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Original Article

Mediterranean Herb Extracts as Natural Antimicrobial and Antibiofilm Agents against Oral Pathogens and Streptococcus Mutans

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ABSTRACT

Rising antibiotic resistance has prompted the search for natural alternatives. This study aimed to assess the antibacterial and antibiofilm effects of 16 extracts derived from Mediterranean herbs. Extracts were characterized by High-Performance Thin Layer Chromatography. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Achillea taygetea, Cistus creticus ssp. creticus, Cistus monspeliensis, Lavandula stoechas, Mentha aquatica, Mentha longifolia, Origanum vulgare, Phlomis cretica, Rosmarinus officinalis, Salvia sclarea, Satureja parnassica, Satureja thymbra, Sideritis euboea, Sideritis syriaca, Stachys spinosa, and Thymus longicaulis were tested against eight oral bacterial species and Candida albicans. Antibiofilm activity against Streptococcus mutans was determined using microtiter plate assays. All extracts strongly inhibited obligate anaerobic bacteria. At ≥0.15 mg/ml, they demonstrated moderate-to-strong antibiofilm activity, similar to chlorhexidine (CHX) against S. mutans. Among the extracts, R. officinalis (MIC: 0.01–0.06 mg/ml) and O. vulgare (MIC: 0.04–1.25 mg/ml) exhibited the greatest antibacterial activity. Furthermore, R. officinalis and L. stoechas effectively suppressed S. mutans biofilm formation at 0.15 mg/ml. These findings suggest that the tested Mediterranean herb extracts may serve as natural agents with both antibacterial and antibiofilm properties. Extracts from Mediterranean herbs could provide a natural approach to controlling oral pathogens and biofilm formation, representing a potential alternative therapy in the context of rising antibiotic resistance.

Keywords: Mediterranean herb extracts, Streptococcus mutans, Oral pathogens, Antimicrobial, Antibiofilm

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Introduction

In recent decades, the emergence of antibiotic-resistant bacteria has made many infections increasingly persistent. This has intensified research into alternative antimicrobial strategies [1, 2]. In dental practice, chlorhexidine (CHX) is widely used for oral disinfection; however, it can be cytotoxic to host tissues and may contribute to bacterial tolerance through mechanisms such as membrane alteration, efflux pump activation, and resistance gene expression [3, 4]. Although CHX effectively targets S. mutans and other oral biofilms, its limitations highlight the need for alternative natural antibiofilm agents [5, 6]. Oral biofilms, which are up to 1,000 times less susceptible to antimicrobial agents than free-floating bacteria, are particularly difficult to eradicate in their deeper layers [7, 8].

The human oral cavity hosts approximately 700 bacterial species colonizing teeth, gingiva, and mucosal surfaces [9]. These species form biofilms that protect them from antimicrobials, reduce oxygen availability, and slow cell division in deeper layers [10, 11]. Biofilm formation begins when early colonizers such as Streptococcus spp., Actinomyces spp., Veillonella spp., and Neisseria spp. attach to the salivary pellicle [12, 13]. Later, Fusobacterium nucleatum creates an anaerobic microenvironment, facilitating colonization by strict anaerobes such as

Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and Prevotella intermedia [14].

Mediterranean plants have long been recognized for their medicinal value [15]. Many bioactive compounds, including phenolic acids, flavonoids, peptides, phenanthrenes, and terpenes, have been isolated and found to possess antimicrobial properties [16-20]. Metabolites such as phenolics, terpenoids, sulfur compounds, coumarins, quinones, and alkaloids are also effective as antibiofilm agents and quorum sensing inhibitors [21]. Plant extracts offer advantages over conventional antibiotics, including lower toxicity to human tissues, multi-targeted action, and reduced likelihood of inducing resistance [22-24]. Additionally, they often have antioxidant and anti-inflammatory properties, which support tissue healing and reduce inflammation during infections [25].

Plant-based remedies remain widely used, particularly in low-income countries, due to affordability and accessibility. Optimizing formulations by targeting specific molecular pathways can enhance their efficacy [26]. Several plant-derived oral care products have been used to manage dental caries, periodontitis, and gingivitis [27, 28]. Because plant extracts often contain complex mixtures of bioactive compounds, they can act synergistically to target pathogenic microbes at vulnerable stages [29]. Given these factors, further investigation into additional plant species, extraction methods, and compound interactions is urgently needed to fully harness the potential of natural antimicrobials for oral health.

Objective

This study aimed to examine the antimicrobial efficacy of sixteen Mediterranean plant extracts against various microorganisms. The ethyl acetate extracts from Achillea taygetea, Cistus creticus, Cistus monspeliensis, Lavandula stoechas, Mentha aquatica, Mentha longifolia, Origanum vulgare, Phlomis cretica, Rosmarinus officinalis, Salvia sclarea, Satureja parnassica, Satureja thymbra, Sideritis euboea, Sideritis syriaca, Stachys spinosa, and Thymus longicaulis were evaluated against eight common oral pathogenic bacteria and the fungus Candida albicans. Additionally, two standard reference strains, Staphylococcus aureus and Escherichia coli, normally found on skin and in the gut, respectively, were included for benchmarking. While some of these extracts have previously been assessed for general antimicrobial activity, their effects on oral pathogens and biofilm formation had not been systematically tested, highlighting the novelty of this research.

The null hypothesis was that the extracts would not show any antimicrobial activity against the tested microbial species. To test this, three assays were employed: minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and biofilm inhibition assay.

Materials and Methods

Plant extraction

Aerial parts of sixteen Mediterranean plants were collected from multiple locations in Greece. Species included: Achillea taygetea Boiss. & Heldr., Cistus creticus L., Cistus monspeliensis L., Lavandula stoechas L., Mentha aquatica L., Mentha longifolia L., Origanum vulgare L., Phlomis cretica C. Presl, Rosmarinus officinalis L., Salvia sclarea L., Satureja parnassica Heldr. & Sart. ex Boiss., Satureja thymbra L., Sideritis euboea Heldr., Sideritis syriaca L., Stachys spinosa L., and Thymus longicaulis C. Presl. Plant material was ground into fine powders using a SCIS grinder (Allenwest-Eac Ltd) and extracted with ultrasound-assisted extraction (UAE) using ethyl acetate as solvent. The plant-to-solvent ratio was 1:10 (w/v), and the extraction ran for 15 minutes at room temperature, repeated twice for completeness. Solvent removal was performed under vacuum at 40°C using a Buchi Rotavapor R-200.

HPTLC profiling

Extracts were analyzed using Camag HPTLC to generate chemical fingerprints. Each extract (10 mg/ml in ethyl acetate) was applied to silica gel 60 F254 plates (20 × 10 cm) via ATS4 sampler, controlled with VisionCats 2.3 software: six tracks, 8 mm bands, 8 mm from the bottom edge, 20 mm margins left and right, and 10.4 mm spacing. Sample volume was 8 µl per track. Plates were developed in an automatic development chamber (ADC2) with 20 min chamber saturation, 10 min plate conditioning at 33% RH using MgCl2, and 5 min drying. Mobile phase was toluene/ethyl acetate/formic acid (80:20:2, v/v/v). Plates were documented at 254 nm and 366 nm using CAMAG Visualizer 2.

Microbial strains

Ten bacterial strains and one fungal strain were selected. Eight bacteria and C. albicans are common oral species. S. aureus and E. coli served as reference organisms. Facultative Gram-positive species included Streptococcus mutans DSM 20523, S. sobrinus DSM 20381, S. oralis ATCC 35037, Enterococcus faecalis ATCC 29212, and S. aureus ATCC 25923. E. coli ATCC 25922 is a Gram-negative facultative anaerobe.

Obligate anaerobic species were Porphyromonas gingivalis W381, Prevotella intermedia MSP34, Fusobacterium nucleatum ATCC 25586, and Parvimonas micra ATCC 23195. C. albicans DSM 1386 is dimorphic. Strains were provided by the Division of Infectious Diseases and the Institute of Medical Microbiology, Albert-Ludwigs-University Freiburg, and stored at −80°C in growth medium with 15% glycerol.

