes. Finally, 70 µL of Na_2CO_3 solution (0.2M) was added to stop the reaction and develop color from the product, p-nitrophenol. Acarbose was used as the positive control, while 96% ethanol was used as the negative control. Each experiment was repeated three times. After the reaction was completed, the

absorbance was measured at 405 nm using a Lambda 25 UV-visible recording spectrophotometer (USA). The α-glucosidase enzyme inhibition capability was calculated based on the percentage of α-glucosidase enzyme inhibition and the IC_{50} value.

Results and discussion

Guava leaves were dried, then finely ground and soaked in ethanol for 72 hours. After extraction, the solution was filtered and centrifuged to obtain the extract. Subsequently, the extract was vacuum evaporated to yield the concentrated extract. To perform fractionation, the concentrated extract was diluted with methanol at a ratio of 1:10 by weight, then sequentially extracted with hexane, chloroform, and ethyl acetate.

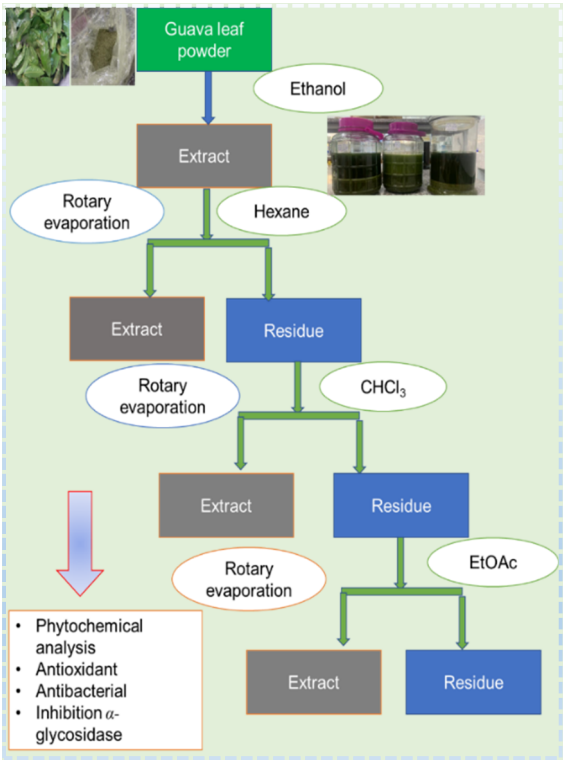


Figure 1. Schematic diagram of extraction.

Fractionated extracts were obtained by vacuum evaporation of the organic phase. Afterwards, the qualitative composition of the ethanol extract was determined. Additionally, the total phenol content in the extracts, as well as the content of quercetin and gallic acid in the ethanol extract, were quantified. Lastly, biological activities such as DPPH free radical scavenging, antibacterial, and α-glucosidase enzyme inhibition were evaluated on each extract obtained from this extraction process.

Mass of extract

Table 1 demonstrates that the EtOH50 solvent system exhibited the highest efficiency, followed by EtOH70, while the lowest was observed in EtOH100. However, the mass obtained during organic fractionation revealed that the EtOH100 solvent yielded the highest value. Precisely, the highest content of the extract in the EtOAc fraction reached 2.17 ± 0.15, followed by the $CHCl_3$ fraction, with the lowest in the hexane fraction. The content of the solvent-extracted and fractionated extracts depends on the polarity of the compounds present in the raw material. This is because different solvents have varying affinities for different types of compounds based on their polarity. Polar compounds tend to dissolve better in polar solvents, while non-polar compounds are more soluble in non-polar solvents. Therefore, the choice of solvent and the fractionation process play a crucial role in extracting specific compounds from the original material.

Table 1. The efficiency extraction (EE%) in various ethanol solvents and their fractions.

Samples	Mass of extract (g/100 g of dried sample)
EtOH100	11.31± 1.24
EtOH100-Hex	1.16 ± 0.03
EtOH100-ChCl	1.28± 0.14
EtOH100-EtOAc	2.17± 0.15
EtOH70	13.56 ± 2.38
EtOH70-Hex	1.07± 0.06
EtOH70-ChCl	1.28± 0.17
EtOH70-EtOAc	1.60 ± 0.19
EtOH50	13.67± 1.24
EtOH50-Hex	1.53± 0.09
EtOH50-ChCl	0.53± 0.02
EtOH50-EtOAc	0.73± 0.05

Phytochemical qualification analysis

Table 2 shows that the qualitative phytochemical screening of different extracts from the formulation confirmed the presence of alkaloids, phenols, tannins, flavonoids, and terpenoids. The analysis of the plant extracts revealed the presence of phytochemicals renowned for their medicinal and physiological attributes. Tannins, for instance, are polyphenolic compounds that bind to proline-rich proteins, disrupting protein synthesis, and have demonstrated antibacterial effects.[9,28,29] Flavonoids, hydroxylated polyphenolic compounds, are synthesized by plants in response to microbial infections, and extensive studies have showcased their antimicrobial activity against various microorganisms in vitro.[30] This antimicrobial efficacy is

ascribed to their ability to form complexes with extracellular and soluble proteins as well as bacterial cell walls.[31] Terpenoids, primarily valued for their aromatic qualities, have also exhibited potential as antibacterial agents.[32] Saponins, and glycosides, have been identified to inhibit gram-positive organisms such as *S. aureus*. Hence, the phytochemical analysis suggests that the ethanol extracts harbor chemical compounds acknowledged for their antibacterial properties, which could contribute to explaining the observed antibacterial outcomes. Moreover, Polyphenols, including compounds like quercetin and catechins, are potent antioxidants that scavenge free radicals, thereby reducing oxidative stress in the body. Flavonoids, such as kaempferol and rutin, also possess strong antioxidant activity, helping to protect cells from damage caused by reactive oxygen species.[34]

Table 2. Phytochemical test results.

