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# Evaluation and Comparison of the Antimicrobial Effects of Portulaca oleracea Seed Fatty Acids and Hydroalcoholic Extracts on Oral Pathogens

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## Abstract

# Background

Dental caries and periodontal disease remain prevalent oral conditions. The increasing prevalence of antimicrobial resistance due to antibiotic overuse underscores the need for alternative agents. *Portulaca oleracea* L. (purslane) exhibits anti-inflammatory, antioxidant, and antibacterial properties. This study compared the in vitro antimicrobial activity of hydroalcoholic extracts from purslane leaves and seeds and seed-derived fatty acids against selected oral pathogens.

# Materials and Methods

Hydroalcoholic extracts were prepared by maceration in 95% ethanol, and fatty acids were extracted with n-hexane. Antimicrobial activity was evaluated against *Streptococcus sobrinus* PTCC 1601, *Streptococcus sanguinis* PTCC 1449, *Candida albicans* ATCC 10231, *Streptococcus mutans* ATCC 35668, and *Lactobacillus acidophilus* ATCC 4356 using agar well diffusion and broth microdilution assays. MIC and MBC/MFC were determined in triplicate. Chlorhexidine (CHX; 0.12%) and nystatin (100,000 units) served as positive controls, and DMSO as a negative control. Principal constituents were identified by HPLC and GC–MS. Data were analyzed using ANOVA and Games–Howell tests.

# Results

HPLC detected caffeic acid in hydroalcoholic extracts, and GC–MS identified linolenic acid methyl ester as the predominant compound in seed fatty acids. All preparations inhibited tested pathogens, although CHX and nystatin were more effective ($P < 0.05$). Seed fatty acids showed the lowest MIC values ($P < 0.05$), with MIC/MFC of 1.4/2.8 mg/mL against *C. albicans*. Leaf extract demonstrated greater activity than seed extract and was most active against *Streptococcus* species, particularly *S. mutans*, and least active against *L. acidophilus*.

# Conclusion

Purslane seed fatty acids exhibit stronger antimicrobial activity than hydroalcoholic extracts but remain less potent than CHX or nystatin, supporting potential adjunctive use in oral hygiene formulations.

## Background

Dental caries and periodontal disease represent major public health challenges worldwide.

(1), particularly in low-income countries, where they constitute a "silent epidemic" affecting both children and adults (2, 3). Effective control of dental plaque is essential to limit gingival inflammation, caries

progression, and halitosis-associated microorganisms (2).

Frequent intake of dietary carbohydrates, especially sugars, elevates oral acidity and disrupts the symbiotic oral microbiome, promoting dysbiotic biofilm formation and enamel demineralization (2). Acidogenic and acid-tolerant species strongly linked to caries include *Streptococcus mutans*, *Lactobacillus spp*., *Actinomyces*, *Bifidobacterium*, and *Scardovia* (2). Microbial composition varies with tooth site, caries extent, and disease progression rate (2). The multifactorial etiology of dental caries complicates prevention and management (2).

Topical fluoride has substantially reduced decay rates, yet access remains limited by cost(2). Chlorhexidine (CHX), the most commonly used mouthwash and a long-standing gold standard in dentistry, has limitations, including taste alteration, tooth staining, burning sensation, mucosal ulcers, xerostomia, and parotid swelling (3). Routine antibiotic administration fosters resistance, necessitating alternatives to target acidogenic bacteria such as *S. mutans* (4). Natural, edible plant-derived agents offer promise for modulating the cariogenic microbiome with fewer adverse effects.

Medicinal and edible plants, especially nutrient-dense superfoods, have attracted growing interest (5). *Portulaca oleracea L.* (purslane) qualifies as a superfood owing to its rich nutritional composition (6).

This widespread annual succulent adapts to varied climates, featuring decumbent stems usually under 30 cm, smooth, rounded leaves, small yellow five-petaled flowers (with minor geographic variation), and a protective waxy cuticle on aerial parts (7, 8). Capsules enclose tiny black seeds. Cultivation succeeds in diverse soils with adequate humidity, sunlight, and temperatures above 25°C (8). It shares a close relation with the ornamental *Portulaca grandiflora*, common across Europe, Asia, and America (8).

Purslane serves as a traditional edible herb (9–12). Moreover, displays multiple pharmacological activities, including hypolipidemic, anti-aging, anti-inflammatory (13), antioxidant, analgesic, wound-healing(14, 15), antibacterial (16, 17), hypoglycemic, hypocholesterolemia, and antitumor effects (18). These derive from key constituents such as unsaturated fatty acids (19), terpenoids (20), coumarins, flavonoids (21), and alkaloids (22–24). *Purslane* is a notable dietary source of omega-3 fatty acids (9, 25).

Medicinal plants have historically been used to treat microbial infections (26). Inappropriate antibiotic prescribing accelerates the development of resistance (27, 28). In dentistry, overuse, poor adherence, and misuse of oral antiseptics contribute to the rising incidence of antimicrobial resistance (29). Plant-based alternatives may help mitigate this trend. The present study examines the in vitro antimicrobial activity of hydroalcoholic extracts from *P. oleracea* seeds and leaves, together with seed-derived fatty acids, against oral pathogens, to support resistance prevention and selective targeting of pathogenic microbes while preserving beneficial flora.

# Materials and Methods

# Plant Material Collection and Authentication

Fresh aerial parts, including leaves and seeds, of *P. oleracea* were obtained from the Medicinal Plant Market in Tehran, Iran, during the flowering season from June to August 2023. Plant material was authenticated at the Herbarium Department, Faculty of Pharmacy, University of Tehran (voucher No. PMP-4614).

## Preparation of Crude Extracts

# Drying and Powdering

Leaves and seeds were separated, rinsed with distilled water, and shade-dried at 25°C for seven days to constant weight (30). Dried samples were ground separately using an IKA® analytical mill equipped with a 0.5 mm sieve and stored in amber glass containers until extraction (31).