Minimum Inhibitory Concentration (MIC) Assessment

For each microbial strain, overnight cultures were prepared in accordance with CLSI standards. Microorganisms were plated on either Columbia blood agar (CBA) or yeast-cysteine blood agar (HCB), depending on their oxygen requirements. Facultative anaerobic bacteria and Candida albicans were incubated on CBA plates at 37°C under 5%–10% CO_2 for 24 hours. Obligate anaerobic species were cultivated on HCB plates at 37°C for 48 hours within an anaerobic chamber (Anaerocult, Merck Chemicals GmbH, Darmstadt, Germany).

Microbial suspensions were adjusted to a 0.5 A (for bacteria) or 1 A (for fungi) McFarland standard using 0.9% saline. For the microdilution procedure, facultative anaerobes and C. albicans were diluted 1:10 in Mueller Hinton II Broth (cation-adjusted, MHB, BD, Heidelberg, Germany), while obligate anaerobes were prepared in Wilkins–Chalgren broth (WCB) at 0.5 A McFarland. ISO 20776-1:2006 recommends inoculum densities of approximately 5×10^5 CFU/ml for facultative anaerobes, 5×10^4 CFU/ml for fungi, and 5×10^6 CFU/ml for obligate anaerobes, which were followed in this study.

Aliquots of microbial suspensions were transferred into 96-well microtiter plates. Plant extracts were pre-dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Germany) at 100 mg/ml. Serial dilutions of extracts ranged from 10 mg/ml down to 0.02 mg/ml, achieving a 10- to 5120-fold dilution series. Each well contained a total volume of 100 µl. DMSO-only controls were included to monitor any solvent effects. Wells with only broth served as negative controls, while 0.1% chlorhexidine (CHX) acted as the positive control. Growth controls consisted of broth with inoculum only. Sterility was maintained throughout using sterile MHB/WCB.

Incubation conditions were strain-specific: E. coli, S. aureus, E. faecalis, and C. albicans at 37°C for 18 hours; streptococci at 37°C under 5%–10% CO_2 for 24 hours; and obligate anaerobes at 37°C for 48 hours in anaerobic conditions. All experiments were performed in duplicate. The MIC was recorded as the lowest extract concentration preventing visible growth. For minor discrepancies between duplicates, the higher MIC was taken; if differences exceeded two dilution steps, the assay was repeated. Any inhibitory effect from DMSO was also considered.

Minimum Bactericidal Concentration (MBC) Assessment

Following MIC determination, MBC testing was conducted as per CLSI guidance. From each well, 10 µl of the extract-microbe mixture was plated onto CBA or HCB agar. E. coli, S. aureus, and E. faecalis were incubated on CBA at 37°C for 24 hours. Streptococci and C. albicans were incubated on CBA at 37°C under 5%–10% CO_2 for 48 hours. Obligate anaerobes were cultured on HCB at 37°C for 5 days in an anaerobic chamber.

The MBC was determined by visually evaluating colony-forming units (CFU). The concentration of extract causing a 3-log reduction in CFU (equivalent to 99.9% inhibition) compared to the growth control was recorded as the MBC.

Biofilm Plate Assay

An overnight culture of Streptococcus mutans R15-8 (clinical isolate) was cultivated at 37°C under aerobic conditions with 5%–10% CO_2 in BMH medium (BD, Heidelberg, Germany) supplemented with 1% sucrose (MH-S). For biofilm experiments, 96-well polystyrene plates (Greiner bio-one, Frickenhausen, Germany) were filled with 100 µl of MH-S containing the plant extracts at ten different concentrations ranging from 0.019 mg/ml to 10 mg/ml. Subsequently, 5 µl of the bacterial overnight culture was added to each well, with the initial inoculum reaching ~10^8 CFU/ml (Log_{10} CFU) on CBA plates. Plates were then incubated for 48 hours at 37°C under the same aerobic conditions with 5%–10% CO_2.

After incubation, the medium was removed, and wells were washed three times with 300 µl of phosphate-buffered saline (PBS, Life Technologies, Darmstadt, Germany) to remove loosely attached cells. No chemical fixation was performed. The adherent biofilm was left to air-dry and stained using Carbol Gentian Violet (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) containing 0.1%–<0.25% methyl violet for 10 minutes. Excess stain was rinsed with distilled water, and plates were dried at 60°C for 10 minutes. Dye resolubilization was achieved by adding 50 µl of 99.9% ethanol to each well. Optical density at 595 nm was measured using a Tecan Infinite 200 plate reader (Tecan, Crailsheim, Germany).

All assays were performed in quadruplicate, and the mean of the replicates was calculated. Extracts demonstrating the highest biofilm inhibition underwent

a secondary screening to confirm reproducibility. Biofilm inhibition was classified into three categories using OD_{595} cut-offs: C1 (no biofilm), C2 (moderate biofilm), and C3 (high biofilm). The low cut-off was determined by the negative control mean plus three standard deviations; the high cut-off was determined by repeating the low cut-off measurement three times. OD_{595} thresholds were 0.143 (low) and 0.428 (high). Values ≤ 0.143 indicated strong biofilm inhibition, and values between 0.143 and 0.428 indicated moderate biofilm formation. DMSO and chlorhexidine (CHX) controls were included for all extract concentrations.

Statistical analysis

Descriptive statistics including mean, median, and standard deviation were calculated. T-tests were applied to Log_{10}-transformed absorbance values comparing extract-treated wells with control groups. Bonferroni correction was applied to account for multiple comparisons. Scatter plots were generated to visualize the results. All analyses were conducted using STATA v17.0 (College Station, TX, USA).

Results and Discussion

Sixteen plant extracts belonging to the families Lamiaceae, Cistaceae, and Asteraceae, native to the Mediterranean region, were selected for evaluation against oral pathogens. Their antimicrobial effects against these bacteria had not been fully explored before.

After extraction with ethyl acetate, HPTLC analysis was used to identify the major bioactive compounds. Lamiaceae species (Lavandula stoechas, Mentha aquatica, Mentha longifolia, Origanum vulgare, Phlomis cretica, Rosmarinus officinalis, Salvia sclarea, Satureja parnassica, Satureja thymbra, Sideritis euboea, Sideritis syriaca, Stachys spinosa, Thymus longicaulis) contained phenolic acids and flavonoid aglycones, including apigenin and luteolin, with rosmarinic acid detected in Lavandula, Mentha, Origanum, Rosmarinus, Salvia, and Thymus.

Achillea taygetea (Asteraceae), endemic to Taygetos and Parnon mountains in southern Peloponnese, contained flavonoids (apigenin/luteolin derivatives) and phenolic acids. Cistus creticus and Cistus monspeliensis (Cistaceae) were rich in flavonoids and phenolic acids, confirming their traditional medicinal use. HPTLC visualization at 254 nm and 366 nm confirmed that most extracts were chemically diverse, demonstrating potential as biofilm inhibitors and antimicrobial agents.

Achillea taygetea

The ethyl acetate extract from *Achillea taygetea* exhibited notable inhibition against obligate anaerobes, with MIC values ranging from 0.04 mg/ml (*P. micra*) to 0.60 mg/ml (*F. nucleatum*). Facultative anaerobic streptococci were affected to varying extents: *S. oralis* was suppressed at 0.60 mg/ml, *S. sobrinus* at 5.00 mg/ml, while effects seen in *S. mutans* were attributed to DMSO. Among the other tested microorganisms **(Table 1),** only *S. aureus* showed susceptibility (MIC = 2.50 mg/ml), whereas the rest were resistant. The MBC values spanned 0.15 mg/ml (*P. micra*) to 5.00 mg/ml (*S. aureus*), except for *S. sobrinus*, which was not fully eliminated under the conditions tested.