Phytochemicals	Sample extracts		
	EtOH100	**EtOH70**	**EtOH50**
Polyphenol	+	+	+
Flavonoid	+	+	+
	+	+	+
Tannin	+	+	+
Alkaloids	+	+	-
Terpenoids	+	-	-

Note: +: presence of constituent (positive); −: absence of constituent (negative).

Total phenolic content

From the results in Table 3, it can be seen that the EtOH70 solvent system yielded the highest total polyphenol content, followed by EtOH50, and the lowest was observed for EtOH100. Meanwhile, in the organic solvent fractions, the Hex fraction was found to exhibit the lowest efficiency.

Conversely, the highest total polyphenol content was noted in the EtOAc fraction, followed by $CHCl_3$. The reason could be attributed to the polar nature of polyphenolic compounds, thus, the extraction efficiency depended on the polarity of the solvent. Specifically, the polarity index of EtOAc, $CHCl_3$, and Hex solvents was 4.3, 4.1, and 0.1, respectively.[35] Therefore, using solvents with moderate polarity during the extraction process, such as in the case of EtOAc, facilitated the optimal extraction of polyphenols from guava leaf extracts.

Table 3. TPC content in extract.

Samples	**TPC content in extract (mg GAE /g)**
EtOH100	19.77 ± 1.23
EtOH100-Hex	3.74 ± 0.62
EtOH100-ChCl	6.53 ± 034
EtOH100-EtOAc	41.22 ± 0.69
EtOH70	24.64± 2.17
EtOH70-Hex	0.79± 0.02
EtOH70-ChCl	4.91± 0.14
EtOH70-EtOAc	20.11± 0.32
EtOH50	21.80± 1.12
EtOH50-Hex	1.33± 0.14
EtOH50-ChCl	10.81± 0.34
EtOH50-EtOAc	15.02± 0.69

Table 4. The content of gallic acid and quercetin in the extract was determined by HPLC-UV analysis.

Samples	Gallic acid, mg/g	Quercetin, mg/g
EtOH100	12.63 ± 0.92	15.15 ± 0.32
EtOH70	13.58 ± 0.35	11.91 ± 0.16
EtOH50	13.15 ± 0.24	13.54 ± 0.53

From the results in Table 4, it is evident that the chemical characterization method has revealed that both the guava leaf extract and its organic fractions contain various groups of naturally occurring compounds with biological activity, such as polyphenols, flavonoids, and tannins. Furthermore, the levels of gallic acid and quercetin have also been quantitatively analyzed using the HPLC-UV method. The chromatogram in Figure 2 displays the appearance of garlic acid and quercetin in the extract. Table 4 shows that the content of Gallic acid ranges from 12.63 ± 0.92 to 13.58 ± 0.35 mg/g, while the content of quercetin ranges from 11.91 ± 0.16 to 15.15 ± 0.32 mg/g. These results indicate that the quercetin content is more dependent on the polarity of the solvent compared to gallic acid.

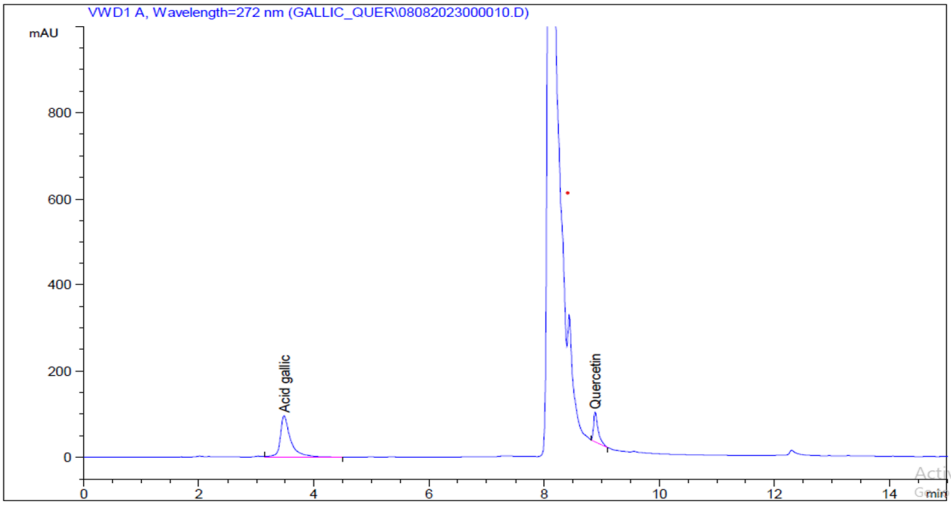


Figure 2. Representative HPLC-UV chromatogram of guava leaf extract.