## Oil extraction

Oil was extracted using n-hexane at a 1:4 (w/v) ratio with continuous agitation in a shaker at room temperature under dark conditions for 36 h. The solvent was removed under reduced pressure using a rotary evaporator at 40°C. Extracted oil was stored at − 18°C until further analysis (32).

## Hydroalcoholic Extraction

Plant metabolites were extracted using a hydroalcoholic solvent composed of 95% ethanol and deionized water at an 80:20 (v/v) ratio, selected based on preliminary optimization trials (Abu-Reidah et al., 2019). Approximately 20 g of powdered plant material was immersed in 60 mL of solvent and agitated at 150 rpm at room temperature for 24 h (Schreck & Melzig, 2021). Suspensions were filtered sequentially through Whatman No. 1 filter paper, followed by a fine membrane filter to obtain clear extracts. Filtrates were concentrated under reduced pressure at 40–50°C using a rotary evaporator. Residual moisture was removed by freeze-drying. Dried extracts were stored at − 20°C until further analysis(33).

# Phytochemical Profiling

**GC–MS Analysis of Purslane Seed Oil**

Gas chromatography–mass spectrometry (GC–MS) analysis of the seed oil was performed on an Agilent 7890 gas chromatograph coupled to an Agilent 5977 mass selective detector. Separation occurred on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Samples were injected in split mode (ratio 1:100). The oven temperature program began at 60°C (no hold), ramped at

3°C $min^{-1}$ to 250°C, and held for 10 min. Injection volume was 1 µL, with immediate vaporization at the inlet (250°C). Helium served as carrier gas at 1 mL $min^{-1}$. Mass spectra were acquired at 70 eV in electron ionization mode.

Compounds were identified by comparing mass spectra with those in the Wiley and NIST MS 2.0 libraries, confirming names, molecular weights, and structural features.

## HPLC Analysis of Purslane Seed and Leaf Extracts

High-performance liquid chromatography (HPLC) was performed using an Agilent 1260 system equipped with a variable-wavelength detector (VWD). Separation was achieved on an Agilent Eclipse C18 column (250 × 4.6 mm, 5 µm, 100 Å). The mobile phase consisted of solvent A (0.1% v/v formic acid in Milli-Q ultrapure water) and solvent B (HPLC-grade methanol). A linear gradient elution was applied: 0–5 min, 30% B; 5–20 min, 30–70% B; 20–30 min, 70% B; 30–35 min, 30% B. Flow rate was maintained at 1.0 mL $min^{-1}$. Detection occurred at 280 nm for gallic acid and caffeic acid. Injection volume was 20 µL; samples were passed through a 0.45 µm PTFE syringe filter prior to analysis. Quantification of major bioactive compounds relied on external calibration with authentic standards.

## Microbial Strains and Culture Conditions

Antimicrobial activity was assessed against oral pathogens obtained from the Persian Type Culture Collection (PTCC), Tehran, Iran, and the American Type Culture Collection (ATCC): *S. sobrinus* PTCC 1601, *S. sanguinis* PTCC 1449, *C. albicans* ATCC 10231, *S. mutans* ATCC 35668, and *L. acidophilus* ATCC 4356.

*L. acidophilus* ATCC 4356 was cultured anaerobically in Man–Rogosa–Sharpe broth (MRSB) at 37°C for 48 h. The culture was then plated on MRS agar and incubated at 37°C for 24 h. Single colonies were re-isolated under identical conditions and stored in Tryptic Soy Broth (TSB) supplemented with 20% glycerol.

Streptococcus strains (*S. mutans* ATCC 35668, *S. sanguinis* PTCC 1449, *S. sobrinus* PTCC 1601) were grown in TSB at 35–37°C for 48 h under microaerophilic conditions (5–7% $CO_2$) in a candle jar. Following incubation, cultures were plated on 5% sheep blood agar and incubated at 37°C in 5–7% $CO_2$ for 24 h. Isolated colonies were subcultured and preserved in TSB with 20% glycerol.

*C. albicans* ATCC 10231 was initially cultured on Sabouraud Dextrose Agar (SDA) aerobically at 37°C for 24 h. Pure colonies were obtained by subculture under the same conditions and stored in Sabouraud Dextrose Broth (SDB) containing 20% glycerol.

# Antimicrobial Assays

# Agar Well Diffusion (Qualitative Screening)

The antimicrobial activity of purslane leaf and seed hydroalcoholic extracts, together with seed-derived fatty acids, was preliminarily screened against selected oral pathogens using the agar well diffusion method (performed in triplicate). Variable zones of growth inhibition were observed, supporting the potential of purslane-derived preparations as natural agents against oral microorganisms.

Standardized microbial suspensions were prepared at $1.5 \times 10^8$ CFU/mL (equivalent to 0.5 McFarland standard) for *L. acidophilus*, *S. mutans*, *S. sanguinis*, *S. sobrinus*, and *C. albicans*. Suspensions were evenly inoculated onto appropriate media: MRS agar for *L. acidophilus*, Mueller-Hinton agar (MHA) supplemented with 5% sheep blood for the Streptococcus species, and SDA for *C. albicans*. Swab inoculation ensured uniform confluent growth suitable for susceptibility testing (34).

Wells of 6 mm diameter were punched into the inoculated agar plates using a sterile sampler head, with adequate spacing between wells. To prevent leakage, each well received 50 µL of molten agar (50°C) as a base sealant. Test samples (100 µL) of purslane leaf extract, seed extract, or seed fatty acids were then added to individual wells (35). Control wells contained 100 µL of 10% dimethyl sulfoxide (DMSO) as a negative control, 0.12% CHX as a positive control for bacteria, or 100,000 IU nystatin as a positive control for *C. albicans*.

Plates were pre-incubated at 25°C for 30–60 min to allow initial diffusion of the test substances. Incubation followed at 37°C for 24 h: bacterial cultures under microaerophilic conditions (candle jars, 5–7% $CO_2$) and yeast cultures aerobically. After incubation, antimicrobial effects were quantified by measuring inhibition zone diameters (in mm) around each well with precision.