Table 1. MIC and MBC (mg/ml) for *Achillea taygetea* ethyl acetate extract

Microorganism	Ethyl acetate extract dissolved in DMSO (%)		Ethyl acetate extract (in acetone, mg/ml)	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	2.50	NA
Streptococcus sobrinus DSM 20381	20.00	NA	5.00	NA
Streptococcus oralis ATCC 35037	10.00	20.00	0.60	0.60
Enterococcus faecalis ATCC 29212	20.00	NA	NA	NA
Candida albicans DSM 1386	10.00	NA	10.00	10.00
Escherichia coli ATCC 25922	20.00	NA	10.00	NA
Staphylococcus aureus ATCC 25923	20.00	NA	2.50	5.00
Porphyromonas gingivalis W381	20.00	20.00	0.15	0.30
Prevotella intermedia MSP 34	5.00	5.00	0.15	0.30

Fusobacterium nucleatum ATCC 25586	10.00	10.00	0.60	0.60
Parvimonas micra ATCC 23195	5.00	20.00	0.04	0.15

NA: no detectable activity; MIC and MBC evaluated at 10.00 mg/ml and DMSO 20%, respectively.
MIC: concentration yielding minimal bacterial growth (OD-based)
MBC: concentration causing ≥99.9% bacterial kill

In the biofilm assay, the *S. mutans* strain demonstrated strong inhibition at 5.00 mg/ml. The low OD_{595} threshold was 0.143. At 2.50 mg/ml, biofilm production was classified as moderate (C2), while lower concentrations had no detectable effect, exceeding the high cut-off of 0.428 **(Figure 2).**

Cistus creticus and Cistus monspeliensis

For the ethyl acetate extract of *Cistus creticus*, MICs ranged from 0.04 mg/ml (*P. gingivalis*, *P. micra*) to 5.00 mg/ml (*S. sobrinus*), and MBCs indicated 99.9% bacterial reduction between 0.04 mg/ml (*P. micra*) and 5.00 mg/ml (*S. aureus*). *E. faecalis* (MIC = 2.50 mg/ml) and *S. sobrinus* were only partially suppressed. *S. mutans*, *E. coli*, and *C. albicans* remained largely unaffected.

Table 2. MIC and MBC (mg/ml) for ***Cistus creticus*** ethyl acetate extract

Microorganism	**Ethyl acetate extract in DMSO (% v/v)**		**Pure ethyl acetate extract (mg/ml)**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	10.00	NA	5.00	NA
Streptococcus sobrinus DSM 20381	20.00	NA	5.00	NA
Streptococcus oralis ATCC 35037	20.00	20.00	0.15	0.60
Enterococcus faecalis ATCC 29212	20.00	NA	2.50	NA
Candida albicans DSM 1386	10.00	NA	10.00	10.00
Escherichia coli ATCC 25922	20.00	NA	NA	NA
Staphylococcus aureus ATCC 25923	20.00	NA	1.25	5.00
Porphyromonas gingivalis W381	20.00	20.00	0.04	0.08
Prevotella intermedia MSP 34	5.00	5.00	0.15	0.30
Fusobacterium nucleatum ATCC 25586	10.00	10.00	0.60	0.60
Parvimonas micra ATCC 23195	5.00	20.00	0.04	0.04

NA: no activity detected; MIC and MBC evaluated at 10.00 mg/ml and DMSO 20%, respectively.
MIC: lowest extract concentration reducing bacterial growth
MBC: concentration causing ≥99.9% kill

Data collection and study tool

Consistent with MIC/MBC outcomes, *S. mutans* biofilm formation remained high (C3) across all tested concentrations **(Figure 3).**

The ethyl acetate extract of *Cistus monspeliensis* also displayed effective antimicrobial activity, with MBC values from 0.04 mg/ml (*P. micra*) to 5.00 mg/ml (*E. faecalis*), including notable inhibition of *C. albicans*. *E. coli* showed no significant sensitivity aside from minor DMSO effects **(Table 3).** Maximum inhibitory effects were seen at 0.04 mg/ml for *P. micra*, followed by 0.08 mg/ml for *S. oralis* and *P. gingivalis*. For *S. aureus*, the mean MIC and MBC were 0.60 mg/ml.

Table 3. MIC and MBC (mg/ml) for *Cistus monspeliensis* ethyl acetate extract

Microorganism	**Ethyl acetate extract in DMSO (% v/v)**		**Pure ethyl acetate extract (mg/ml)**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	2.50	2.50
Streptococcus sobrinus DSM 20381	10.00	NA	2.50	2.50
Streptococcus oralis ATCC 35037	20.00	20.00	0.08	0.08

Enterococcus faecalis ATCC 29212	20.00	NA	5.00	5.00
Candida albicans DSM 1386	10.00	20.00	5.00	5.00
Escherichia coli ATCC 25922	20.00	NA	NA	NA
Staphylococcus aureus ATCC 25923	20.00	NA	0.60	0.60
Porphyromonas gingivalis W381	20.00	20.00	0.08	0.08
Prevotella intermedia MSP 34	2.50	2.50	0.30	0.30
Fusobacterium nucleatum ATCC 25586	5.00	10.00	0.30	0.30
Parvimonas micra ATCC 23195	5.00	10.00	0.04	0.04

NA: no activity detected; MIC and MBC evaluated at 10.00 mg/ml and DMSO 20%, respectively.
MIC: extract concentration limiting bacterial growth
MBC: concentration causing ≥99.9% bacterial kill

Figure 3 Biofilm testing showed that *S. mutans* formation was unaffected at the concentrations examined.

Lavandula stoechas

Table 4 reports the antimicrobial readings for the ethyl acetate fraction of *L. stoechas*. In these tests, obligate anaerobes were highly sensitive: the MIC needed to limit *P. gingivalis* was 0.04 mg/ml, whereas *F. nucleatum* required 1.25 mg/ml. Facultative oral microbes were likewise affected, needing between 0.15 mg/ml (*S. oralis*, *S. mutans*) and 1.25 mg/ml (*S. sobrinus*) for inhibition. Neither *E. coli* nor *C. albicans* responded to the extract itself; only DMSO produced any observable effect.

Table 4. Antimicrobial activity (mg ml−1) of *L. stoechas* ethyl acetate extract.

Microorganism	**Ethyl acetate extract in DMSO (% v/v)**		**Pure ethyl acetate extract (mg/ml)**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	0.15	NA
Streptococcus sobrinus DSM 20381	20.00	NA	1.25	NA
Streptococcus oralis ATCC 35037	10.00	20.00	0.15	0.30
Enterococcus faecalis ATCC 29212	20.00	NA	0.60	1.25
Candida albicans DSM 1386	10.00	NA	10.00	NA
Escherichia coli ATCC 25922	20.00	20.00	NA	NA
Staphylococcus aureus ATCC 25923	20.00	NA	1.25	1.25
Porphyromonas gingivalis W381	20.00	20.00	0.04	0.15
Prevotella intermedia MSP 34	5.00	5.00	0.30	1.25
Fusobacterium nucleatum ATCC 25586	5.00	10.00	1.25	2.50
Parvimonas micra ATCC 23195	2.50	20.00	0.08	0.60

NA = absence of detectable inhibition. Extract values were evaluated up to 10.00 mg ml−1**,** while DMSO was used at **20%**.
MIC = concentration that kept OD changes minimal.
MBC = concentration that caused a 99.9% drop in viable cells.

Most oral isolates displayed some level of killing, with MBC requirements stretching from 0.15 mg/ml (*P. gingivalis*) up to 2.50 mg/ml (*F. nucleatum*). No MBC endpoints could be measured for *S. mutans* or *S. sobrinus*. *S. aureus* was fully eliminated (99.9%) at 1.25 mg/ml. **Figure 1** shows that biofilm assembly was sharply reduced by the extract even at 0.04 mg/ml, and the smallest dose tested (0.02 mg/ml) still produced a moderate reduction (category C2)

