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Research Paper

Antimicrobial Activity and Phytochemistry of *Psidium guajava* Essential Oil From Palestine: Targeting Oral Microbial Strains

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ABSTRACT

Introduction: The prevalence of oral diseases has been rising, presenting a significant challenge exacerbated by the increasing resistance of bacteria due to the inappropriate use of antibiotics. Given the typical financial challenges encountered in developing nations, there is an urgent requirement for alternative approaches and materials to address and manage these diseases. This study aims to investigate the chemical composition and antimicrobial properties of *Psidium guajava* leaf essential oil (PGL-EO) against specific oral bacterial species.
Methods: The extraction of PGL-EO was conducted using a Clevenger apparatus, and the chemical composition of PGL-EO was determined using gas chromatography-mass spectrometry (GC-MS) analysis. Various microbial strains from the American Type Culture Collection (ATCC) and clinically obtained oral strains were selected, and the antibacterial activity was assessed using the microdilution broth technique.
Results: The GC-MS analysis indicated the existence of 28 chemicals in PGL-EO, representing 97.76% of the total oil composition. The primary constituents found in this material consisted of l-limonene (31.70%), caryophyllene (27.31%), and caryophyllene oxide (6.90%). In addition, the major phytochemical class was sesquiterpene hydrocarbons (42.35%), followed by monoterpene hydrocarbons (32.30%) and oxygenated sesquiterpenoids (21.51%). PGL-EO exhibited remarkable antimicrobial efficacy against *Streptococcus mutans*, *Streptococcus mitis, Streptococcus sanguinis*, *Enterococcus faecalis, Lactobacillus acidophilus, and Candida albicans*..
Conclusions: The findings of this study strongly suggest that PGL-EO holds potential as an effective anti-plaque and anticary agent. Furthermore, the results obtained in this study consider PGL-EO a promising anticandidal agent in that it can contribute to treating and preventing oral diseases.

Introduction

Oral diseases pose significant challenges to public health, given the complexity of the oral microbiota, which comprises an average of 50–100 billion bacteria in adults, including approximately 200 prevalent bacterial species (Chandra Shekar et al., 2014; Krishnan et al., 2017).

Medicinal plants have long been recognised as valuable sources of raw materials for developing preventive and curative drugs (Qneibi et al., 2020). Herbal preparations are known for their mild yet effective nature, offering a favourable safety profile compared to synthetic pharmaceutical agents (Jaradat and Zaid, 2019; Kochikar Pai et al., 2015). The increasing incidence of adverse drug reactions and cost considerations has fuelled growing interest in traditional medicine from academia, the public, and government entities (Atanasov et al., 2021). Numerous natural products and their derivatives exhibit potent antimicrobial properties, making them popular in oral medicine (Jaradat et al., 2016; Koychev et al., 2017; Ukrainets et al., 2006). This is particularly relevant considering the financial burden and unintended side effects of dental and oral cavity inflammation treatments. Moreover, the rising prevalence of multidrug-resistant bacteria necessitates the exploration of novel infection prevention strategies for oral microbial infections (Khalil et al., 2019; Kochikar Pai et al., 2015).

Several plant extracts, including the EOs, have been extensively employed as antibacterial therapeutic agents. Examples include cinnamon,

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turmeric, cloves, ginger, black seed, eucalyptus, and garlic (Chandra Shekar et al., 2018; Elgamily et al., 2019).

Psidium guajava L. (Myrtaceae), commonly known as guava, is a perennial fruit-bearing tree with a rich therapeutic history. The bark and leaves of *P guajava* have been traditionally employed for their antidiarrheal and antidysenteric properties and their application in managing gum bleeding, sore throats, and mouth ulcers (Gutiérrez et al., 2008). Phytochemical studies on *P guajava* leaves have revealed the presence of various bioactive substances, including triterpenes, tannins, essential and fixed oils, flavonoids, saponin, lectins, carotenoids, vitamins, glycosides, alkaloids, and reducing sugar (Nayak et al., 2019; Seo et al., 2014).

Therefore, the present study aims to investigate the chemical composition of *Psidium guajava* L. leaf essential oil (**PGL-EO**) from Palestine for the first time and assess its antibacterial and antifungal activities against pathogenic microorganisms such as *Streptococcus mutans*, *Enterococcus faecalis*, *Streptococcus sanguinis*, *Pseudomonas aeruginosa*, *Lactobacillus acidophilus*, *Streptococcus mitis/oralis*, and *Candida albicans*. These microorganisms play a substantial role in developing severe gingivitis, dental caries, and plaque formation.

