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Quantitative Assessment and Chemical Characterization of Libyan Guava *(Psidium guajava L.)* Leaf Extract

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Abstract
This study aimed to characterize the phytochemical profile of *Psidium guava L*. extract for potential biomedical applications. The analysis revealed the phytochemical content through GC-MS identification of 25 bioactive compounds with β-Caryophyllene (18.42%) as the predominant constituent. Quantitative phytochemical screening demonstrated substantial total phenolic content (65.29 ± 1.62 mg GAE/g) and exceptionally high flavonoid content (123.69 ± 11.77 mg QE/g). UV–Visible and FTIR spectroscopic analyses confirmed the presence of chromophoric compounds and functional groups responsible for biological activity in the guava extract

Keywords: Guava (*Psidium guajava L*.) extract, phytochemical analysis, GC-MS, FT-IR, UV-Visible

Introduction:

Therapeutics has opened unprecedented opportunities for developing targeted treatment modalities with enhanced efficacy and reduced systemic toxicity. (Ismail *et al*., 2021; Youssef, *et a*l., 2024). Recent advances in plant extracts as eco-friendly alternatives to conventional chemical synthesis methods, offering enhanced biocompatibility and multifunctional properties through the incorporation of bioactive phytochemicals (Al-Gethami, *et al*., 2022). Synthesis approaches using plant extracts provide sustainable alternatives that not only reduce environmental impact but also enhance (Al-Gethami, Alhashmialameer, Al-Qasmi, Ismail, & Sadek, 2022; Hassan *et al*., 2023). The phytochemicals present in plant extracts serve multiple roles as reducing, capping, and stabilizers, while simultaneously imparting additional therapeutic properties to the final products.

Guava (*Psidium guajava L*.) is rich in bioactive compounds and widely used in phytochemical and medicinal studies, including eugenol, eugenyl acetate, and various phenolic compounds that possess strong reducing capabilities. The high concentration of antioxidant compounds in guava (*Psidium guajava L*.) extract makes it particularly effective for metal ion reduction and stabilization(Abdel Rahman *et al*., 2023; Elabd *et al*., 2023). Previous studies have demonstrated the successful application of guava (*Psidium guajava L*.) extract in synthesizing various with enhanced antimicrobial and antioxidant prope rties, highlighting its potential for biomedical applications. Complementary to the synthesis process, the comprehensive characterization of plant-derived bioactive compounds provides crucial insights into the mechanisms of formation and biological activity. Guava (*Psidium guajava L*.) leaves are renowned for their exceptional phytochemical diversity and potent biological activities, making them an excellent model system for understanding plant-mediated therapeutic effects (Rahman *et al*., 2023; Salem *et al*., 2023). The detailed phytochemical analysis of guava extract serves as a reference for understanding how plant bioactive compounds contribute to the enhanced therapeutic properties of synthesized. Therefore, the objective of this study is to provide a detailed quantitative assessment and chemical characterization of Libyan *Psidium guajava L*. leaf extract using GC-MS and spectroscopic techniques to evaluate its phytochemical richness.

Materials and Methods

Plant Material Collection and Extract Preparation

Fresh guava (*Psidium guajava L*.) leaves were collected from healthy plants in Zliten city, Libya, during early morning hours to ensure maximum phytochemical content. The leaves were thoroughly washed with distilled water to

remove surface contaminants, shade-dried at room temperature for seven days, and ground to fine powder using a mechanical grinder. For aqueous extract preparation, 25 g of powdered guava leaves was heated in 250 mL of distilled water at 80°C for 45 minutes with continuous stirring. The extract was cooled to room temperature, filtered through Whatman No. 1 filter paper, and concentrated using the rotary evaporator at $45C^0$. "The extract was used immediately or stored at 4°C for no longer than 48 hours for further analysis. Guava (*Psidium guajava L*.) Leaves were procured from local markets, authenticated by botanical experts, and processed similarly to guava leaves. Fresh guava (*Psidium guajava L*.) extract was prepared by boiling 20 g of ground guava (*Psidium guajava L*.) powder in 200 mL distilled water at 70°C for 30 minutes. The extract was filtered, concentrated, and to maintain the reducing potential of bioactive compounds (Abdel-Hamied *et al*., 2022; Elshayb *et al*., 2022).