Nano liposome loanding extract

The nano liposome system containing guava leaf extract has been successfully prepared, with particle sizes ranging from 80 to 250 nm and an average size of 159 nm (Figure 3A). An important parameter is the polydispersity index (PI), which is measured at 0.384, indicating that the system exhibits good dispersion. Furthermore, the Zeta potential is measured at -11.5 mV, showing that the system carries a negative charge (Figure 3B). This implies that the particles will repel each other, thereby enhancing the stability of the system. All of these factors together demonstrate that the nano liposome system containing guava leaf extract is not only successfully fabricated but also possesses ideal physical characteristics for applications in pharmaceutical and food industries

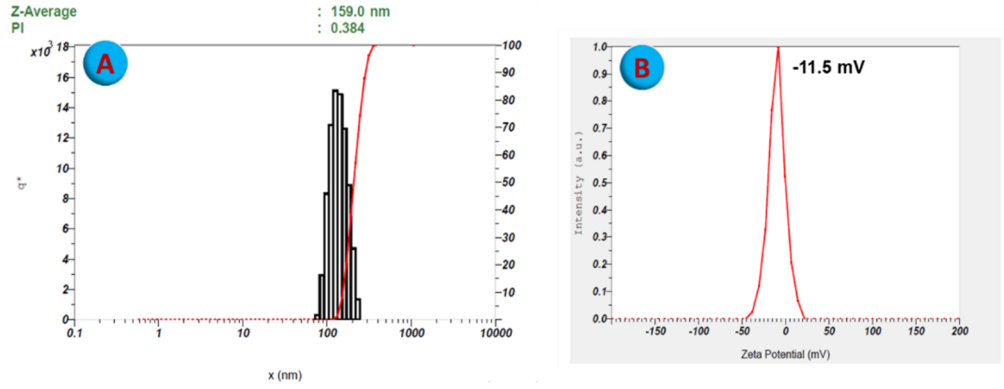


Figure 3. Properties of nano liposome loading extract, (A): Diameter by DLS, (B): Zeta potential.

Bioactivities

DPPH scavaging activity

The antioxidant effectiveness of guava leaf extract was assessed based on its ability to neutralize DPPH free radicals, which showed a proportional relationship with the extract concentration: higher extract concentrations demonstrated greater free radical scavenging activity, and conversely.[20,23,36]

As illustrated in Figure 4, the antioxidant activity assays indicated that guava leaf extract exhibited the capacity to inhibit free radicals at a concentration of 100 µg/mL, depending on the solvent system used for extraction. Specifically, the EtOH50 extract yielded the highest inhibition percentage (I%), followed by EtOH70, while EtOH100 exhibited the lowest I%. Moreover, regarding the DPPH free radical scavenging ability across different extract fractions, the Hexane fraction demonstrated the lowest activity, while the EtOAc extract exhibited the highest. Notably, the antioxidant activity of the EtOH50-EtOAc extract combination reached an I% value of 78.36%, with an IC50 of 55.61 µg/mL. This was only marginally lower than the positive control, vitamin C, which showed an I% of 90.1% and an IC50 of 65.09 µg/mL. Overall, the findings underscore the potent antioxidant potential of guava leaf extract, particularly the EtOH50-EtOAc combination, suggesting its promising role in combating oxidative stress.

As stated earlier, guava leaf extract comprises polyphenols, flavonoids, alkaloids, and other compounds with the capability to counteract free radicals by offering electrons or hydrogen atoms. These compounds are known for their ability to neutralize free radicals by donating electrons or hydrogen atoms. Polyphenols and flavonoids act as antioxidants by scavenging these free radicals, thereby preventing or reducing oxidative damage. By donating electrons or hydrogen atoms to unstable free radicals, they stabilize them and render them less harmful to cells and tissues.[37] Furthermore, the alkaloids present in guava leaf extract may also contribute to its antioxidant properties, although their specific mechanisms of action may differ from those of polyphenols and flavonoids. Overall, the combination of these bioactive compounds in guava leaf extract provides powerful antioxidant protection.[38]

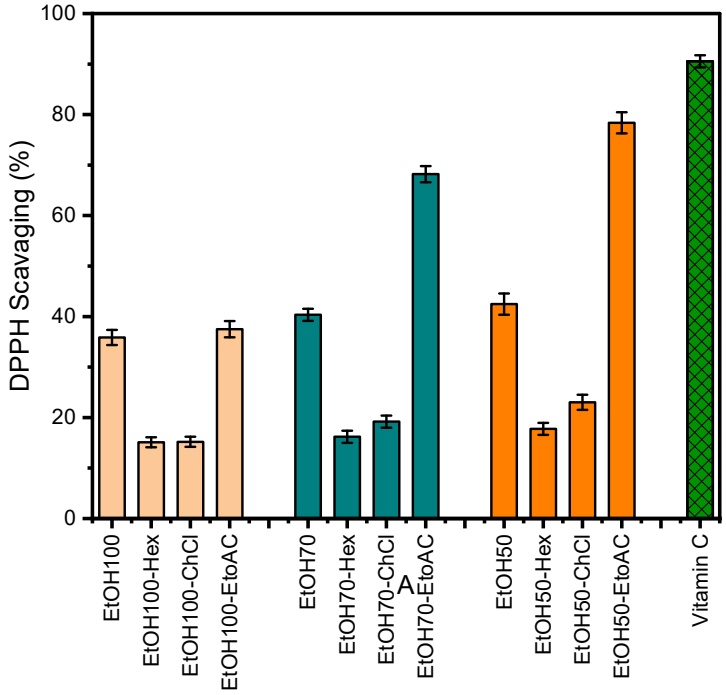


Figure 4. DPPH scavaging (%) of extract samples.

The results also indicated that the nano liposome system containing the extract demonstrates excellent antioxidant capacity, significantly surpassing that of the EtOH 70 extract at the same concentration. This enhanced antioxidant activity can be attributed to the superior bioavailability and stability of the active compounds within the nano liposomes. By encapsulating the extract in nano liposomes, the compounds are better protected from degradation and can be delivered more effectively to biological systems. Consequently, this suggests that the nano liposome formulation could be a more effective strategy for harnessing the antioxidant properties of the guava leaf extract, implying potential applications in food preservation and therapeutic interventions aimed at oxidative stress

Antibacterial activity

Table 5 indicates that ethanol extracts in all three formulations exhibited inhibitory activity against Gram-positive bacteria *P. aeruginosa* and *S. aureus*, while no inhibition was observed against any Gram-negative.