Materials and Methods

Collection of Plant Material

P guajava leaves were collected from the Tulkarm region of Palestine during the plant's flowering time in September 2020. Taxonomical characterisation was determined by the pharmacognosist Prof Nidal Jaradat in the Pharmacognosy Laboratory, Faculty of Medicine and Health Sciences, An-Najah National University, and kept within a voucher specimen number of Pharm-PCT-2720. Before the PGL-EO extraction, the green leaves were washed with distilled water and then dried for 14 days in the shade at room temperature. Finally, the dried leaves were saved for further use.

Extraction Method

The PGL-EO from leaves was extracted utilising the hydrodistillation technique connected with a Clevenger apparatus (Merck, USA) for 3 hours based on the method described in the British Pharmacopoeia. The obtained oil was chemically dried using calcium carbonate and stored at 4 °C in a dark chamber until further use (Dadalioğlu and Evrendilek, 2004). The obtained oil yield was 1.55% from the dried plant sample.

Gas Chromatography/Mass Spectrometry

Gas chromatography–mass spectrometry (**GC–MS**) analysis was completed using an HP 5890 series II gas chromatograph equipped with a Perkin Elmer Elite-5-MS (Perkin Elmer, USA) fused-silica capillary column (0.25 mm × 30 m, film thickness of 0.25 μm). Helium was used at a flow rate of 1.1 ml/min. The injector temperature was set at 250 °C, the oven temperature was programmed at 50 °C for 5 minutes, followed by a ramp of 4.0 °C/min–280 °C, and the detector flame ionisation detector was adjusted at 250 °C. The total running time was 62.50 minutes, and the solvent delay was from 0 to 4.0 minutes. The MS scan time was from 4 to 62.5 minutes, covering a mass range of 50.00–300.00 *m/z*. The mass spectra were collected under electronic ionisation conditions at 70 eV. In brief, retention indices (RIs) were calculated according to the injected standard mixture of normal alkanes (C_6–C_{27}) under the mentioned conditions using the following well-known equation approved by the International Union of Pure and Applied Chemistry (https://goldbook.iupac.org/terms/view/R05360).

The identification was also confirmed by comparison of their mass spectra with those stored in the Wiley7n.l MS computer library. The linear temperature-programmed RIs of all the constituents were calculated from the gas chromatogram by interpolation between bracketing n-alkanes using equation $RI = 100 \times (((tRi - tRz)/(tR(z + 1) - tRz)) + z)$, where z is the number of carbon atoms in the smaller n-alkane and tR(i), tR(z), and tR(z + 1) are the retention times of the desired compound, the smaller n-alkane, and the larger n-alkane, respectively.

Antimicrobial Activity

In this study, the broth microdilution method was employed to evaluate the antimicrobial activity of PGL-EO against six bacterial and one fungal strains, namely *S mutans* (American Type Culture Collection [**ATCC**] 25175), *E faecalis* (ATCC 29212), *S sanguinis* (ATCC 10556), *P aeruginosa* (ATCC 27853), *L acidophilus* (ATCC 4356), and *C albicans* (ATCC 90028) that were selected from the ATCC of Manassas, Virginia, USA. In addition to one clinical strain, *S mitis/oralis* was isolated from An-Najah National University Hospital and identified there using an automated microbiology bacterial identification and antimicrobial susceptibility system, VITEK2. The microbes were revived in the laboratory for the antimicrobial assay. Each strain was cultured in aerobic and anaerobic conditions using Brain Heart Infusion Agar and Broth media. A 96-microwell plate was used. Briefly, 50 μl of Brain Heart Infusion media were added per well and mixed with 50 μl of 200 mg/ml PGL-EO in the first well, and then serial dilution was applied to obtain a concentration gradient for the tested essential oil. Positive control (well 11) contains bacteria and media, and negative control (well 12) contains media alone. The tested oil control contains extracted oil and media to be sure there is no contamination or turbidity and changes are not due to the oil itself, so the oil was serially diluted in this control. The plate was then incubated at 37 °C for 24–48 hours. After inoculation, 50 μl of microbial suspension was added to each well (1–11) except for the tested oil control. The experiment was repeated three times, and the results were evaluated. The lowest concentration of PGL-EO, in which no visible microbial growth was observed, was considered the minimum inhibitory concentration value of the examined PGL-EO. Ciprofloxacin, vancomycin, and fluconazole were positive controls for antimicrobial activities (Balouiri et al., 2016; Jaradat et al., 2022).