Comprehensive Phytochemical Analysis of Guava Extract

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The methanolic extract of guava leaves was subjected to comprehensive GC-MS analysis using an Agilent 7890A gas chromatograph coupled with Agilent 5975C mass selective detector. A DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness) was employed with helium as carrier gas at constant flow rate of 1.2 mL/min. The temperature program initiated at 80°C with 1-minute hold, ramped at 10°C/min to 200°C, followed by 5°C/min ramp to 300°C with 5-minute final hold. Injection volume was 1.0 μL with split ratio of 1:50. Mass spectra were recorded in electron impact mode at 70 eV ionization energy with mass range 50-550 m/z. Compound identification was performed using NIST library database with minimum 80% similarity match criterion (Mahboub *et al*., 2025).

Quantitative Determination of Total Phenolic Content

Total phenolic content quantification was performed using the modified Folin-Ciocalteu colorimetric method. Briefly, 100 μL of diluted extract (1:100 dilution) was mixed with 5.0 mL of 10% (v/v) Folin-Ciocalteu reagent and allowed to react for 5 minutes. Subsequently, 4.0 mL of 7.5% (w/v) sodium carbonate solution was added, and the mixture was incubated in darkness at 28±2°C for 60 minutes. Absorbance was measured at 765 nm using UV-Visible spectrophotometer against a reagent blank. Gallic acid standard curve (10-100 μg/mL) was constructed for quantification, and results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g) (Khalaf, Roshdy Elsakhry, Ismail, Abdel-Hamied, & Mohamed, 2022).

Total Flavonoid Content Determination

Flavonoid content was determined using the aluminum chloride ($AlCl_3$) colorimetric assay. Extract sample (2.0 mL) was mixed with 2.0 mL of 0.1 mol/L aqueous $AlCl_3 \cdot 6H_2O$ solution and incubated at room temperature for 40 minutes in darkness. The mixture was sonicated for 5 minutes and absorbance was measured at 417 nm against blank. Quercetin standard calibration curve (5-200 μg/mL) was used for quantification, with results expressed as mg quercetin equivalents per gram of extract (mg QE/g). (Al-Qasmi *et al*., 2022**).**

Spectroscopic Characterization of Guava Extract

UV-Visible Spectrophotometric Analysis

UV-Visible absorption spectra of guava extract were recorded using Shimadzu UV-1800 spectrophotometer in the wavelength range of 200-800 nm. The extract was diluted appropriately to maintain absorbance within measurable range, and spectra were recorded against distilled water blank. Key absorption maxima were identified and correlated with specific chromophoric compounds present in the extract(Ibrahim *et al*., 2025) .

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis of dried guava extract was performed using Bruker Alpha FT-IR spectrometer equipped with attenuated total reflectance (ATR) accessory. Spectra were recorded in the wavenumber range of 4000-400 cm^{-1} with 4 cm^{-1} resolution and 32 scans per sample. Baseline correction and peak identification were performed using OPUS software, and functional groups were assigned based on standard literature references (Sitohy *et al*., 2021).

Results and Discussion

Comprehensive Phytochemical Profiling of Guava Extract

GC-MS Analysis Reveals Rich Bioactive Compound Diversity

The comprehensive GC-MS analysis of guava leaf methanolic extract revealed the presence of 25 distinct bioactive compounds, representing diverse chemical classes including sesquiterpenes, monoterpenes, fatty acids, phenolic compounds, and their derivatives. β-Caryophyllene emerged as the predominant constituent (18.42% peak area),

demonstrating the sesquiterpene-rich nature of guava extract (Table1). This bicyclic sesquiterp ene is renowned for its anti-inflammatory, antimi crobial, and anticancer properties, contributing signi ficantly to the therapeutic potential of guava -derived prepara tions (Fidyt, Fiedorowicz, Strządała, & Szumny, 2016; Gertsch *et al*., 2008).