As presented in Figure 5, among the guava leaf extracts, EtOH70 demonstrated the highest antibacterial efficacy against *P. aeruginosa* and *S. aureus* with zones of inhibition measuring 12.5 mm and 11.5 mm, respectively. Figure 5 indicates the minimum inhibitory concentrations (MIC) of 1000 µg/mL for *P. aeruginosa* and 1400 µg/mL for *S. aureus*. The antibacterial activity of the segmented extracts decreased in the order of solvents Hexane, EtOAc, and $CHCl_3$.

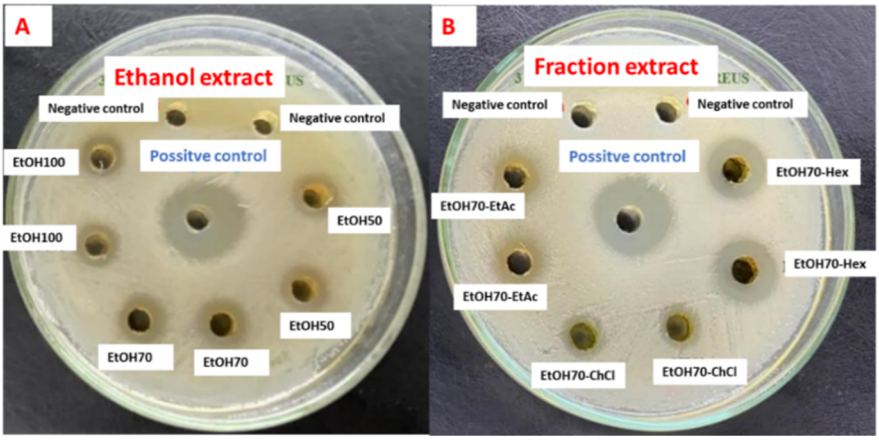


Figure 5: Antibacterial activity against *S. aureus* of ethanol extracts (A), and three fraction extracts of EtOH70 (B).

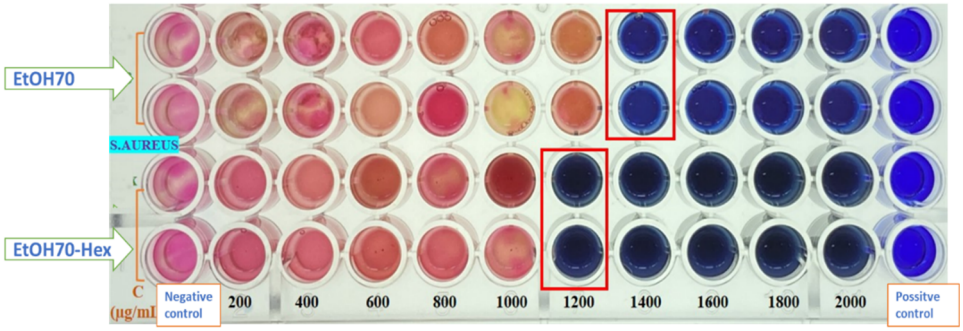


Figure 6: The minimum inhibitory concentration (MIC) of EtOH70 extract and EtOH70-Hex extract fraction on *S. aureus.*

Overall, these results suggest that the use of guava leaves in the treatment of *P. aeruginosa* and *S. aureus* infections may yield positive outcomes. The resistance of Gram-negative bacteria may be attributed to their

cell wall structure, which includes an effective permeability barrier such as a thin outer membrane of lipopolysaccharide, limiting the penetration of plant extracts. Previous reports have indicated that Gram-negative bacteria often exhibit resistance to plant-derived antimicrobial agents and may even be unaffected, unlike gram-positive bacteria. Conversely, gram-positive bacteria possess a peptidoglycan layer resembling a hexagonal mesh, which is more accessible to the penetration of plant extracts. These findings are consistent with data reported in previous studies.[9] Nascimento et al.,[39] reported similar results, demonstrating the inhibitory effects of guava extracts against Staphylococcus and Bacillus species but no effect against *Escherichia* and *Salmonella*. However, Mahfuzul Hoque et al.[40] found no antibacterial activity of ethanol extracts of guava against *E. coli* and *S. enteritidis*. Nevertheless, some studies have reported contradictory findings, such as Vieira et al.[41] discovering effective inhibition of *E. coli* by guava bud extracts, and other authors reporting similar results.[42,43]

Table 5. Antibacterial zone diameter measurement and MIC values of guava leaf extracts.

Samples	Inhibition zone diameter (mm) *P. aeruginosa*			MIC (µg/mL)	Inhibition zone diameter (mm) *S. Aureus*			MIC (µg/mL)
		Positive	Negative			Positive	Negative	
EtOH50	10.0			1600	8.5			1800
EtOH100	11.5	24.0		1400	10.0	20.0		1600
EtOH70	12.5		6.0	1000	11.5		6.0	1400
EtOH70-Hex	15.0			800	13.0			1200
EtOH70-ChCl	11.5	28.0		1200	10.0	13.0		1400
EtOH70-EtOAc	12.0			1000	11.0			1400

Note: 6 mm: No antibacterial activity detected

α-Glucosidase enzyme inhibition activity

Inhibiting the enzyme α-glucosidase is an important way for the control and treatment of diabetes. This enzyme participates in the breakdown of carbohydrates from food into glucose in the intestine, playing a crucial role in controlling blood sugar levels after meals. When the α-glucosidase enzyme is inhibited, the process of carbohydrate breakdown and absorption of glucose from food into the bloodstream slows down or is prevented. This leads to reduced absorption of glucose from food into the bloodstream, lowering blood sugar levels after meals, and preventing sudden spikes in blood sugar, which is significant in diabetes control. Therefore, inhibiting α-glucosidase enzyme is considered an effective means of managing type 2 diabetes and can be used to reduce the risk of disease for individuals at high risk.