# Broth Microdilution

*Purslane* leaf and seed extracts (3 mL each) were diluted 1:2 in distilled water containing 10% DMSO, yielding final concentrations of 25 mg/mL for seed extract and 50 mg/mL for leaf extract. Seed-derived fatty acids were tested at 100 mg/mL. Five sterile 96-well microplates were prepared with 90 µL of pathogen-specific broth in each well, except for the last row, which was reserved for controls. Mueller–Hinton broth was used for streptococci, Man–Rogosa–Sharpe broth for *L. acidophilus*, and SDB for *C. albicans*.

The first row of each plate, comprising 12 wells, was divided into four triplicate groups: 90 µL of seed-derived fatty acids, 90 µL of leaf extract, 90 µL of seed extract, and 90 µL of control solution consisting of 0.12% CHX for bacteria or 100,000 IU/mL nystatin for yeast. Two-fold serial dilutions were prepared by transferring 90 µL from each well to the subsequent well within the same row to generate dilution series of extracts, fatty acids, and controls. The final 90 µL from each series was discarded into the last row. The final two wells served as controls: a growth control containing inoculated media without test substances and a sterility control containing uninoculated media.

To obtain a final microbial concentration of approximately $5 \times 10^5$ CFU/mL, the initial suspension of $1–2 \times 10^8$ CFU/mL was diluted 1:20 to approximately $1 \times 10^6$ CFU/mL. Subsequently, 10 µL of microbial suspension at $5 \times 10^6$ CFU/mL was added to each well, except sterility controls, resulting in a final concentration of $5 \times 10^5$ CFU/mL. Final concentrations of leaf extract in the wells were 22.5, 11.25, 5.62, 2.81, 1.40, and 0.70 mg/mL. Microplates, excluding those containing yeast, were incubated in a candle jar to maintain microaerophilic conditions. All plates were incubated at 37°C for 20 h. Wells were examined visually for turbidity and measured using an enzyme-linked immunosorbent assay reader.

Minimum bactericidal concentration (MBC**)** and minimum fungicidal concentration (MFC) were determined by subculturing 100 µL from clear wells onto microorganism-specific agar: blood agar for streptococci, Man–Rogosa–Sharpe agar for *L. acidophilus*, and SDA for *C. albicans*. After 24 h of incubation, the lowest concentration demonstrating no visible growth, corresponding to ≥ 99.9% microbial reduction, was recorded as the minimum bactericidal or fungicidal concentration (36).

# Statistical Analysis

Each experiment was performed in triplicate. Results were compared with control groups using one-way analysis of variance followed by the Games–Howell post hoc test. Statistical significance was defined as $p < 0.05$.

# Results

### HPLC Analysis of Purslane Leaf and Seed Extracts

HPLC equipped with a VWD set at 280 nm was used to identify and quantify major phenolic compounds in ethanol extracts of *P. oleracea* leaves and seeds. Extracts were diluted before injection to optimize peak resolution and prevent column overload. Gallic acid and caffeic acid standards were analyzed under identical conditions for calibration.

In the leaf extract chromatogram, distinct peaks were observed at 2.754 minutes for gallic acid and 10.040 minutes for caffeic acid. In the seed extract, corresponding peaks were observed at 2.701 minutes for gallic acid and 9.997 minutes for caffeic acid (Figs. 1 and 2).

Quantitative analysis, including standard deviations from replicate measurements, showed gallic acid concentrations of 0.91 ± 1.05 mg/g in leaves and 0.22 ± 1.93 mg/g in seeds. Caffeic acid concentrations were lower, measuring 0.12 ± 0.09 mg/g in leaves and 0.09 ± 1.21 mg/g in seeds (Table 1). These results are consistent with the reported phenolic composition of purslane, in which gallic acid represents a predominant antioxidant associated with anti-inflammatory and antimicrobial activity (37). The higher gallic acid content in leaves compared with seeds may reflect tissue-specific biosynthesis and may warrant further evaluation in future extraction optimization studies.

Table 1
Phenolic compound content (gallic and caffeic acids) in *purslane* leaf and seed extracts (mg/g dry weight)

| Solvent | Gallic acid (mg/g) | Caffeic acid (mg/g) |
|---|---|---|
| Leaf | 0.91 ± 1.05 | 0.12 ± 0.09 |
| Seed | 0.22 ± 1.93 | 0.09 ± 1.21 |

**GC-MS Analysis of Purslane Seed Oil**

GC–MS was used to profile fatty acid methyl esters (FAMEs) in purslane seed oil and characterize lipid composition. Thirteen major compounds were identified, with retention times (RT) ranging from 41.121 to 63.926 minutes, molecular masses of 256.24 to 382.381 Da, and area percentages totaling approximately 100% (Table 2 and Fig. 3).

Table 2
Relative percentage composition of fatty acid methyl esters in purslane oil identified by GC-MS.

| Systematic Name | Common Name | R. T | Formula | Molecular weight | Area (%) |
|---|---|---|---|---|---|
| 7-Hexadecenoic acid, methyl ester, (Z)- | | 41.121 | $C_{17}H_{32}O_2$ | 268.24 | 0.17 |
| Hexadecanoic acid, methyl ester | Palmitic acid, methyl ester | 42.392 | $C_{17}H_{34}O_2$ | 270.256 | 24.7 |
| Hexadecanoic acid | Palmitic acid | 43.368 | $C_{16}H_{32}O_2$ | 256.24 | 0.54 |
| Hexadecanoic acid, 14-methyl-, methyl ester | | 44.179 | $C_{18}H_{36}O_2$ | 284.272 | 0.06 |
| Heptadecanoic acid, methyl ester | Margaric acid, methyl ester | 45.053 | $C_{18}H_{36}O_2$ | 284.272 | 0.13 |
| 9,12-Octadecadienoic acid methyl ester, (Z, Z)- | Linoleic acid, methyl ester | 47.612 | $C_{19}H_{34}O_2$ | 294.256 | 16.93 |
| 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- | Linolenic acid, methyl ester | 48.260 | $C_{19}H_{32}O_2$ | 292.24 | 49.33 |
| Octadecanoic acid, methyl ester | Stearic acid, methyl ester | 48.572 | $C_{19}H_{38}O_2$ | 298.287 | 3.48 |
| 9,12-Octadecadienoic acid (Z, Z)- | Linoleic acid | 49.75 | $C_{18}H_{32}O_2$ | 280.24 | 3.97 |
| Eicosanoic acid, methyl ester | Arachidic acid, methyl ester | 53.854 | $C_{21}H_{42}O_2$ | 326.318 | 0.43 |
| 9,17-Octadecadienal, (Z)- | | 56.42 | $C_{18}H_{32}O$ | 264.245 | 0.15 |
| Docosanoic acid, methyl ester | Behenic acid, methyl ester | 59.042 | $C_{23}H_{46}O_2$ | 354.35 | 0.11 |
| Tetracosanoic acid, methyl ester | Lignoceric acid, methyl ester | 63.926 | $C_{25}H_{50}O_2$ | 382.381 | 0.07 |
| . | | | | | |