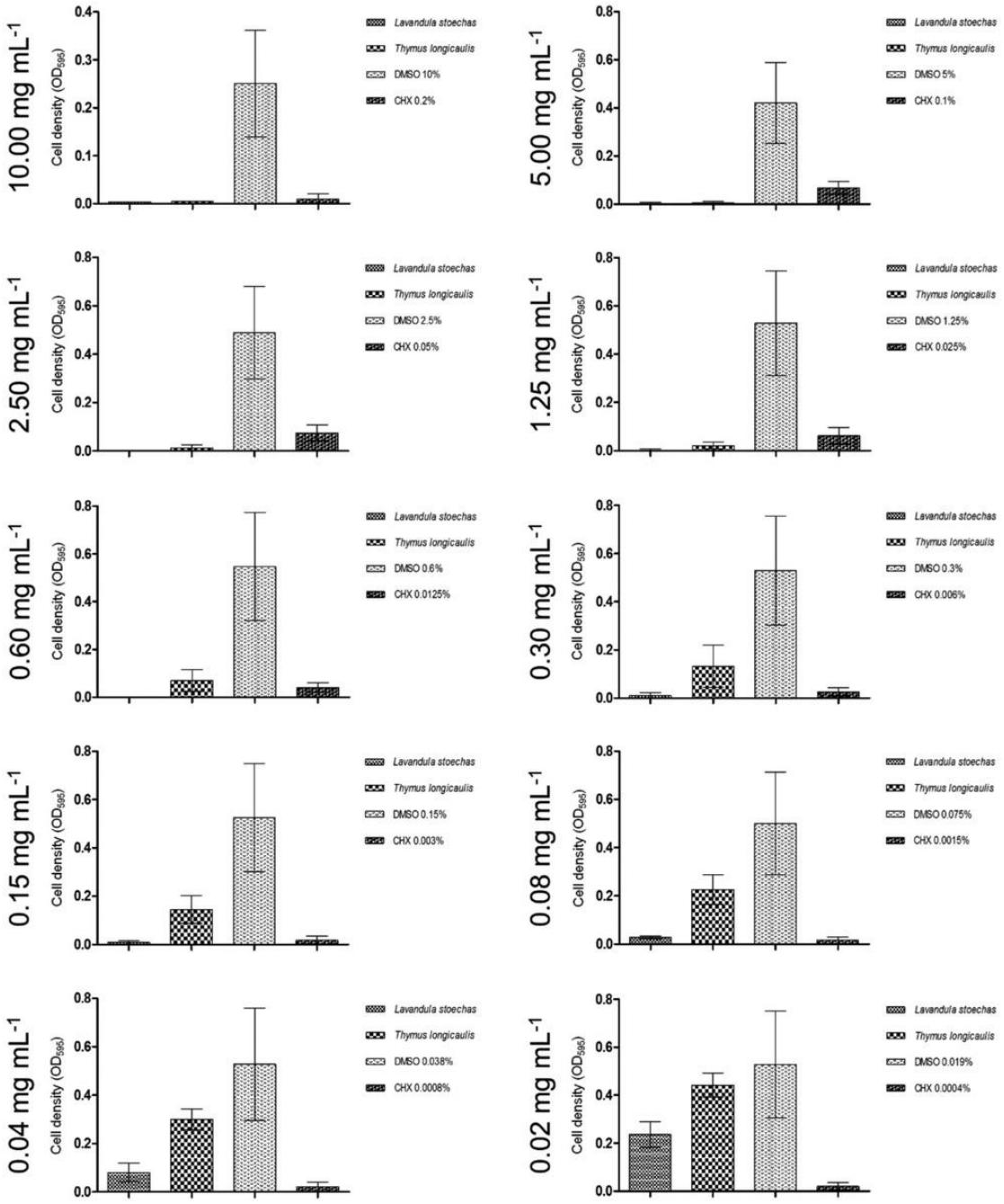

Figure 1. Biofilm-inhibition profiles produced by ethyl acetate extracts.

Mentha aquatica and Mentha longifolia

Table 5 contains the MIC and MBC data for *M. aquatica* ethyl acetate extract. Obligately anaerobic strains responded strongly, requiring only 0.30 mg/ml (*P. gingivalis*) to 1.25 mg/ml (*F. nucleatum*) for inhibition. Except for *S. oralis* and *C. albicans*, other organisms showed only weak responses, with MIC ranges from 2.50 mg/ml (*S. mutans*, *E. faecalis*) to 5.00 mg/ml (*S. sobrinus*, *E. coli*, *S. aureus*). Killing activity was measurable mainly for *P. micra*, *P. gingivalis*, and *S. oralis*, requiring 1.25 mg/ml to 5.00 mg/ml**.** DMSO accounted for the limited effects seen in the remaining strains.

Table 5. Antimicrobial activity (mg ml−1) of *M. aquatica* ethyl acetate extract.

Microorganism	**Ethyl acetate extract in DMSO (% v/v)**		**Pure ethyl acetate extract (mg/ml)**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	10.00	NA	2.50	NA

Streptococcus sobrinus DSM 20381	20.00	NA	5.00	NA
Streptococcus oralis ATCC 35037	10.00	20.00	5.00	5.00
Enterococcus faecalis ATCC 29212	20.00	NA	2.50	NA
Candida albicans DSM 1386	10.00	20.00	5.00	NA
Escherichia coli ATCC 25922	20.00	NA	5.00	NA
Staphylococcus aureus ATCC 25923	20.00	NA	5.00	NA
Porphyromonas gingivalis W381	20.00	20.00	0.30	2.50
Prevotella intermedia MSP 34	5.00	5.00	0.60	2.50
Fusobacterium nucleatum ATCC 25586	10.00	10.00	1.25	5.00
Parvimonas micra ATCC 23195	5.00	20.00	0.60	1.25

NA = no recorded activity. Maximum extract concentrations were 10.00 mg ml−1; DMSO was 20%.
MIC = point where OD growth no longer increased.
MBC = concentration that reduced viable cells by 99.9%.

Figure 3 demonstrates that *S. mutans* biofilms were greatly restricted up to 1.25 mg/ml, which places these doses in group C1 (strong inhibition). At 0.60 mg/ml, biofilm accumulation was still moderate, and levels higher than 1.25 mg/ml met the definition for C3 (no suppression detected).

The ethyl acetate extract from *M. longifolia* was active toward all tested organisms, as shown in **Table 6**. MIC values ranged between 0.08 mg/ml (*P. micra*) and 5.00 mg/ml (*E. coli*). Among Streptococcus species, *S. oralis* and *S. mutans* were the most responsive (MIC 0.60 mg/ml). MBCs extended from 0.08 mg/ml (*P. micra*) to 5.00 mg/ml (*S. oralis*). However, no lethal effect was observed for *S. mutans*, *S. sobrinus*, *E. faecalis*, *C. albicans*, or *E. coli*.

Table 6. Antimicrobial activity (mg ml−1) of *M. longifolia* ethyl acetate extract.

Microorganism	**Ethyl acetate extract in DMSO (% v/v)**		**Pure ethyl acetate extract (mg/ml)**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	0.60	NA
Streptococcus sobrinus DSM 20381	20.00	NA	1.25	NA
Streptococcus oralis ATCC 35037	10.00	20.00	0.60	5.00
Enterococcus faecalis ATCC 29212	20.00	NA	2.50	NA
Candida albicans DSM 1386	10.00	NA	2.50	NA
Escherichia coli ATCC 25922	20.00	NA	5.00	NA
Staphylococcus aureus ATCC 25923	20.00	NA	1.25	2.50
Porphyromonas gingivalis W381	20.00	20.00	0.15	0.60
Prevotella intermedia MSP 34	5.00	5.00	0.30	0.60
Fusobacterium nucleatum ATCC 25586	10.00	10.00	0.60	1.25
Parvimonas micra ATCC 23195	5.00	20.00	0.08	0.08

NA = absence of detectable inhibition. Extracts tested up to 10.00 mg ml−1; DMSO used at 20%.
MIC = concentration halting OD rise.
MBC = dose responsible for 99.9% loss of viable bacteria.

S. mutans Biofilm Assay

In the biofilm experiment using the S. mutans strain **(Figure 2)**, the ethyl acetate fraction produced a moderate decline in biofilm buildup. At concentrations down to 1.25 mg/ml, absorbance values matched the classification for absence of biofilm development.