The greater the inhibition percentage and the smaller the IC_{50} value, the stronger the inhibition activity against the α-glucosidase enzyme. The results illustrated the inverse relationship between the inhibition capacity against the α-glucosidase enzyme of guava leaf extracts and the water content in the solvent extraction system. Among the total leaf extracts, the EtOH100 extract exhibits the highest inhibition activity, followed by EtOH50, and the lowest is EtOH70. Conversely, in the fractionated leaf extracts, the inhibition activity against the α-glucosidase enzyme decreases in the following order: EtOAc, Hex, $CHCl_3$. Remarkably, Figure 7 indicates that the EtOH100-EtOAc extract demonstrates the best inhibition activity against the α-glucosidase enzyme with an IC50 value of 2.98 µg/mL, surpassing acarbose by 28.16 times (IC50 = 83.91 µg/mL), a drug commonly used to treat diabetes by inhibiting α-glucosidase (more detail in Table S2). This suggests the potential utilization of EtOAc solvent for guava leaf extract isolation and application in diabetes therapy. Notably, the nano liposome system containing guava leaf extract also demonstrates the ability to inhibit the

enzyme α-glucosidase, with an IC_{50} value that is two times lower than that of Acarbose and four times lower than that of the initial extract. This significant enhancement in enzyme inhibition can be attributed to the nano liposome formulation, which likely increases the bioavailability and targeted delivery of the active components within the extract. By encapsulating the extract in nano liposomes, the therapeutic effects can be amplified, making it a promising candidate for managing postprandial hyperglycemia in diabetic patients. The ability to achieve a lower IC50 compared to both acarbose, and the original extract illustrates the potential of nano liposomes in enhancing the efficacy of natural compounds for therapeutic applications.

Inhibition of α-glucosidase can lead to reduced glucose absorption, thus helping to regulate blood sugar levels, which is crucial in diabetes management. This result shows the potential of using guava leaf extract as a natural alternative or supplement to existing diabetes therapies. Previous studies have also indicated the inhibition activity of ethanol guava leaf extracts against α-glucosidase enzyme and α-amylase enzyme.[44–46]

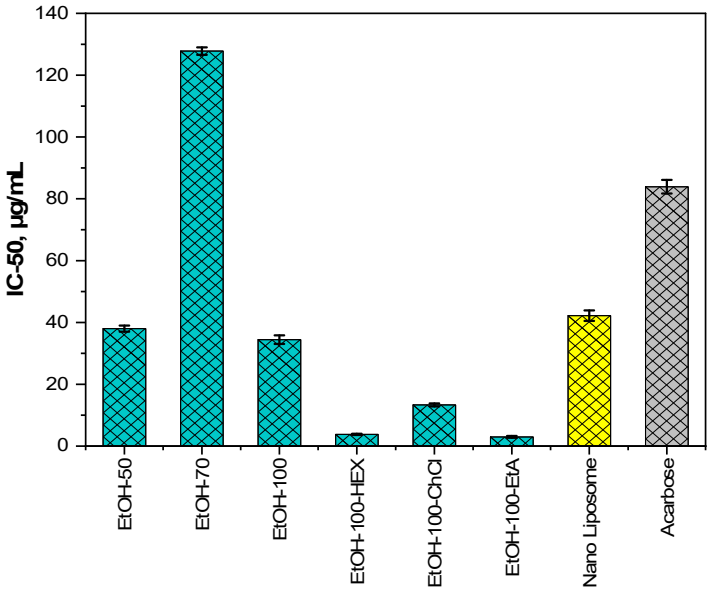


Figure 7. IC_{50} values of the extracts inhibiting α-glucosidase enzyme.

Conclusion

In this study, we extracted phytochemicals from Vietnamese guava (*Psidium guajava*) leaves. The results demonstrated that the guava leaf extracts contained notable amounts of polyphenols and flavonoids, such as gallic acid and quercetin. Among the extracts, the EtOAc fraction exhibited the best antioxidative activity, comparable to vitamin C. Additionally, all extracts effectively inhibited the growth of *P. aeruginosa* and *S. aureus*, with the hexane fraction showing the most potent activity. The ability of extracts to inhibit α-glucosidase also suggested their applicability in diabetes management, with their IC_{50} value being significantly lower than that of the conventional drug acarbose. These findings underscore the therapeutic potential of guava leaf extracts in the health, food, and pharmaceutical sectors, meriting further clinical evaluation and safety assessments.

Conflicts of Interest

There are no conflicts of interest that the authors can disclose regarding this paper's publication.

Author contributions

Conceptualization, Huynh Cong Phuc.; synthesis, Pham Thanh Phong, Nguyen Minh Khoi, validation, and investigation, Huynh Cong Phuc; methodology, software, Tran Quang Hieu; writing - original draft preparation, Tran Quang Hieu; writing - review and editing, Huynh Cong Phuc, Tran Quang Hieu; supervision, project administration, Huynh Cong Phuc. All authors have read and agreed to the published version of the manuscript.