The predominant fatty acid was 9,12,15-octadecatrienoic acid methyl ester (α-linolenic acid methyl ester, Z, Z, Z), accounting for 49.33% of the total area at an RT of 48.260 minutes ($C_{19}H_{32}O_2$, 292.24 Da). This omega-3 polyunsaturated fatty acid supports purslane as a source of essential lipids (38). Hexadecanoic acid methyl ester (palmitic acid methyl ester) was the second most abundant compound at 24.70% (RT 42.392 minutes, $C_{17}H_{34}O_2$, 270.256 Da). Other prominent constituents included 9,12-octadecadienoic acid methyl ester (linoleic acid methyl ester, Z, Z) at 16.93% (RT 47.612 minutes, $C_{19}H_{34}O_2$, 294.256 Da) and 9,12-octadecadienoic acid (linoleic acid, Z, Z) at 3.97% (RT 49.75 minutes, $C_{18}H_{32}O_2$, 280.24 Da). These omega-6 polyunsaturated fatty acids together comprised 20.90% of the total composition.

Minor constituents included octadecanoic acid methyl ester (stearic acid methyl ester, 3.48%), hexadecanoic acid (palmitic acid, 0.54%), eicosanoic acid methyl ester (arachidic acid methyl ester, 0.43%), and compounds present at < 0.2%, including 7-hexadecenoic acid methyl ester (Z) at 0.17% and tetracosanoic acid methyl ester (lignoceric acid methyl ester) at 0.07%. The profile indicates a polyunsaturated-to-saturated fatty acid ratio of approximately 4:1, with predominance of omega-3 fatty acids, consistent with reported hypolipidemic and anti-inflammatory effects of purslane (39).

**Antimicrobial Effects of Purslane Leaf and Seed Extracts and Seed-Derived Fatty Acids**

# Agar Well Diffusion Results

Agar well diffusion assays demonstrated distinct inhibition patterns. Purified fatty acids inhibited only *C. albicans*, producing a growth inhibition zone diameter of 16 mm (Fig. 4). Leaf extract showed broader anti-streptococcal activity, inhibiting *S. mutans* with a 15 mm inhibition zone (well diameter 6 mm), *S. sanguinis* with a 9 mm zone, and *S. sobrinus* with an 8 mm zone, each measured around 6 mm wells. These findings indicate selective antifungal activity of fatty acids and broader antibacterial activity of leaf extract against oral pathogens.

# MIC, MBC, and MFC Results

Leaf and seed hydroalcoholic extracts, together with seed-derived fatty acids, demonstrated antimicrobial activity against *S. mutans*, *L. acidophilus*, *S. sanguinis*, *S. sobrinus*, and *C. albicans* in the broth microdilution assay (Tables 3 and 4).

Table 3
Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of *purslane* leaf and seed extracts and seed fatty acids against bacteria

| **Microorganisms** | **MIC/MBC (mg/ml)** | | | | |
|---|---|---|---|---|---|
| | **Fatty acids** | **Hydroalcoholic extract** | | **CHX** | **DMSO** |
| | | **Leaf** | **Seed** | | |
| *S. mutans* | 2.8/2.8 | 2.8/2.8 | 11.2/11.2 | 0.5/1 | NA |
| *S. sanguinis* | 2.8/2.8 | 5.6/5.6 | 5.6/11.2 | 0.5/1 | NA |
| *S. sobrinus* | 2.8/2.8 | 5.6/5.6 | 5.6/11.2 | 0.5/1 | NA |
| *L. acidophilus* | 11.2/11.2 | 11.2/11.2 | 11.2/11.2 | 0.5/1 | NA |

Table 4
Minimum inhibitory (MIC) and fungicidal (MFC) concentrations of *purslane* leaf and seed extracts and seed fatty acids against yeast

| **Microorganisms** | **MIC/MBC (mg/ml)** | | | | |
|---|---|---|---|---|---|
| | **Fatty acid** | **Hydroalcoholic extract** | | **Nystatin** | **DMSO** |
| | | **Leaf** | **Seed** | | |
| *C. albicans* | 1.4/2.8 | 5.6/5.6 | 5.6/5.6 | 0.4/0.8 | NA |

# Results of Analysis of Variance for MIC

Analysis of variance (ANOVA) assessed differences among minimum inhibitory concentration (MIC) means for leaf extract, seed extract, and fatty acids. The F statistic of 5.783 reflected between-group variability relative to within-group variability. The *p*-value of 0.017 indicated a statistically significant difference among the group means. Post hoc Games–Howell analysis, performed due to unequal variances confirmed by Levene test ($p = 0.044$), showed significantly higher MIC values for seed extract compared with fatty acids (mean difference 5.35, 95% CI − 10.18 to − 0.51, $p = 0.036$). No other pairwise comparisons reached statistical significance (all $p > 0.05$). Leaf extract showed intermediate MIC values (Table 5 and Fig. 5).