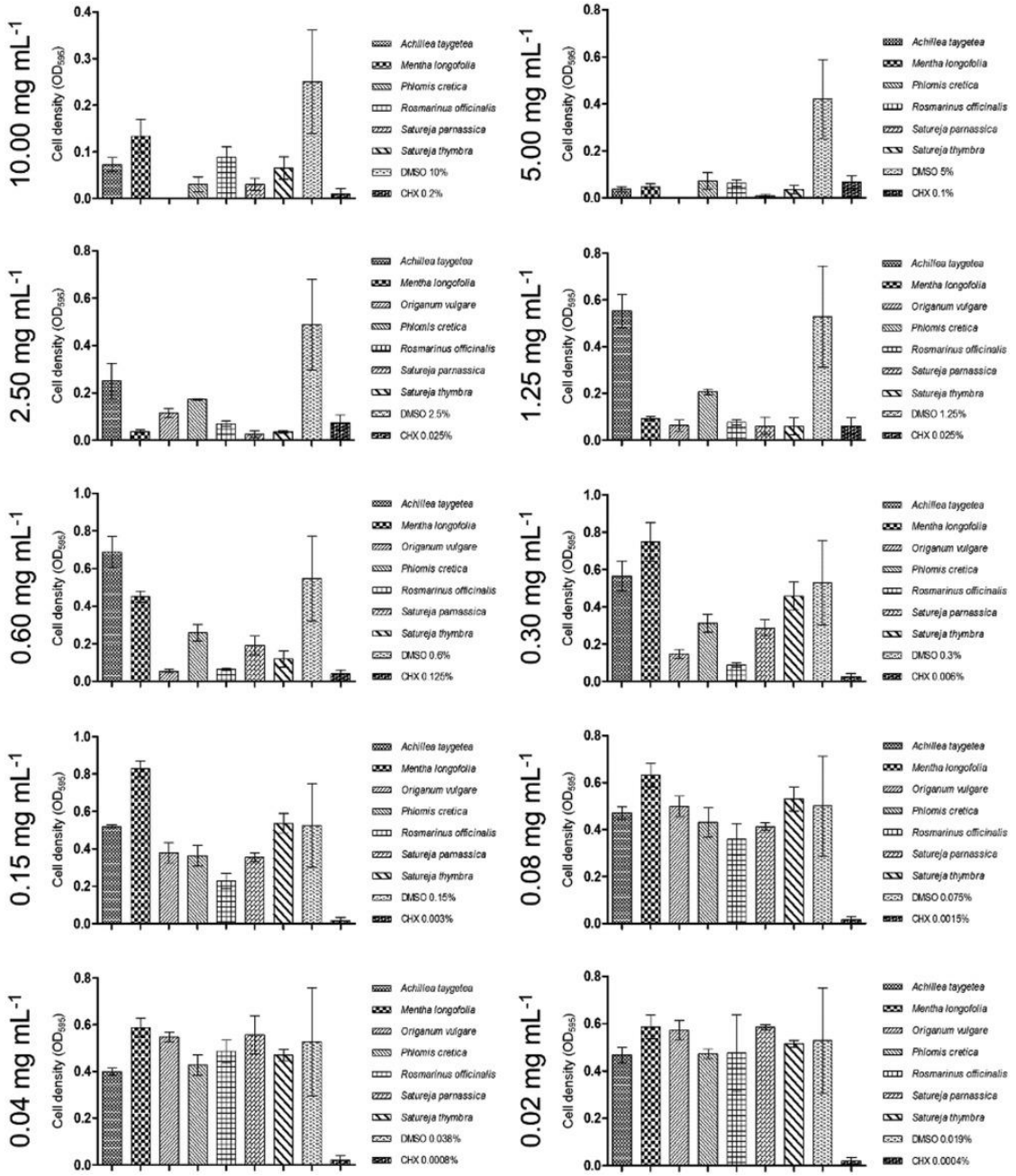

Figure 2. Representation of extracts showing moderate suppression of biofilm formation.

Origanum vulgare

The ethyl acetate extract displayed broad antimicrobial coverage, with notable effects on oral microbes **(Table 7).** MIC values spanned from 0.04 mg/ml (*P. gingivalis*, *P. micra*) to 2.50 mg/ml (*E. coli*). Among facultative anaerobes, inhibitory concentrations ranged between 0.15 mg/ml (*S. mutans*, *S. sobrinus*, *S. oralis*) and 2.50 mg/ml (*E. coli*). MBC values varied from 0.08 mg/ml (*P. micra*) to 2.50 mg/ml (*E. coli*, *C. albicans*). Aside from *C. albicans* and *E. coli* (both MBC = 2.50 mg/ml), all other organisms were eliminated at lower levels, between 0.08 mg/ml (*P. micra*) and 1.25 mg/ml (*E. faecalis*).

Table 7. Antimicrobial activity (mg ml−1) of *O. vulgare* ethyl acetate extract.

Microorganism	**Ethyl acetate extract in DMSO (% v/v)**		**Pure ethyl acetate extract (mg/ml)**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	0.15	0.30
Streptococcus sobrinus DSM 20381	20.00	20.00	0.15	0.15

Streptococcus oralis ATCC 35037	5.00	20.00	0.15	0.15
Enterococcus faecalis ATCC 29212	20.00	NA	1.25	1.25
Candida albicans DSM 1386	10.00	NA	1.25	2.50
Escherichia coli ATCC 25922	20.00	20.00	2.50	2.50
Staphylococcus aureus ATCC 25923	20.00	NA	0.30	0.30
Porphyromonas gingivalis W381	20.00	20.00	0.04	0.15
Prevotella intermedia MSP 34	5.00	5.00	0.30	0.60
Fusobacterium nucleatum ATCC 25586	10.00	10.00	0.15	0.15
Parvimonas micra ATCC 23195	2.50	10.00	0.04	0.08

NA = no effect observed; extracts tested up to 10.00 mg ml−1, DMSO at 20%.
MIC = minimal concentration preventing OD increase.
MBC = dose inducing 99.9% reduction in bacterial growth.

In the plate-based biofilm assay **(Figure 2),** only a moderate decrease in biomass was detected. The transition point for reduced biofilm was placed between 0.30 mg/ml and 0.60 mg/ml, while concentrations at 0.08 mg/ml and below allowed high biofilm levels.

Phlomis cretica

Table 8 summarizes the inhibitory actions of the ethyl acetate extract, which mainly affected bacterial strains. After factoring in DMSO's contribution, the extract showed no substantial inhibition of *E. faecalis*, *E. coli*, or *C. albicans*. MIC values extended from 0.04 mg/ml (*P. micra*) to 2.50 mg/ml (*S. sobrinus*, *S. aureus*). MBC data revealed that *S. mutans*, *S. sobrinus*, *C. albicans*, *E. faecalis*, and *E. coli* remained viable, whereas other organisms were eliminated at concentrations from 0.08 mg/ml (*P. micra*) to 2.50 mg/ml (*S. aureus*).

Table 8. Antimicrobial activity (mg ml−1) of *P. cretica* ethyl acetate extract.

Microorganism	**Ethyl acetate extract in DMSO (% v/v)**		**Pure ethyl acetate extract (mg/ml)**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	1.25	NA
Streptococcus sobrinus DSM 20381	20.00	NA	2.50	NA
Streptococcus oralis ATCC 35037	10.00	20.00	0.30	0.30
Enterococcus faecalis ATCC 29212	20.00	NA	NA	NA
Candida albicans DSM 1386	10.00	NA	10.00	NA
Escherichia coli ATCC 25922	20.00	NA	NA	NA
Staphylococcus aureus ATCC 25923	20.00	NA	2.50	2.50
Porphyromonas gingivalis W381	20.00	20.00	0.15	0.60
Prevotella intermedia MSP 34	5.00	5.00	0.60	1.25
Fusobacterium nucleatum ATCC 25586	10.00	10.00	1.25	1.25
Parvimonas micra ATCC 23195	5.00	20.00	0.04	0.08

NA = lack of measurable activity; MIC/MBC tested to 10.00 mg ml−1; DMSO at 20%.
MIC = lowest concentration maintaining minimal OD.
MBC = concentration causing a three-log (99.9%) reduction.

Within the biofilm assay, no detectable biofilm was present at 5.00 mg/ml of the extract. Across more than five dilution steps—including 0.15 mg/ml **(Figure 2)**—moderate formation was seen, while concentrations below this threshold fell under C3, meaning no inhibitory influence.