References

1. Díaz-de-Cerio, E.; Verardo, V.; Gómez-Caravaca, A.; Fernández-Gutiérrez, A.; Segura-Carretero, A. Health effects of *Psidium guajava* L. leaves: an overview of the last decade. *Int. J. Mol. Sci.* **2017**, *18*(4), 897. https://doi.org/10.3390/ijms18040897
2. Kumar, M.; Tomar, M.; Amarowicz, R.; Saurabh, V.; Nair, M. S.; Maheshwari, C.; Sasi, M.; Prajapati, U.; Hasan, M.; Singh, S.; Changan, S.; Prajapat, R. K.; Berwal, M. K.; Satankar, V. Guava (*Psidium guajava* L.) Leaves: nutritional composition, phytochemical profile, and health-promoting bioactivities. *Foods* **2021**, *10*(4), 752. https://doi.org/10.3390/foods10040752
3. Park, H.; Kim, B.; Kang, Y.; Kim, W. Study on chemical composition and biological activity of *Psidium guajava* leaf extracts. *Curr. Issues Mol. Biol.* **2024**, *46*(3), 2133 – 2143. https://doi.org/10.3390/cimb46030137
4. Nguyễn, T. B. T.; Cao, L. N. H.; Đoàn, V. H. T.; Trần, T. M. Research on the chemical composition and biological activity of *Psidium guajava* L. leaf extract. *Can Tho Univ. J. Sci.* **2023**, *59* (ETMD), 129 – 133. https://doi.org/10.22144/ctu.jvn.2023.037
5. Weli, A.; Al-Kaabi, A.; Al-Sabahi, J.; Said, S.; Hossain, M. A.; Al-Riyami, S. Chemical composition and biological activities of the essential oils of *Psidium guajava* leaf. *J. King Saud Univ. - Sci.* **2019**, *31*(4), 993 – 998. https://doi.org/10.1016/j.jksus.2018.07.021
6. Ryu, N. H.; Park, K.-R.; Kim, S.-M.; Yun, H.-M.; Nam, D.; Lee, S.-G.; Jang, H.-J.; Ahn, K. S.; Kim, S.-H.; Shim, B. S.; Choi, S.-H.; Mosaddik, A.; Cho, S. K.; Ahn, K. S. A hexane fraction of guava leaves (*Psidium guajava* L.) induces anticancer activity by sppressing AKT/Mammalian target of Rapamycin/Ribosomal P70 S6 kinase in human prostate cancer cells. *J. Med. Food* **2012**, *15*(3), 231 – 241. https://doi.org/10.1089/jmf.2011.1701
7. Shao, M.; Wang, Y.; Huang, X.-J.; Fan, C.-L.; Zhang, Q.-W.; Zhang, X.-Q.; Ye, W.-C. Four new triterpenoids from the leaves of *Psidium guajava*. *J. Asian Nat. Prod. Res.* **2012**, *14* (4), 348 – 354. https://doi.org/10.1080/10286020.2011.653964
8. Szu, C. S.; Fang, C. C.; Ning, J. Wu. Effect of guava (*Psidium guajava Linn*.) leaf soluble solids on glucose metabolism in type 2 diabetic rats. *Phytother. Res.* **2008,** *22(11)*, 1458-1464. https://doi.org/10.1002/ptr.2476

9. Biswas, B.; Rogers, K.; McLaughlin, F.; Daniels, D.; Yadav, A. Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. *Int. J. Microbiol.* **2013**, *2013*, 1 – 7. https://doi.org/10.1155/2013/746165

10. Lok, B.; Babu, D.; Tabana, Y.; Dahham, S. S.; Adam, M. A. A.; Barakat, K.; Sandai, D. The anticancer potential of *Psidium guajava* (guava) extracts. *Life* **2023**, *13*(2), 346. https://doi.org/10.3390/life13020346

11. Braga, T. V.; Dores, R. G. R. das; Ramos, C. S.; Evangelista, F. C. G.; Tinoco, L. M. da S.; Varotti, F. de P.; Carvalho, M. das G.; Sabino, A. de P. Antioxidant, antibacterial and antitumor activity of ethanolic extract of the *Psidium guajava* leaves. *Am. J. Plant Sci.* **2014**, *5*(23), 3492 – 3500. https://doi.org/10.4236/ajps.2014.523365

12. Naseer, S.; Hussain, S.; Naeem, N.; Pervaiz, M.; Rahman, M. The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clin. Phytosci.* **2018**, *4*(1), 32. https://doi.org/10.1186/s40816-018-0093-8

13. Gutierrez-Montiel, D.; Guerrero-Barrera, A. L.; Chávez-Vela, N. A.; Avelar-Gonzalez, F. J.; Ornelas-García, I. G. *Psidium Guajava* L.: From Byproduct and use in traditional Mexican medicine to antimicrobial agent. *Front. Nutr.* **2023**, *10*, 1108306. https://doi.org/10.3389/fnut.2023.1108306

14. Sampath Kumar, N. S.; Sarbon, N. M.; Rana, S. S.; Chintagunta, A. D.; Prathibha, S.; Ingilala, S. K.; Jeevan Kumar, S. P.; Sai Anvesh, B.; Dirisala, V. R. Extraction of bioactive compounds from *Psidium guajava* leaves and its utilization in preparation of jellies. *AMB Express* **2021**, *11*(1), 36. https://doi.org/10.1186/s13568-021-01194-9

15. Zoghi, A.; Khosravi-Darani, K.; Omri, A. Process Variables and design of experiments in liposome and nanoliposome research. *Mini Rev. Med. Chem.* **2018**, *18*(4), 324 – 344. https://doi.org/10.2174/1389557516666161031120752