Table 5
Pairwise comparisons of MIC values using Games-Howell test

| **Comparison** | **Mean Difference** | **95% CI** | **p-value** |
|---|---|---|---|
| Faty acid vs. Leaf | -3.66 | [-8.50, 1.18] | 0.115 |
| Faty acid vs. Seed | -5.35 | [-10.18, -0.51] | 0.036 |
| Leaf vs. Seed | -1.69 | [-7.25, 3.88] | 0.675 |
| **Notes.** CI: Confidence Interval; Values rounded to 2 decimals. | | | |

# Discussion

*P. oleracea* leaf and seed hydroalcoholic extracts, together with seed-derived fatty acids, demonstrated antimicrobial activity against *S. mutans*, *S. sanguinis*, *S. sobrinus*, *L. acidophilus*, and *C. albicans* in agar well diffusion and broth microdilution assays. CHX and nystatin showed significantly greater efficacy ($P < 0.05$). The MIC of seed fatty acids was significantly lower than that of hydroalcoholic extracts ($P < 0.05$).

GC–MS analysis indicated a favorable fatty acid composition in purslane seed oil, characterized by high levels of linolenic acid (omega-3, 49.33%) and linoleic acid (omega-6, 20.9%), along with saturated fatty acids (29.3%), predominantly palmitic acid (24.7%). This composition yields an omega-6 to omega-3 ratio of approximately 0.42, supporting its nutritional relevance and anti-inflammatory potential, with

possible applications in food, pharmaceutical, and cosmetic fields (40). The predominance of linolenic acid methyl ester (49.33%) aligns with previous reports (41, 42). Linolenic acid methyl ester represents a naturally occurring omega-3 polyunsaturated FAME present in plant oils and animal fats. This compound is used in biodiesel production and as an ingredient in dietary supplements (43). Seed-derived fatty acids demonstrated marked antifungal activity against C. albicans, consistent with findings reported by Petropoulos et al. (41) and Liu et al. (19).

Experimental evidence from in vitro and in vivo studies indicates that linolenic acid and its derivatives, administered alone or in combination with conventional agents, exhibit antimicrobial activity (40). Linolenic acid serves as a precursor of omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are considered essential dietary components (WHO) (40, 44). The human body metabolizes linolenic acid through enzymatic pathways involving desaturases and elongases. Fatty acids are initially synthesized in saturated form, followed by desaturation through insertion of double bonds at carbon positions 5, 6, and 9, and subsequent elongation by addition of two carbon units(40, 45). These metabolic reactions generate EPA and DHA (40).

Although desaturases and elongases are absent in the human oral cavity, the present findings indicate that linolenic acid, linoleic acid, and other fatty acids derived from purslane seeds exhibit antimicrobial activity against oral pathogens. Previous investigations suggest that bacteria modify membrane lipid composition by altering fatty acid profiles through endogenous biosynthetic pathways (40, 46). Fatty acid biosynthesis produces phospholipids required for electron transport, solute transport, initiation of DNA replication, and cell division (40, 46). In contrast to mammals, which rely on a multifunctional type I enzyme complex, bacteria use discrete type II enzymes for fatty acid synthesis (40, 47). Enoyl–acyl carrier protein reductase (FabI) mediates the final elongation step in this pathway. Inhibition of FabI reduces fatty acid elongation (46, 47) and may disrupt phospholipid function. Exogenous fatty acids, including DHA, oleic acid, linoleic acid, arachidonic acid, and palmitoleic acid, influence FabI activity (40, 47, 48). Unsaturated fatty acids at concentrations ranging from 0.05 to 0.40 mmol/L inhibit *Staphylococcus aureus* and *Streptococcus pyogenes* growth through FabI inhibition (40, 47).

Sun et al. reported antimicrobial effects of DHA and EPA against periodontal pathogens, including Porphyromonas gingivalis and Fusobacterium nucleatum, in both planktonic and biofilm forms, supporting their potential as adjuncts in periodontal disease management (49). Huang et al. further demonstrated that omega-3 polyunsaturated fatty acids and their ester derivatives, including EPA and DHA, exhibit antibacterial activity against oral pathogens such as *S. mutans*, *C. albicans*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*, suggesting benefits for oral health through combined antibacterial and anti-inflammatory mechanisms (50).

The stronger antimicrobial activity of purslane seed fatty acids compared with hydroalcoholic extracts of leaves and seeds suggests that linolenic acid may represent a principal active component, consistent with previous reports. Purslane seed oil, characterized by high linolenic acid content, ranks below

flaxseed and perilla seed oils as a source of alpha-linolenic acid and shows comparable levels to camelina seed oil (51).

Liu et al. reported that an organic acid extract of *P. oleracea* accelerated cutaneous wound healing, reduced bacterial load, and decreased inflammatory cell infiltration and cytokine expression, indicating potential activity against *Staphylococcus aureus* infections (10). In the present study, hydroalcoholic extracts of purslane leaves and seeds showed comparable antimicrobial activity against the tested oral pathogens, with the leaf extract exhibiting a slightly lower MIC. HPLC confirmed the presence of gallic acid and caffeic acid in both leaf and seed extracts.

Previous studies indicate that phenolic compounds, including quercetin, myricetin, luteolin, tannic acid, gallic acid, ellagic acid, epigallocatechin gallate, and caffeic acid, exert activity against dental caries. Their roles in dentin remineralization, anti-inflammatory responses, and antibacterial effects have supported investigation in oral diseases (52). Inhibition of glucosyltransferase activity during biofilm formation represents a principal antibacterial mechanism of these compounds against cariogenic pathogens (53, 54).

Gallic acid, derived from the secondary metabolite shikimic acid, forms part of hydrolysable tannins in plants (55, 56). Evidence suggests that gallic acid enhances antibiotic efficacy and may exert synergistic effects (57). Lattanzio et al. reported that gallic acid inhibited sporulation and germination in saprophytic fungi (58). Lima et al. observed maximal growth inhibition of certain *C. albicans* strains at 500 µg/mL of pure gallic acid. Caffeic acid also demonstrates antimicrobial and antioxidant activity (55). Differences between the MICs reported in the present study and those described by Lima et al. may reflect the use of crude extracts with variable active compound content rather than purified gallic acid.