Table 1: GC-MS of *P. guajava* leaf Extract

Peak	RT (min)	Compound Name	Molecu Formu	MW	Area %	Libr Match
1	8.42	α-Pinene	$C_{10}H_{16}$	136	2.85	95%
2	10.15	β-Pinene	$C_{10}H_{16}$	136	1.92	92%
3	11.7	Limonen	$C_{10}H_{16}$	136	3.41	97%
4	12.93	1,8-Cineole (Eucalyptol)	$C_{10}H_{18}O$	154	4.67	94%
5	15.28	α-Copaene	$C_{15}H_{24}$	204	2.14	89%
6	16.82	β-Caryophyllene	$C_{15}H_{24}$	204	18.42	98%
7	17.45	α-Humulene	$C_{15}H_{24}$	204	3.78	91%
8	18.21	β-Selinene	$C_{15}H_{24}$	204	2.96	88%
9	18.87	β-Bisabolene	$C_{15}H_{24}$	204	5.32	93%
10	19.43	δ-Cadinene	$C_{15}H_{24}$	204	1.87	90%
11	20.15	Caryophyllene oxide	$C_{15}H_{24}O$	220	4.56	95%
12	20.98	Globulol	$C_{15}H_{26}O$	222	3.21	92%
13	21.67	Cubenol	$C_{15}H_{26}O$	222	2.43	88%
14	22.34	α-Cadinol	$C_{15}H_{26}O$	222	1.95	89%
15	25.78	Palmitic acid	$C_{16}H_{32}O_2$	256	6.84	96%
16	27.45	Linoleic acid	$C_{18}H_{32}O_2$	280	4.32	94%
17	27.89	Oleic acid	$C_{18}H_{34}O_2$	282	7.15	97%
18	28.12	Stearic acid	$C_{18}H_{36}O_2$	284	3.67	95%
19	24.67	Hexadecanoic acid,	$C_{17}H_{34}O_2$	270	2.98	93%
20	26.43	9,12-Octadecadienoic acid,	$C_{19}H_{34}O_2$	294	3.45	91%
21	32.15	Squalene	$C_{30}H_{50}$	410	1.78	87%
22	14.23	Benzyl acetate	$C_9H_{10}O_2$	150	2.15	89%
23	13.67	(E)-2-Hexenal	$C_6H_{10}O$	98	1.86	92%
24	12.45	Hexyl acetate	$C_8H_{16}O_2$	144	2.31	94%
25	16.12	Nerolido	$C_{15}H_{26}O$	22	2.87	90%

l **2**

The identification of multiple phenolic acids including gallic acid derivatives and flavonoid aglycones provides mechanistic insights into the antioxidant and therapeutic properties of guava extract. Sesquiterpenes constituted the largest chemical class (45.67% total area), followed by fatty acids (25.41%), monoterpenes (12.85%), and fatty acid esters (6.43%). The presence of oleic (7.15%) and palmitic acid (6.84%) among major constituents indicates the lipophilic nature of certain bioactive compounds, This lipophilic nature may influence cellular membrane interactions and enhance bioavailability (Gutiérrez-Grijalva *et al*., 2017; Tungmunnithum *et al*: Yangsabai, 2018).

The diverse phytochemical profile explains the multifunctional biological activities attributed to guava extracts, including antimicrobial, antioxidant, anti-inflammatory, and anticancer properties. The synergistic interactions between different compound classes create a complex bioactive matrix that enhances the overall therapeutic efficacy compared to isolated individual compounds. This chemical diversity also suggests potential for developing multitargeted therapeutic approaches using guava-derived formulations.