Results

Chemical Compositions of Psidium guajava L. Leaf Essential Oil

The chemical composition of PGL-EO was established employing GC–MS apparatus; the analysis revealed the presence of 28 compounds in PGL-EO, accounting for 97.76% of the total oil content (Fig. 1), while two compounds were not identified and represented 2.24% of the total oil. Among this content, the major compounds were identified as limonene (31.70%), caryophyllene (27.31%), and caryophyllene oxide (6.90%). In addition, the major phytochemical class was sesquiterpene hydrocarbons (42.35%), followed by monoterpene hydrocarbons (32.30%), and oxygenated sesquiterpenoids (21.51%). A comprehensive overview of the identified components of PGL-EO, including their retention time, RI, area, percentages, and classifications, is provided in Table 1.

Antimicrobial Activity

The antimicrobial activity of PGL-EO was tested against *S mutans*, *S mitis*, *S sanguinis*, *E faecalis*, *L acidophilus,* and *C albicans*. The effect of PGL-EO on bacterial and fungal growth was examined at different concentrations ranging from 200 to 0.2 mg/ml. The antimicrobial screening results revealed that PGL-EO was effective against most of the tested microbial strains except *P aeruginosa,* as shown in Table 2. The PGL-EO demonstrates strong antimicrobial efficacy against most of the

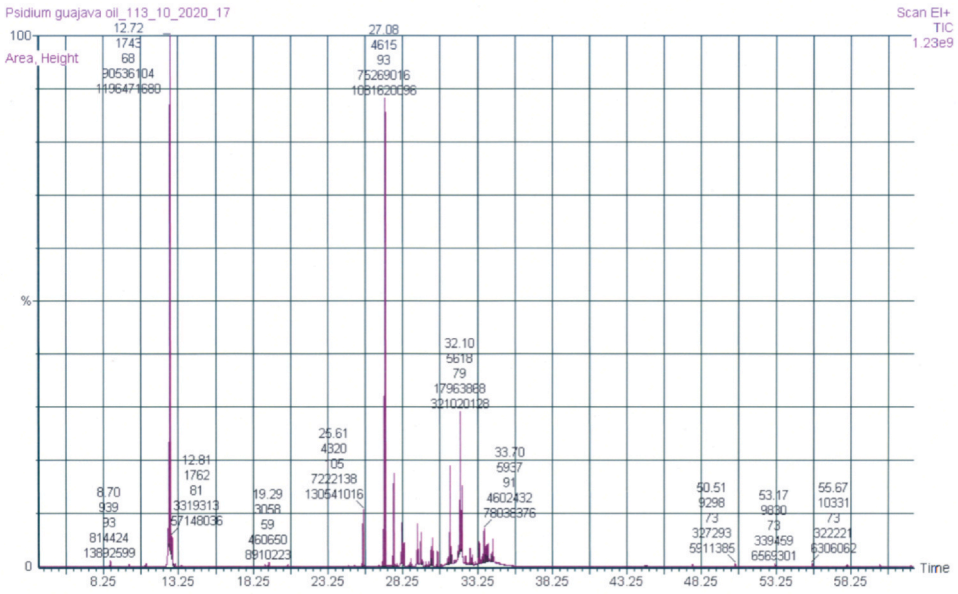

Fig. 1. Gas chromatography–mass spectrometry chromatogram of *Psidium guajava* leaf essential oil.

Table 1
The phytochemical compositions of *Psidium guajava* leaf essential oil.