In this study, leaf and seed extracts showed minimum inhibitory and MBCs of 5.63 and 11.25 mg/mL, respectively, against *C. albicans*. These values exceed the 10 µg/mL MIC reported by Soliman et al. in 2017 (59). Variations in the extraction method and solvent concentration, including 95% ethanol versus 80% ethanol, as well as differences in plant origin and growth conditions, may account for these discrepancies. Differences in fungal strain may also contribute, as Soliman et al. evaluated *C. albicans* SC5314, whereas the present study used *C. albicans* ATCC 10328.

Leaf extract MICs against Gram-positive bacteria were 2.8 mg/mL for *S. mutans*, 5.6 mg/mL for *S. sobrinus*, 5.6 mg/mL for *S. sanguinis*, and 11.25 mg/mL for *L. acidophilus*. Yong-Kai Du et al. reported MICs of purslane leaf extract ranging from 10 to 25 mg/mL against *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* ATCC 10240, *Klebsiella pneumoniae* ATCC 10031, *Salmonella enterica* ATCC 14028, and *Enterobacter cloacae* ATCC 13047 (60). MICs were higher for Gram-negative species than for Gram-positive species. Values reported for Gram-positive bacteria, including *S. aureus* and *M. luteus*, were comparable to those observed in the present study for *S. sanguinis*, *S. sobrinus*, and *L. acidophilus* (5.6–11.25 mg/mL). Du et al. proposed that flavonoid extract penetration into the cytoplasm of *S. aureus* through membrane pores increased reactive oxygen species levels and induced bacterial DNA fragmentation, leading to cell death, which may explain the greater susceptibility of Gram-positive

bacteria. The outer membrane of Gram-negative bacteria may reduce extract permeability and contribute to higher MIC (60).

Liu et al. (2022) reported that purslane organic acid extract inhibited methicillin-resistant *S. aureus* in vitro and in vivo. Their study, conducted in China, showed an MIC of 12.5 mg/mL and an inhibition zone of 13.5 mm at 25 mg/mL, findings comparable to those observed in the present study. Minor differences in MIC values may be due to variations in Gram-positive bacterial genera and species, as well as differences in extraction methods (10).

Bae et al., in a 2004 Korean study titled “Antimicrobial Effect of Purslane Leaf Extracts on Foodborne Pathogens,” reported an MIC of 4000 ppm for ethyl acetate–extracted purslane against *S. aureus*, which is comparable to the MIC observed in the present study. Differences may reflect variations in the extraction solvent and bacterial species (61).

Mousavi et al. demonstrated significant antibacterial activity of purslane leaf and seed hydroalcoholic extracts and proposed potential use against drug-resistant bacteria. Their study, conducted in the Sistan region of southeast Iran, reported a highest MIC of 200 ppm for leaf extract and 100 ppm for seed extract. In contrast, the present study observed the lowest MIC value for the leaf extract. The MIC against *S. mutans* was 50 ppm, differing from values reported by Mousavi et al. and other investigations (62).

Leaf and seed extracts exhibited higher MIC and MBC values against *L. acidophilus* than against *S. mutans* and other streptococci. Given the strong adhesion capacity of *S. mutans* to tooth surfaces and its role in biofilm and dental plaque formation, together with evidence that *L. acidophilus* reduces *S. mutans* adhesion (63), this observation warrants consideration. The lower sensitivity of *L. acidophilus* to purslane extracts may reflect prior colonization of adhesion sites, limiting subsequent streptococcal attachment (63). Selective activity against oral pathogens, with limited effect on Lactobacillus, suggests potential application in mouthwashes or topical formulations to reduce streptococcal adhesion while preserving microbial balance.

These findings support further clinical evaluation and formulation studies targeting oxidative stress and microbial infections (12).

The agar well diffusion method, widely used for preliminary antimicrobial screening due to its simplicity, was applied to measure inhibition zones of seed fatty acids against C. albicans and of a hydroalcoholic leaf extract against *S. mutans*, *S. sanguinis*, and *S. sobrinus*. The largest inhibition zones were observed for seed fatty acids against *C. albicans* and for leaf extract against *S. mutans*. The absence of inhibition zones for the seed hydroalcoholic extract may reflect its lower concentration compared to the leaf extract. The lack of bacterial inhibition by fatty acids may relate to limited diffusion in blood agar, despite adequate diffusion in SDA. Evidence indicates that agar well diffusion is more suitable for preliminary screening than definitive antimicrobial assessment. Outcomes are influenced by lipophilicity, agar concentration, medium pH, microbial strain, and extract concentration and stability (64). Although agar

well diffusion provided qualitative screening, MIC and MBC determinations yielded more reliable quantitative data under controlled conditions (64).

This study has limitations. The absence of standardized extract concentrations limits direct comparisons with other investigations. The in vitro design restricts extrapolation to the oral environment. The agar diffusion method may have underestimated fatty acid activity because of their lipophilicity. The proposed mechanism of FabI inhibition was not experimentally validated. A comprehensive toxicity evaluation was not performed, which is essential for therapeutic development.

# Conclusion

*Purslane* extracts, particularly seed-derived fatty acids enriched in linolenic acid, demonstrated antimicrobial activity against oral pathogens in vitro with comparatively limited effects on beneficial bacteria. These findings suggest potential application as a natural component in oral health formulations; however, caution is warranted in interpretation. Study limitations include the absence of clinical validation, lack of standardization of extracts, reliance on agar-based screening methods, and the omission of detailed toxicity and mechanistic analyses. Further investigation of complex biological systems, along with standardized extraction procedures and comprehensive immunological assessment, is required before therapeutic development can be considered.