16. Hazmi, S. A. A.; Sarah Amira Ismail, N.; Mohamad, M.; Wan Osman, W. H. Extraction of phenolic and flavonoids compounds from kenaf (*Hibiscus Cannabinus* L.) using ultrasound assisted extraction. *Mater. Today: Proc.* **2023**, in press. https://doi.org/10.1016/j.matpr.2023.02.285

17. Fransina, E. G.; Tanasale, M. F. J. D.; Latupeirissa, J.; Malle, D.; Tahapary, R. Phytochemical screening of water extract of gayam (*Inocarpus edulis*) Bark and its amylase inhibitor activity assay. *IOP Conf. Ser.: Mater. Sci. Eng.* **2019**, *509*, 012074. https://doi.org/10.1088/1757-899X/509/1/012074

18. Osukoya, O. A.; Adegbenro, D.; Onikanni, S.; Ojo, O. A.; Onasanya, A. Antinociceptive and antioxidant activities of the methanolic extract of *Telfairia occidentalis* seeds. *Anc. Sci. Life* **2016**, *36*(2), 98. https://doi.org/10.4103/asl.ASL_142_16

19. Krongrawa, W.; Limmatvapirat, S.; Pongnimitprasert, N.; Meetam, P.; Limmatvapirat, C. Formulation and evaluation of gels containing coconut kernel extract for topical application. *Asian J. Pharm. Sci.* **2018**, *13*(5), 415 – 424. https://doi.org/10.1016/j.ajps.2018.01.005

20. Thang, N. Q.; Hoa, V. T. K.; Van Tan, L.; Tho, N. T. M.; Hieu, T. Q.; Phuong, N. T. K. Extraction of cynarine and chlorogenic acid from Artichoke leaves (*Cynara scolymus* L.) and evaluation of antioxidant activity, antibacterial activity of extract. *Vietnam J. Chem.* **2022**, *60* (5), 571 – 577. https://doi.org/10.1002/vjch.202100117

21. Tran, Q.-H.; Chu, H.-K. T.; Nguyen, P.-T.; Nguyen, V.-M.; Nguyen, Q.-T.; Tran, C.-D.; Nguyen, T.-D. Double nanoemulsion loading betalains extract of beetroot (*Beta vulgaris* L.): ultrasound-assisted synthesis, storage stability, and antioxidant activity. *ACS Food Sci. Technol.* **2023**, *3*(12), 2229 – 2237. https://doi.org/10.1021/acsfoodscitech.3c00430

22. Tran, Q.-H.; Thuy, T. T.-H.; Nguyen, T. T.-T. Fabrication of a narrow size nano curcuminoid emulsion by combining phase inversion temperature and ultrasonication: preparation and bioactivity. *New J. Chem.* **2021**, *45*(21), 9658 – 9667. https://doi.org/10.1039/D1NJ01241J

23. Tran, Q.; Le Thi, T.; Nguyen, T.; Tran, T.; Le, Q.; Luu, T.; Dinh, V. Facile Synthesis of novel nanocurcuminoids–Sacha Inchi Oil using the phase inversion temperature method: characterization and antioxidant activity. *J. Food Process. Preserv.* **2021**, *45*(5), e15402. https://doi.org/10.1111/jfpp.15402

24. Elshikh, M.; Ahmed, S.; Funston, S.; Dunlop, P.; McGaw, M.; Marchant, R.; Banat, I. M. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotechnol. Lett.* **2016**, *38*(6), 1015 – 1019. https://doi.org/10.1007/s10529-016-2079-2

25. Wang, H.; Du, Y.-J.; Song, H.-C. α-Glucosidase and α-amylase inhibitory activities of guava leaves. *Food Chem.* **2010**, *123*(1), 6 – 13. https://doi.org/10.1016/j.foodchem.2010.03.088

26. Roheem, F. O.; Ahmed, Q. U.; Mat So'ad, S. Z.; Shah, S. A. A.; Latip, J.; Alhassan, A. M.; Syed Mohammad, S. N. A. Assessment of free radical scavenging and digestive enzyme inhibitory activities of extract, fractions and isolated compounds from *Tetracera macrophylla* leaves. *J. Herb. Med.* **2020**, *22*, 100351. https://doi.org/10.1016/j.hermed.2020.100351

27. Kazeem, M. I.; Adamson, J. O.; Ogunwande, I. A. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* benth leaf. *BioMed Res. Int.* **2013**, *2013*(1), 527570. https://doi.org/10.1155/2013/527570

28. Oulahal, N.; Degraeve, P. Phenolic-rich plant extracts with antimicrobial activity: An alternative to food preservatives and biocides? *Food Microbiol.* **2022**, *12*. https://doi.org/10.3389/fmicb.2021.753518

29. de Melo, L. F. M.; Aquino-Martins, V. G. de Q.; da Silva, A. P.; Oliveira Rocha, H. A.; Scortecci, K. C. Biological and pharmacological aspects of tannins and potential biotechnological applications. *Food Chem.* **2023**, *414*, 135645. https://doi.org/10.1016/j.foodchem.2023.135645

30. Gutiérrez-Venegas, G.; Gómez-Mora, J. A.; Meraz-Rodríguez, M. A.; Flores-Sánchez, M. A.; Ortiz-Miranda, L. F. Effect of flavonoids on antimicrobial activity of microorganisms present in dental plaque. *Heliyon* **2019**, *5*(12), e03013. https://doi.org/10.1016/j.heliyon.2019.e03013