Rosmarinus officinalis

The ethyl acetate extract of *R. officinalis* showed strong antibacterial activity toward both facultative and obligate anaerobic oral microorganisms **(Table 9)**. For facultative bacteria, MIC values ranged from 0.02 mg/ml (*S. mutans*) to 0.60 mg/ml (*E. faecalis*). Among obligate anaerobes, the MIC values varied from 0.01

mg/ml (*P. micra*) to 0.15 mg/ml (*F. nucleatum*). The extract also reduced the growth of *S. aureus*, with MIC/MBC both equal to 0.30 mg/ml. However, *E. coli* and *C. albicans* were unaffected. Bactericidal concentrations for oral species extended from 0.02 mg/ml (*P. micra*) to 1.25 mg/ml (*E. faecalis*).

Table 9. Antimicrobial activity (mg ml−1) of *R. officinalis* ethyl acetate extract.

Microorganism	Ethyl acetate extract in DMSO (% v/v)		Pure ethyl acetate extract (mg/ml)	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	0.02	0.08
Streptococcus sobrinus DSM 20381	20.00	20.00	0.08	0.15
Streptococcus oralis ATCC 35037	10.00	20.00	0.15	0.30
Enterococcus faecalis ATCC 29212	20.00	NA	0.60	1.25
Candida albicans DSM 1386	5.00	NA	10.00	10.00
Escherichia coli ATCC 25922	20.00	NA	10.00	10.00
Staphylococcus aureus ATCC 25923	20.00	NA	0.30	0.30
Porphyromonas gingivalis W381	20.00	20.00	0.04	0.08
Prevotella intermedia MSP 34	2.50	5.00	0.04	0.04
Fusobacterium nucleatum ATCC 25586	10.00	10.00	0.15	0.15
Parvimonas micra ATCC 23195	10.00	20.00	0.01	0.02

NA = no activity measured; extract limits set at 10.00 mg ml−1, DMSO at 20%.

MIC = concentration preventing OD rise.

MBC = three-log (99.9%) cell reduction threshold.

In the *S. mutans* biofilm assay **(Figure 2)**, biofilm accumulation was suppressed at 0.30 mg/ml. A dose of 0.08 mg/ml allowed moderate production, while 0.04 mg/ml and below were categorized as C3, indicating no observable inhibition.

Salvia sclarea

Table 10 summarizes how the ethyl acetate fraction influenced obligate anaerobes. Minimum inhibitory concentrations (MICs) extended from 0.04 mg/ml for *P. gingivalis* and *P. micra* up to 0.15 mg/ml for *F. nucleatum*. Several additional species showed suppression at levels between 0.15 mg/ml (*S. oralis*) and 5.00 mg/ml (*S. sobrinus*). Minimum bactericidal concentration (MBC) outcomes showed lethal action ranging from 0.08 mg/ml (*P. gingivalis*, *P. intermedia*, *P. micra*) to 10.00 mg/ml (*S. sobrinus*), while *E. faecalis* displayed no measurable loss of viability. Adjusting for the effect of DMSO, the extract did not exert meaningful activity toward *S. mutans*, *E. coli,* or *C. albicans*.

Table 10. Antimicrobial activity (mg ml−1) of *Salvia sclarea* ethyl acetate extract.

Microorganism	DMSO solution (%)		Ethyl acetate extract (c/mg ml^{-1})	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	10.00	NA	5.00	NA
Streptococcus sobrinus DSM 20381	20.00	NA	5.00	10.00
Streptococcus oralis ATCC 35037	20.00	20.00	0.15	0.15
Enterococcus faecalis ATCC 29212	20.00	NA	2.50	NA
Candida albicans DSM 1386	10.00	NA	10.00	10.00
Escherichia coli ATCC 25922	20.00	NA	NA	NA
Staphylococcus aureus ATCC 25923	20.00	NA	0.60	1.25
Porphyromonas gingivalis W381	20.00	20.00	0.04	0.08
Prevotella intermedia MSP 34	5.00	5.00	0.08	0.08
Fusobacterium nucleatum ATCC 25586	10.00	10.00	0.15	0.15
Parvimonas micra ATCC 23195	5.00	20.00	0.04	0.08

NA indicates absence of activity; MIC and MBC values were assessed at 10.00 mg ml−1 with 20% DMSO.

MIC = concentration producing minimal OD-detected growth.

MBC = concentration responsible for a three-log (99.9%) decline in bacterial count

Figure 3 shows that the *S. sclarea* ethyl acetate extract did not produce any observable reduction in biofilm formation.

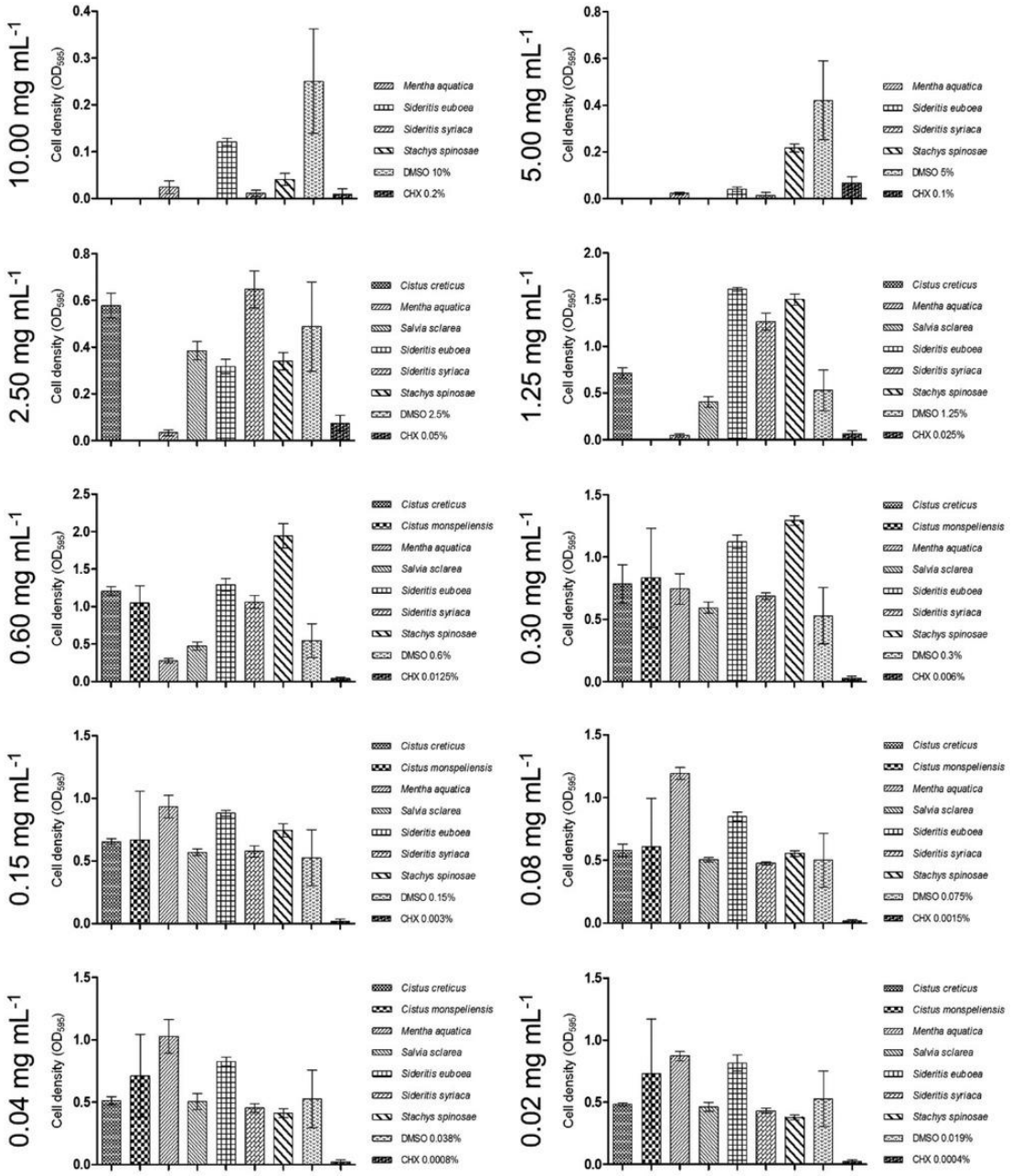

Figure 3. Plots illustrating the limited biofilm-inhibitory activity of ethyl acetate extracts.