# Abbreviations

AA
Arachidonic acid
CHX
Chlorhexidine
DHA
Docosahexaenoic acid
DMSO
Dimethyl sulfoxide
EPA
Eicosapentaenoic acid
GC-MS
Gas chromatography mass spectrometry
HPLC
High-performance liquid chromatography
MBC/MFC
Minimum bactericidal/fungicidal concentration
MIC
Minimum inhibitory concentration
MHA

Mueller-Hinton agar
MRSB
Man-Rogosa-Sharpe broth
SDA
Sabouraud Dextrose Agar
TSB
Tryptic Soy Broth
MBC
Minimum bactericidal concentration
MFC
Minimum fungicidal concentration
*P. oleracea*
*Portulaca oleracea*
*S. mutans*
*Streptococcus mutans*
*S. sobrinus*
*Streptococcus sobrinus*
*S. sanguinis*
*Streptococcus sanguinis*
*C. albicans*
*Candida albicans*
*L. acidophilus*
*Lactobacillus acidophilus*
FabI
Enoyl–acyl carrier protein reductase
VWD
Variable Wavelength Detector
PTCC
Persian Type Culture Collection
ATCC
American Type Culture Collection
SDB
Sabouraud Dextrose Broth

# Declarations

### Author Contributions

A.H.K.: Investigation; methodology; writing, original draft.

M.I.: Conceptualization; methodology; investigation; formal analysis; data curation; writing, review, and editing.

Z.F.A.: Methodology; investigation; formal analysis; data curation; writing, original draft.

M.H.A.: Methodology; investigation; validation; writing, review, and editing.

A.H.: Conceptualization; project administration; supervision; methodology; investigation; data curation; writing, review, and editing.

M.H.: Methodology; investigation; formal analysis; writing, review, and editing.

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**Data Availability**

All data generated or analyzed during this study are presented in the tables and figures within this manuscript.

**Ethical Approval**

The Research Ethics Committee of Shahed University approved this study (Approval ID: IR.SHAHED.REC.1403.034).

**Consent for Publication**

Not applicable.

**Competing Interests**

The authors declare no conflicts of interest.

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# Figures

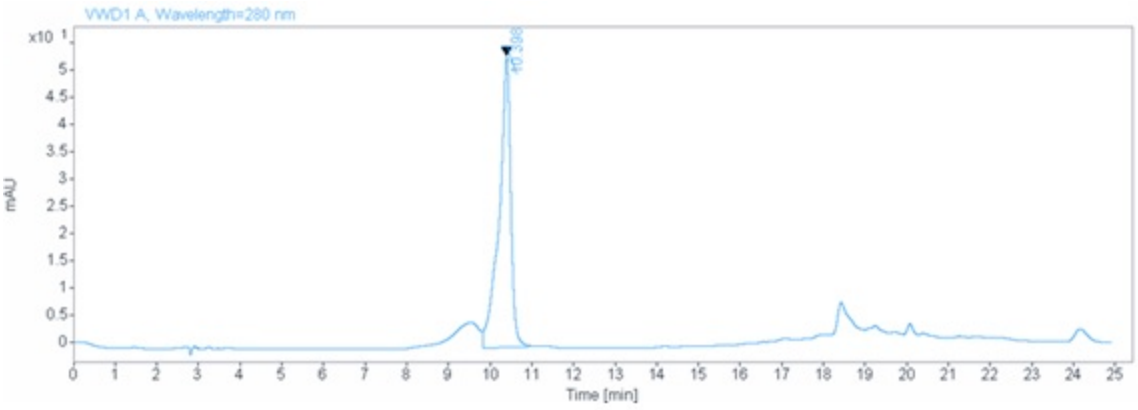



**Figure 1**

HPLC chromatogram of caffeic acid standard.

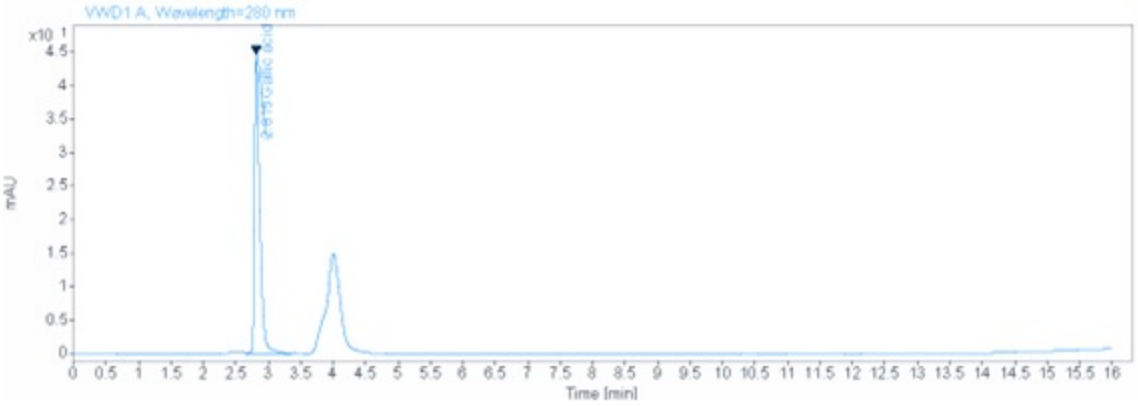



**Figure 2**

HPLC chromatogram of gallic acid standard.