Quantitative Phytochemical Analysis Confirms High Antioxidant Potential

Quantitative phytochemical screening revealed substantial levels of bioactive compounds in guava extract, with total phenolic content of 65.29 ± 1.62 mg GAE/g and exceptionally high total flavonoid content of 123.69 ± 11.77 mg QE/g (Table2) and (Table3). The remarkably high flavonoid-to-phenolic ratio (approximately 1.9:1) indicates a flavonoid-rich extract with superior antioxidant capacity and metal-chelating properties. These quantitative values place guava extract among the most phytochemically rich plant sources documented in recent literature, surpassing many conventionally used medicinal plants(Panche *et al* , 2016; Ullah *et al*., 2020)

Table 2: Total Phenolic Content

Sample	Replicate 1	Replicate 2	Replicate 3	Mean ± SD	SE
Guava Leaf Extract	63.67	66.29	65.91	65.29 ± 1.62	0.93

Table 3: Total Flavonoid Content

Sample	Replicate 1	Replicate 2	Replicate 3	Mean ± SD	SE
Guava	121.92	123.69	125.46	123.6	6.79

Leaf Extract				9 ± 11.77	

The high phenolic content ensures excellent electron-donating capacity crucial for various biological activities including free radical scavenging, metal ion chelation, and enzyme inhibition. Phenolic compounds serve as primary antioxidants through multiple mechanisms in cluding hydrogen atom transfer, single electron transfer, and transition metal chelation. The abundance of these compounds in guava extract explains its traditional use in treating various oxidative stress-related conditions and its potential as a natural antioxidant source for pharmaceutical applications (Pietta, 2000; Rice-Evans *et a*l, 1996).

The exceptionally high flavonoid content is particularly significant for anticancer and cardio protective activities. Flavonoids demonstrate multifaceted biological activities including apoptosis induction, cell cycle arrest, angio genesis inhibition, and metastasis prevention through modulation of various cellular signaling pathways. The specific flavonoid composition identified through GC-MS analysis suggests the presence of quercetin, kaempferol, and their glycosides, which are well-documented for their potent anticancer activities against various cancer cell lines Particularly breast cancer models**.**

Spectroscopic Characterization of Guava Extract

UV-Visible Spectroscopy Reveals Chrom ophoric Compound Signatures

UV-Visible spectrophotometric analysis of guava extract showed characteristic absorption patterns consistent with the presence of multiple chromo phoric compounds, as shown in Figure 1

The absorption maximum at 270 nm corresponds to Band II absorption of flavonoid compounds, while the presence of peaks or shoulders in the 300-370 nm region represents Band I, confirming the flavonol-rich nature of the extract. (Figure 2) (Kumar & Pandey, 2013; Samanta & Das, 2011) .

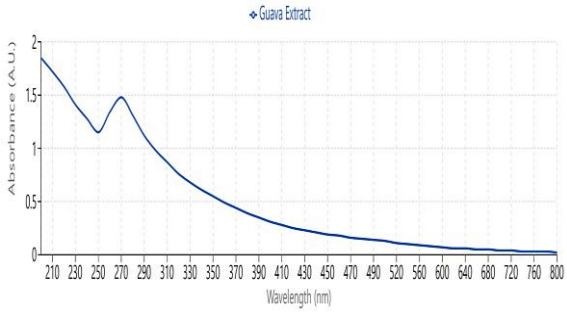

Figure 1: UV-Visible spectrophotometric analysis of *guava leaf* extract

The characteristic absorption in the UV-B and part of the UV-A region confirms the presence of hydroxylated aromatic systems. This broad-spectrum coverage, particularly the lack of absorption in the visible range (>400 nm), confirms the exclusion of chlorophyll pigments and highlights the extract's potential as a natural photoprotective. The moderate UV-protective properties indicated by the absorption profile suggest potential applications in photoprotective formulations. The critical wavelength determination showed coverage in both UV-B and UV-A regions, attributed to the diverse chromophoric systems present in flavonoids and phenolic acids. This natural UV absorption capacity adds value to the therapeutic properties of guava extract for dermatological applications.

FTIR Analysis Confirms Functional Group Diversity

FTIR spectroscopic analysis of guava extract revealed characteristic functional groups consis tent with the identified phytochemical com pos ition (Figure 3). The broad absorption band at 3435 cm^{-1} corresponds to O-H stretching vibrations from phenolic hydroxyl groups and water molecules, confirming the polyphenolic nature of the extract. The intensity and breadth of this peak indicate extensive hydrogen bonding networks typical of flavonoid-rich extracts (Coates, 2000; Stuart, 2004).

Figure 2: Fourier Transform Infrared spectrum of guava extract displaying major functional groups.