31. Górniak, I.; Bartoszewski, R.; Króliczewski, J. Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochem. Rev.* **2019**, *18* (1), 241 – 272. https://doi.org/10.1007/s11101-018-9591-z

32. Masyita, A.; Mustika Sari, R.; Dwi Astuti, A.; Yasir, B.; Rahma Rumata, N.; Emran, T. Bin; Nainu, F.; Simal-Gandara, J. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chem. X* **2022**, *13*, 100217. https://doi.org/10.1016/j.fochx.2022.100217

33. Tagousop, C. N.; Tamokou, J.-D.; Kengne, I. C.; Ngnokam, D.; Voutquenne-Nazabadioko, L. Antimicrobial activities of saponins from *Melanthera elliptica* and their synergistic effects with antibiotics against pathogenic phenotypes. *Chem. Cent. J.* **2018**, *12*(1), 97. https://doi.org/10.1186/s13065-018-0466-6

34. Čižmárová, B.; Hubková, B.; Tomečková, V.; Birková, A. Flavonoids as promising natural compounds in the prevention and treatment of selected skin diseases. *Int. J. Mol. Sci.* **2023**, *24*(7), 6324. https://doi.org/10.3390/ijms24076324

35. Freed, B. K.; Biesecker, J.; Middleton, W. J. Spectral Polarity index: a new method for determining the relative polarity of solvents [1]. *J. Fluorine Chem.*

1990, *48*(1), 63 – 75. https://doi.org/10.1016/S0022-1139(00)82602-0

36. Thuy, H. T. T.; Ngoc, P. T. K.; Tu, N. T. T.; Truc, H. T. T.; Hai, N. Van; Hieu, T. Q. Comparision of curcumin nanoemulsion drops size between hominization and ultrasonication supporting. *J. Sci. Technol.* **2019**, *39,* 3. https://doi.org/10.46242/jst-iuh.v39i03.277

37. Yan, Z.; Zhong, Y.; Duan, Y.; Chen, Q.; Li, F. Antioxidant mechanism of tea polyphenols and its impact on health benefits. *Anim. Nutr.* **2020**, *6*(2), 115 – 123. https://doi.org/10.1016/j.aninu.2020.01.001

38. Mazumder, M. A. R.; Tolaema, A.; Chaikhemarat, P.; Rawdkuen, S. Antioxidant and anti-cytotoxicity effect of phenolic extracts from *Psidium guajava* Linn. leaves by novel assisted extraction techniques. *Foods* **2023**, *12*(12), 2336. https://doi.org/10.3390/foods12122336

39. Nascimento, G. G. F.; Locatelli, J.; Freitas, P. C.; Silva, G. L. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol.* **2000**, *31*(4), 247 – 256. https://doi.org/10.1590/S1517-83822000000400003

40. Mahfuzul Hoque, M. D.; Bari, M. L.; Inatsu, Y.; Juneja, V. K.; Kawamoto, S. Antibacterial activity of guava (*Psidium guajava* L.) and neem (*Azadirachta indica* A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathog. Dis.* **2007**, *4* (4), 481 – 488. https://doi.org/10.1089/fpd.2007.0040

41. Vieira, R. H. S. Dos F.; Rodrigues, D. Dos P.; Gonçalves, F. A.; Menezes, F. G. R. De; Aragão, J. S.; Sousa, O. V. Microbicidal effect of medicinal plant extracts (*Psidium guajava* Linn. and *Carica papaya* Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. *Rev. Inst. Med. Trop. Sao Paulo* **2001**, *43*(3), 145 – 148. https://doi.org/10.1590/S0036-46652001000300005

42. Sanches, N. R.; Garcia Cortez, D. A.; Schiavini, M. S.; Nakamura, C. V.; Dias Filho, B. P. An evaluation of antibacterial activities of *Psidium guajava* (L.). *Braz. Arch. Biol. Technol.* **2005**, *48* (3), 429 – 436. https://doi.org/10.1590/S1516-89132005000300014

43. Lin, J.; Puckree, T.; Mvelase, T. Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. *J. Ethnopharmacol.* **2002**, *79*(1), 53 – 56. https://doi.org/10.1016/S0378-8741(01)00353-1

44. Beidokhti, M. N.; Eid, H. M.; Villavicencio, M. L. S.; Jäger, A. K.; Lobbens, E. S.; Rasoanaivo, P. R.; McNair, L. M.; Haddad, P. S.; Staerk, D. Evaluation of the antidiabetic potential of *Psidium guajava* L. (Myrtaceae) using assays for α-glucosidase, α-amylase, muscle glucose uptake, liver glucose production, and triglyceride accumulation in adipocytes. *J. Ethnopharmacol.* **2020**, *257*, 112877. https://doi.org/10.1016/j.jep.2020.112877

45. Rahayu, I.; Heng, P. H.; Timotius, K. H. In vitro antioxidant properties and α-glucosidase inhibition of combined leaf infusions from *Psidium guajava* L., *Syzygium polyanthum* L., and *Annona muricata* L. *Pharmacogn. J.* **2019**, *11*(6), 1269 – 1277. https://doi.org/10.5530/pj.2019.11.197

46. Wang, L.; Liu, Y.; Luo, Y.; Huang, K.; Wu, Z. Quickly screening for potential α-glucosidase inhibitors from guava leaves tea by bioaffinity ultrafiltration coupled with HPLC-ESI-TOF/MS method. *J. Agric. Food Chem.* **2018**, *66*(6), 1576 – 1582. https://doi.org/10.1021/acs.jafc.7b05280