	Name	RT	RI	% Area
1.	α-Pinene	8.7	933	0.31
2.	Benzaldehyde	9.95	963	0.11
3.	Myrcene	11.07	990	0.17
4.	Limonene	12.71	1 030	31.70
5.	1,8-Cineole	12.81	1 033	1.14
6.	(Z)-β-Ocimene	13.02	1 038	0.12
7.	2-Allyl phenol	19.03	1 190	0.09
8.	α-Terpineol	19.29	1 196	0.16
9.	cis-ρ-Mentha-1(7),8-dien-2-ol	20.57	1 232	0.10
10.	α-Copaene	25.61	1 378	2.78
11.	β-Caryophyllene	27.08	1 423	27.31
12.	Aromadendrene	27.66	1 442	4.88
13.	Alloaromadendrene	28.33	1 463	1.08
14.	Germacrene D	28.8	1 478	0.45
15.	β-Selinene	29.248	1 492	1.94
16.	Valencene	29.47	1 498	1.78
17.	δ-Cadinene	30.15	1 522	0.56
18.	cis-Calamenene	30.24	1 525	0.87
19.	trans-Cadina-1,4-diene	30.57	1 536	0.71
20.	E-Nerolidol	31.41	1 565	5.50
21.	Caryophyllene oxide	32.1	1 588	6.90
22.	Globulol	32.21	1 592	2.78
23.	Humulene epoxide II	32.65	1 604	0.75
24.	Muurola-4,10(14)-dien-1-β-ol	33.38	1 633	1.78
25.	Caryophylla-4(12),8(13)-dien-5-β-ol	33.69	1 644	1.11
26.	Cubenol	33.82	1 653	0.49
27.	Himachalol	33.95	1 653	0.51
28.	Intermedeol	34.28	1 665	1.69
Total identified				97.76
Phytochemical classifications				
Monoterpene hydrocarbons				32.30
Oxygenated monoterpenoid				1.40
Sesquiterpene hydrocarbons				42.35
Oxygenated sesquiterpenoids				21.51
Others				0.20
ND				2.24
Total				100.00

NI, not identified; RI, retention index; RT, retention time.

tested microbial strains, having the highest antimicrobial potential detected against *L acidophilus* and *S mitis/oralis*, with MIC values of 0.39 mg/ml.

Discussion

Periodontitis and gingivitis are inflammatory disorders caused by the accumulation and persistence of microbial biofilms within the oral cavity. The initial expression of this inflammatory response is observed in gingivitis (Matthews, 2014). While the causal link between dental plaque and gingivitis is well-established, the persistent prevalence of gingivitis underscores the inadequacy of current mechanical interventions in managing oral biofilms (Tonetti et al., 2015). This inadequacy has led to the widespread use of antimicrobial mouth rinses as adjuncts to clinical prescriptions. However, the long-term use of chemotherapeutic agents has faced scrutiny due to their unfavourable effects, highlighting the need for alternatives that can be employed over extended periods (Figuero et al., 2019).

Given the accessibility, feasibility, and ease of harvesting plant-derived materials like leaves, oils, and extracts, they hold immense potential for supporting traditional therapeutic pharmaceuticals.

In this study, we specifically selected microorganisms that play a role in the development and progression of dental caries. *S mutans* is the predominant microorganism associated with the onset of human dental caries (Krishnakumar et al., 2002). Conversely, *E faecalis* is frequently implicated in endodontic infections, while *L acidophilus* primarily contributes to the development of caries rather than its initiation (Cogulu et al., 2007). Our investigation commenced by evaluating the efficacy of PGL-EO.

The antibacterial efficacy of organic and aqueous PGL extracts has been extensively documented (Amaliya et al., 2018; Díaz-de-Cerio et al., 2017). Previous studies have reported the therapeutic effects of ethanolic and aqueous guava extracts in treating conditions such as peritonitis and gingivitis (Seo et al., 2014; Shetty et al., 2018). It is worth noting that a study by Wang et al. (2017) delved into PGL-EO in China, revealing differences in the identified compounds. Notably, their finding indicated β-caryophyllene (17.17–31.38%), ϒ-gurjunene (9.17–15.22%), and τ-cadinol (1.352–10.02%) as major constituents. Similarly, in Brazil, de Souza et al. (2018) observed a distinct composition ratio in the Brazilian PGL-EO. Similarly, de Souza et al. (de Souza et al., 2018) observed a distinct composition ratio in the Brazilian PGL-EO as well as β-selinene (13.83%), α-humulene (10.9%), caryophyllene oxide (9.09%), α-selinene (8.32%), β-caryophyllene (7.61%), and humulene epoxide II (7.26%) were the major oil components. Moreover, the GC–MS profile of the PGL-EO from Nepal revealed the presence of seventeen compounds, with limonene (51.3%), eucalyptol (21.3%),

Table 2
Antimicrobial effects (MICs) of *Psidium guajava* leaf essential oil, ciprofloxacin, vancomycin, and fluconazole (mg/ml).

Samples	Bacterial isolates						Fungus (Yeast)
	S sanguinis	*S mutans*	*L acidophilus*	*E faecalis*	*P aeruginosa*	*S mitis/oralis*	*C albicans*
	ATCC 10556	ATCC 25175	ATCC 4356	ATCC 29212	ATCC 27853	Clinical isolate	ATCC 90028
PGL-EO	3.125	0.39	3.125	3.125	Resistance	0.39	12.5
Vancomycin	0.0005	0.001	0.001	0.002	0.0002	0.0005	-
Ciprofloxacin	-	-	-	-	0.0005	-	-
Fluconazole	-	-	-	-	-	-	0.0005

ATCC, American Type Culture Collection; PGL-EO, *Psidium guajava L. leaf essential oil.*

caryophyllene oxide (6.2%), β-caryophyllene (5.6%), and nerolidol (4.5%) being the trending constituents (Mandal et al., 2022).