Satureja parnassica and Satureja thymbra

Table 11 details the suppressive effects of the *S. parnassica* ethyl acetate extract across all bacterial species listed in **Table 12.** MIC values ranged between 0.08 mg/ml (*P. gingivalis*) and 5.00 mg/ml (*E. coli*, *S. aureus*). *C. albicans*, however, was unaffected by this extract. Bactericidal activity (99.9% reduction) was recorded for obligate anaerobes, *S. aureus*, *S. oralis*, and *S. sobrinus*, at doses spanning 0.60 mg/ml (*P. micra*) to 5.00 mg/ml (*S. aureus*).

Table 11. Antimicrobial activity (mg ml−1) of *Satureja parnassica* ethyl acetate extract.

Microorganism	**DMSO solution (%)**		**Ethyl acetate extract (c/mg ml^{-1})**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	10.00	NA	0.60	NA
Streptococcus sobrinus DSM 20381	20.00	NA	2.50	2.50

Streptococcus oralis ATCC 35037	10.00	20.00	0.60	1.25
Enterococcus faecalis ATCC 29212	20.00	NA	2.50	NA
Candida albicans DSM 1386	10.00	20.00	5.00	NA
Escherichia coli ATCC 25922	20.00	NA	5.00	NA
Staphylococcus aureus ATCC 25923	20.00	NA	5.00	5.00
Porphyromonas gingivalis W381	20.00	20.00	0.08	1.25
Prevotella intermedia MSP 34	5.00	5.00	0.60	1.25
Fusobacterium nucleatum ATCC 25586	10.00	10.00	1.25	2.50
Parvimonas micra ATCC 23195	5.00	20.00	0.30	0.60

NA: no observable inhibition at 10.00 mg ml−1 extract or 20% DMSO.
MIC = concentration producing minimal OD growth.
MBC = concentration resulting in a 99.9% decrease in viable cells.

Table 12. Antimicrobial activity (mg ml−1) of *Satureja thymbra* ethyl acetate extract**.**

Microorganism	**DMSO solution (%)**		**Ethyl acetate extract (c/mg ml^{-1})**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	1.25	5.00
Streptococcus sobrinus DSM 20381	20.00	NA	0.60	1.25
Streptococcus oralis ATCC 35037	10.00	20.00	0.30	0.30
Enterococcus faecalis ATCC 29212	20.00	NA	1.25	5.00
Candida albicans DSM 1386	10.00	NA	5.00	10.00
Escherichia coli ATCC 25922	20.00	20.00	10.00	10.00
Staphylococcus aureus ATCC 25923	20.00	NA	0.60	0.60
Porphyromonas gingivalis W381	20.00	20.00	0.08	0.15
Prevotella intermedia MSP 34	5.00	5.00	0.60	0.60
Fusobacterium nucleatum ATCC 25586	5.00	10.00	0.30	0.30
Parvimonas micra ATCC 23195	2.50	20.00	0.04	0.08

NA: no measurable inhibition at 10.00 mg ml−1 extract or 20% DMSO.
MIC = concentration producing minimal OD growth.
MBC = concentration resulting in three-log (99.9%) microbial reduction.

In the biofilm assay, *S. parnassica* extract blocked biofilm synthesis at 1.25 mg/ml. Values between 0.60 mg/ml and 0.08 mg/ml yielded moderate biofilm formation **(Figure 2).**

The ethyl acetate extract of *S. thymbra* showed broad activity against nearly every microorganism evaluated. MIC values ranged from 0.04 mg/ml (*P. micra*) to 1.25 mg/ml (*S. mutans*, *E. faecalis*). Among facultative anaerobes, MICs fell between 0.30 mg/ml (*S. oralis*) and 1.25 mg/ml (*E. faecalis*, *S. mutans*). MBC values varied from 0.08 mg/ml (*P. micra*) to 5.00 mg/ml (*S. mutans*, *E. faecalis*). Neither *E. coli* nor *C. albicans* exhibited susceptibility once solvent effects were considered.

In the *S. mutans* biofilm microplate assay **(Figure 2)**, the extract resulted in a moderate reduction of biofilm mass. Concentrations below 0.60 mg/ml corresponded to the category characterized by a lack of detectable biofilm accumulation.

Sideritis euboea and Sideritis syriaca

Table 13 lists MIC and MBC readings for the ethyl acetate extracts against all tested organisms. MIC values were recorded between 0.15 mg/ml (*P. gingivalis*, *P. intermedia*, *P. micra*) and 2.50 mg/ml (*S. mutans*, *S. sobrinus*). For a 99.9% decline in viability, MBC values ranged from 0.15 mg/ml (*P. gingivalis*) to 2.50 mg/ml (*S. aureus*). The extract exhibited no notable influence on *E. coli*, *E. faecalis*, or *C. albicans*, and neither *S. mutans* nor *S. sobrinus* was eliminated at the concentrations assessed for *S. euboea*.

Table 13. Antimicrobial activity (mg ml−1) of *Sideritis euboea* ethyl acetate extract.

Microorganism	**DMSO solution (%)**		**Ethyl acetate extract (c/mg ml^{-1})**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	10.00	NA	2.50	NA
Streptococcus sobrinus DSM 20381	20.00	NA	2.50	NA
Streptococcus oralis ATCC 35037	10.00	20.00	0.60	0.60
Enterococcus faecalis ATCC 29212	20.00	NA	NA	NA
Candida albicans DSM 1386	10.00	NA	10.00	10.00

Escherichia coli ATCC 25922	20.00	NA	NA	NA
Staphylococcus aureus ATCC 25923	20.00	NA	1.25	2.50
Porphyromonas gingivalis W381	20.00	20.00	0.15	0.15
Prevotella intermedia MSP 34	5.00	5.00	0.15	0.30
Fusobacterium nucleatum ATCC 25586	10.00	10.00	0.60	0.60
Parvimonas micra ATCC 23195	5.00	20.00	0.15	0.30

NA: no detectable MIC or MBC at 10.00 mg ml−**1** extract or 20% DMSO.
MIC = concentration associated with minimal OD-based growth.
MBC = concentration producing a three-log (99.9%) decline in bacterial population.

Biofilm plate assay

Figure 3 indicates that biofilm development ceased only when the extract reached 5.00 mg/ml. Amounts of 2.50 mg/ml and above were assigned to C2, while weaker doses showed no detectable influence and were grouped as C3.

Compared with *S. euboea*, the *S. syriaca* extract produced far stronger interference with the growth of *S. sobrinus, E. faecalis, P. gingivalis, P. micra, S. aureus,* and *E. coli* (**Table 14**). MIC values extended from 0.08 mg/ml (for *P. gingivalis* and *P. micra*) to 2.50 mg/ml (for *E. coli* and *E. faecalis*). No inhibition occurred for *S. mutans* or *C. albicans*. Strict anaerobes together with *S. oralis* required much lower extract levels for removal, spanning 0.08 mg/ml (*P. micra*) up to 10.00 mg/ml (*S. mutans, S. sobrinus*).

Table 14. Antimicrobial activity of *Sideritis syriaca* ethyl acetate extract (mg ml^{-1}).

Microorganism	**DMSO solution (%)**		**Ethyl acetate extract (c/mg ml^{-1})**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	2.50	10.00
Streptococcus sobrinus DSM 20381	20.00	NA	1.25	10.00
Streptococcus oralis ATCC 35037	10.00	20.00	0.60	0.60
Enterococcus faecalis ATCC 29212	20.00	NA	2.50	NA
Candida albicans DSM 1386	10.00	NA	5.00	NA
Escherichia coli ATCC 25922	20.00	NA	2.50	NA
Staphylococcus aureus ATCC 25923	20.00	NA	0.60	2.50
Porphyromonas gingivalis W381	20.00	20.00	0.08	0.15
Prevotella intermedia MSP 34	5.00	5.00	0.15	0.30
Fusobacterium nucleatum ATCC 25586	10.00	10.00	0.60	0.60
Parvimonas micra ATCC 23195	5.00	20.00	0.08	0.08

NA = no measurable effect. All extracts were evaluated up to 10.00 mg ml^{-1}**,** and DMSO at 20%**.**
MIC = lowest concentration showing minimal OD change.
MBC = dose producing a 99.9% reduction **in** viable cells.