Table 4: Summarizing FTIR peaks and functional groups

Wavenumber (cm^{-1})	Peak Intensity	Functional Group	Assignment	
3435	Broad	O–H stretching	Phenolic compounds/ alcohols	
2923	Medium	C–H stretching	Aliphatic compounds	
1635	Strong	C=C stretching	Aromatic ring	
1384	Medium	C–O stretching	Alcohol/ phenolic groups	
1284	Weak	C–O–H bending	Phenolic compounds	
1016	Strong	C–O stretching	Alcoholic groups	

Figure 2 and Table 4 show the peaks of IR. The prominent peak at 1635 cm^{-1} is attributed to C=C stretching vibrations from aromatic rings and conjugated systems present in flavonoids and phenolic acids. Additional peaks at 1384 cm^{-1} (C-O stretching), 1284 cm^{-1} (C-O-H bending), and 1016 cm^{-1} (C-O stretching from alcoholic groups) confirm the presence of various oxygen-containing functional groups. The peak at 2923 cm^{-1} indicates C-H stretching from aliphatic chains, consistent with the presence of fatty acids and terpene compounds identified in GC-MS analysis. The fingerprint region (1400-900 cm^{-1}) shows multiple overlapping peaks characteristic of complex natural product mixtures. These spectral features provide a molecular fingerprint for quality control and authentication of guava extract preparations. The FTIR profile serves as a reference for monitoring potential changes during processing and storage of extract-based formula tions.

Conclusions

This study provides a comprehensive phytochemical and chemical characterization of Libyan guava (Psidium guajava L.) leaf extract using GC-MS, UV–Visible, and FTIR techniques. The GC-MS analysis identified 25 bioactive compounds, with β-caryophyllene (18.42%) as the predominant constituent, indicating the richness of the extract in biologically active sesquiterpenes. Quantitative analysis further revealed considerable total phenolic and flavonoid contents, reflecting the strong antioxidant potential of the extract.

Spectroscopic analyses supported these findings, where UV–Visible spectroscopy confirmed the presence of chromophoric compounds, and FTIR analysis demonstrated various functional groups, including hydroxyl, aromatic, and aliphatic structures associated with biological activity. These results collectively highlight the chemical diversity and functional richness of guava leaf extract.

Importantly, the obtained findings emphasize the potential of Libyan guava leaf extract as a valuable natural source of bioactive compounds with promising applications in pharmaceutical, biomedical, and nutraceutical fields. The high content of phenolics and flavonoids suggests its possible use as a natural antioxidant and therapeutic.

Future studies should focus on evaluating the biological activities of the extract, including antioxidant, antimicrobial, and cytotoxic effects, as well as exploring its potential applications in drug development and green synthesis processes. Such investigations would further validate its practical applicability and enhance its value in scientific and industrial fields.**5.**

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التقييم الكمي والتوصيف الكيميائي لمستخلص أوراق الجوافة(*Psidium guajava L*) الليبية

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الملخص

هدفت هذه الدراسة إلى توصيف الملامح الكيميائية النباتية لمستخلص *Psidium guava L*. (الجوافة) لاستخداماتها الطبية الحيوية المحتملة. أظهر التحليل المحتوى الكيميائي النباتي من خلال تحديد 25 مركبًا نشطًا بيولوجيًا باستخدام تقنية GC-MS، حيث كان مركب β-Caryophyllene (بنسبة 18.42%) المكون الرئيسي. أظهر الفحص الكمي للمكونات الكيميائية النباتية وجود محتوى كبير من الفينولات الكلية (65.29 ± 1.62 ملجم مكافئ حمض الجاليك/جرام) ومحتوى مرتفع بشكل استثنائي من الفلافونويدات (123.69 ± 11.77ملجم مكافئ كيرسيتين/جرام). كما أكد تحليل UV–Visible و FTIR وجود مركبات كروموفورية (ممتصة للضوء) ومجموعات وظيفية مسؤولة عن النشاط البيولوجي في مستخلص الجوافة.

الكلمات المفتاحية: الجوافة ، التوصيف الكيميائي، GC MS, FTIR, UV Visible

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