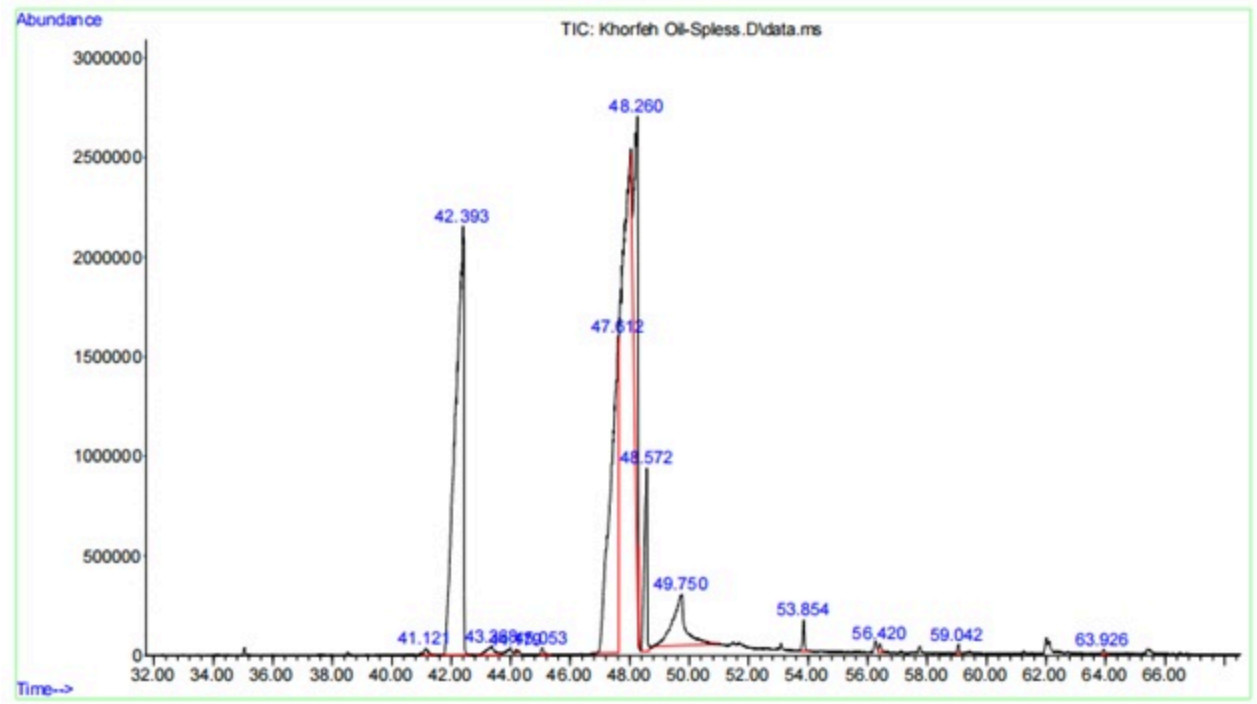



**Figure 3**

GC–MS chromatogram of purslane oil.

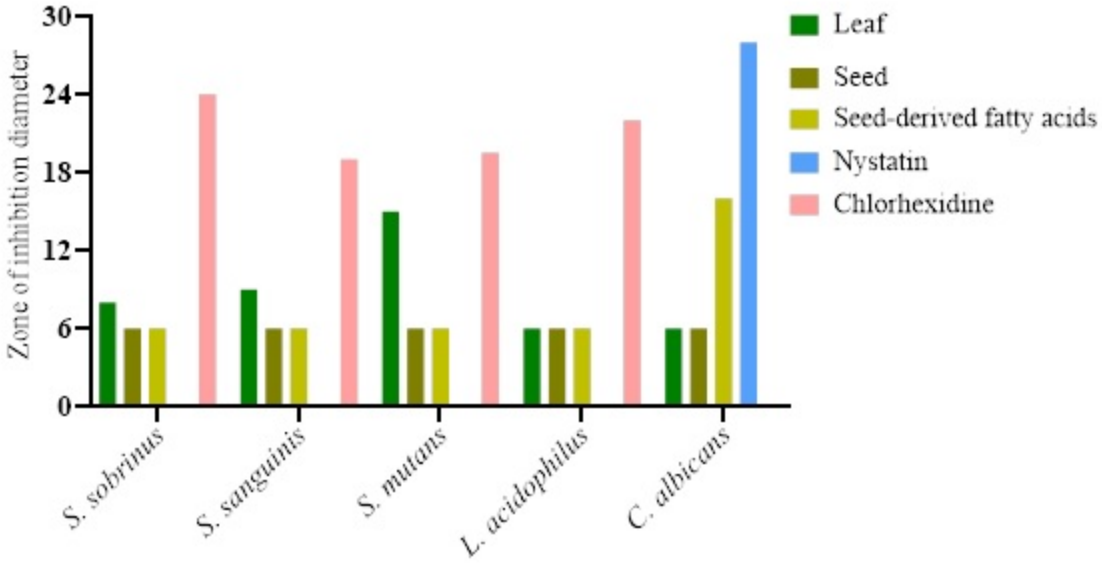



**Figure 4**

Growth inhibition zones of purslane leaf and seed hydroalcoholic extracts and seed-derived fatty acids against oral pathogens, compared with chlorhexidine and nystatin.

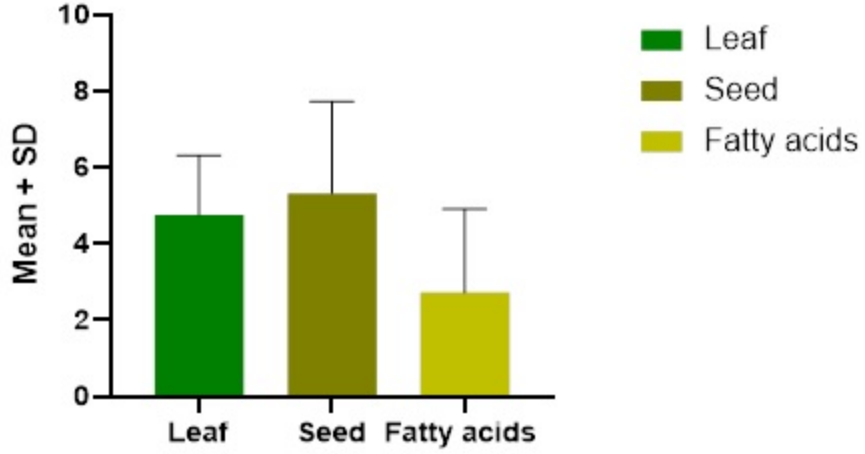



**Figure 5**

Mean ± standard deviation of MIC values for purslane leaf and seed extracts and seed-derived fatty acids against bacterial and yeast strains.