In contrast to the two prior studies, our research identified the major compounds in Palestinian PGL-EO as limonene (31.70%), β-caryophyllene (27.31%), and caryophyllene oxide (6.90%).

These variations in chemical composition within the same plant species from different regions are commonplace and attributed to factors such as chemotaxonomy, physiological age, harvesting seasons, geographical location, and extraction methods (de Souza et al., 2018).

The antimicrobial activity of PGL extract has been shown to reduce the hydrophobicity of oral pathogenic bacteria, thereby inhibiting their adhesion to tooth surfaces. These properties position PGL-EO as potential natural antiplaque agents, as they can hinder bacterial adherence, co-aggregation, and the growth of dental plaque bacteria without disrupting oral cavity homoeostasis. Thus, PGL holds promise as a valuable supplement to periodontal therapy (Ravi and Divyashree, 2014).

Numerous studies have assessed the antibacterial efficacy of PGL aqueous and methanol extracts and demonstrated antibacterial properties, inhibiting the growth of various bacteria strains, including *Staphylococcus aureus*, *Escherichia coli,* and *P aeruginosa* (Vignesh et al., 2017). Additionally, methanolic, aqueous, acetone-water, spray-dried extracts, and PGL-EO have exhibited inhibitory activity against both Gram-positive and Gram-negative bacteria (Biswas et al., 2013), as well as fungi (Díaz-de-Cerio et al., 2017).

Due to their broad-spectrum bactericidal activity, safety, and low toxicity (Kim et al., 2013), limonene isomers have broad application prospects in antibacterial and food preservation. In addition, limonene isomers can significantly inhibit the activity of gram-negative and gram-positive bacteria and fungi (Hsouna et al., 2011). Previous research has confirmed that limonene damages the cell membranes of both Gram-positive and Gram-negative bacterial cells, resulting in the leakage of intracellular materials and, eventually, cell death (Gupta et al., 2021).

Our research findings indicate that Palestinian PGL-EO has potent antimicrobial properties, with the highest inhibitory effect noted against clinical and ATCC strains of *S mitis* and *S mutans* (0.39 mg/ml). Actually, Palestinian PGL-EO showed potent activity against *S sanguinis*, *L acidophilus,* and *E faecalis* (3.125 mg/ml), along with notable antifungal activity against *C albicans* (12.5 mg/ml), but *P aeruginosa* was highly resistant to PGL-EO.

Conclusion

We identified several key compounds in PGL-EO through qualitative and quantitative analysis, including limonene, β-caryophyllene, and caryophyllene oxide. Our findings demonstrated that PGL-EO exhibited potent antibacterial and antifungal activities even at low concentrations. It was particularly effective against bacterial strains such as *S mutans*, *S sanguinis, L acidophilus*, and *E faecalis*. Furthermore, it demonstrated robust antifungal activity against *C albicans*. Based on these results, we conclude that PGL-EO contains highly active components that protect against bacteria and fungi. This suggests its potential application as an antiplaque and anticary agent, offering promising possibilities for treating and preventing oral diseases. In addition, these findings support the growing interest in natural products and herbal extracts as alternative approaches to oral healthcare, particularly in resource-limited settings. Future studies, including clinical trials involving a broader population, are recommended to evaluate the efficacy and safety of PGL-EO. Its potential use extends beyond mouthwashes and toothpaste, as it effectively inhibits bacteria and fungi associated with pathologies such as caries, tooth decay, gum disease, and periodontitis. Additionally, PGL-EO holds promise as a potential treatment for drug-resistant bacterial infections, serving as an additional therapeutic option.

Ethical approval

Not applicable.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

CRediT authorship contribution statement

Manar Abdalrazeq and **Nabil Massad:** Writing – review & editing. **Nawaf Al-Maharik:** Writing – review & editing, Formal analysis. **Nidal Jaradat:** Writing – review & editing, Methodology, Conceptualization. **Mohammad Qadi:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Masa Batanjeh** and **Shatha Rabaya:** Writing – review & editing, Investigation. **Hammam Tomeh:** Writing – original draft, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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