As presented in **Figure 3**, *S. mutans* biofilm disappeared completely once 5.00 mg/ml of the *S. syriaca* extract was applied (C1).

Stachys spinosa

All oral pathogens and *S. aureus* responded to this extract with MIC values from 0.15 mg/ml (*P. micra*) to 2.50 mg/ml (*S. oralis, E. faecalis, S. aureus*). Bactericidal concentrations, however, were confined to strict anaerobes (0.30 mg/ml for *P. micra*), *S. oralis* (5.00 mg/ml), and *S. aureus* (10.00 mg/ml) according to **Table 15**.

Table 15. Antimicrobial activity of *Stachys spinosa* ethyl acetate extract (mg ml^{-1}).

Microorganism	**DMSO solution (%)**		**Ethyl acetate extract (c/mg ml^{-1})**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	10.00	NA	0.60	NA
Streptococcus sobrinus DSM 20381	20.00	NA	0.60	NA
Streptococcus oralis ATCC 35037	10.00	20.00	2.50	5.00
Enterococcus faecalis ATCC 29212	20.00	NA	2.50	NA
Candida albicans DSM 1386	10.00	NA	NA	NA
Escherichia coli ATCC 25922	20.00	NA	10.00	NA
Staphylococcus aureus ATCC 25923	20.00	NA	2.50	10.00
Porphyromonas gingivalis W381	20.00	20.00	0.30	0.60
Prevotella intermedia MSP 34	5.00	5.00	1.25	1.25
Fusobacterium nucleatum ATCC 25586	10.00	10.00	1.25	1.25

Parvimonas micra ATCC 23195	5.00	20.00	0.15	0.30

NA = no activity at 10.00 mg ml^{-1}**;** DMSO assessed at 20%**.**
MIC = minimal OD-indicated growth.
MBC = concentration generating **a** three-log (99.9%) loss of cells.

Biofilm formation was absent at 10.00 mg/ml, and 2.50 mg/ml already limited biofilm accumulation to the C2 category **(Figure 3)**.

Thymus longicaulis

All organisms except *E. coli* and *C. albicans* were inhibited, with MIC measurements between 0.04 mg/ml (*P. gingivalis*) and 2.50 mg/ml (*S. sobrinus, S. aureus*). MBC data reflected fewer susceptible targets: 1.25 mg/ml for *S. oralis*, 0.30–2.50 mg/ml for obligate anaerobes, and 10.00 mg/ml for *S. aureus* **(Table 16)**.

Table 16. Antimicrobial activity of *Thymus longicaulis* ethyl acetate extract (mg ml^{-1}).

Microorganism	**DMSO solution (%)**		**Ethyl acetate extract (c/mg ml^{-1})**	
	MIC	MBC	MIC	MBC
Streptococcus mutans DSM 20523	5.00	NA	0.30	NA
Streptococcus sobrinus DSM 20381	20.00	NA	2.50	NA
Streptococcus oralis ATCC 35037	10.00	20.00	0.30	1.25
Enterococcus faecalis ATCC 29212	20.00	NA	1.25	NA
Candida albicans DSM 1386	10.00	20.00	10.00	NA
Escherichia coli ATCC 25922	20.00	20.00	NA	NA
Staphylococcus aureus ATCC 25923	20.00	NA	2.50	10.00
Porphyromonas gingivalis W381	20.00	20.00	0.04	1.25
Prevotella intermedia MSP 34	5.00	5.00	1.25	2.50
Fusobacterium nucleatum ATCC 25586	10.00	10.00	1.25	2.50
Parvimonas micra ATCC 23195	2.50	10.00	0.15	0.30

NA = no effect at 10.00 mg ml^{-1}**;** DMSO evaluated at 20%**.**
MIC = minimal OD-based growth.
MBC = concentration causing a 99.9% decline in viability.

The biofilm findings mirrored the inhibitory profile against *S. mutans*: no detectable biofilm appeared up to 0.30 mg/ml. A dose of 0.04 mg/ml curtailed biofilm to a moderate extent, while 0.02 mg/ml—as shown in **Figure 1**—was classified as C3.

This work assessed the antimicrobial and antibiofilm potential of 16 ethyl acetate extracts from Mediterranean herbs against eight oral bacterial species plus *C. albicans*. Most available studies concentrate on essential oils affecting non-oral microbes, with only occasional reports addressing antibiofilm behavior in a single oral bacterium [30]. Ethyl acetate was selected for extraction because it captures bioactive molecules with reduced toxicity relative to solvents such as ethanol or methanol. No earlier investigation has evaluated these particular extracts across such a broad panel of oral pathogens.

The *A. taygetea* extract strongly suppressed obligate anaerobes. When compared with its essential oil, the ethyl acetate preparation displayed greater activity toward Gram-positive facultative anaerobes than toward Gram-negative strains [31].

Previous studies [32-34] also document that species of *Cistus* exert extensive antimicrobial effects on numerous non-oral bacterial and fungal groups.

Gram-negative organisms possess an exterior membrane layer that forms a compact diffusion shield, greatly limiting the passage of lipophilic substances. Because of this architectural feature, they display higher tolerance to Cistus spp. preparations [35] than Gram-positive species [36]. In contrast, Gram-positive bacteria rely on protective tactics that include releasing extracellular proteases and altering the chemistry of their membranes or walls, which ultimately strengthen their defenses against antimicrobial substances [37, 38]. Using Cistus tea as an oral rinse has been reported to lessen bacterial adherence to enamel surfaces under in-situ conditions [39]. Fungal cells, however, persisted despite the application of highly concentrated extracts. Specifically, C. albicans, serving as a reference fungus, remained largely unaffected even when exposed to strong Cistus spp. formulations [32-34].

S. euboea exhibited only modest antimicrobial potential when compared with other representatives of Sideritis spp. In contrast, S. syriaca has been examined for its antibacterial properties both as a water-based decoction and as an essential oil. Although their chemical profiles differ because of polarity, each preparation displayed detectable activity. The more polar extract contains compounds such as hypoelatin,

isoscutellarein diglucosides, and chlorogenic acid, which collectively contribute to the inhibition of both Gram-positive and Gram-negative strains.

The essential oil of L. stoechas has been evaluated repeatedly for activity against bacteria from both categories [40-42]. Nevertheless, research focused specifically on oral taxa reported limited antimicrobial strength, indicated by an MIC of 4 µl/ml [43].

A comparison of several preparations of O. vulgare—including decoction, infusion, and methanol–water extract, each with different proportions of luteolin O-glucuronide, luteolin 7-O-glucoside, and rosmarinic acid—showed that the ethyl acetate fraction produced stronger inhibition of Gram-negative organisms than of Gram-positive ones [44]. Leaves of O. vulgare harvested in Mexico displayed higher quantities of α-pinene and terpinen-4-ol than of thymol and carvacrol [45].

The oral isolates examined in this study responded to rosemary extract, echoing the report of Takarada *et al*. [46], who worked with rosemary essential oil (EO). The foliage of the plant contained greater amounts of active inhibitory molecules than the stems. The principal constituents, carnosic acid and carnosol, presented MIC values of 0.09 mg/ml and 0.08 mg/ml toward S. mutans and S. sobrinus, respectively, and achieved elimination of E. faecalis at 0.07 mg/ml and 0.10 mg/ml [47]. A polyherbal mouthwash composed in part of R. officinalis extract showed antibacterial performance comparable to 0.2% (w/v) chlorhexidine (CHX) in a randomized, double-blind, placebo-controlled gingivitis trial [48]. A separate clinical evaluation in periodontitis demonstrated that a rinse enriched with essential oils from Rosmarinus spp., including rosemary, facilitated clearance of subgingival biofilms dominated by obligate anaerobic bacteria [49], possibly through interference with quorum-sensing (QS) communication.

In the current investigation, the S. sclarea ethyl acetate fraction produced stronger inhibition of obligate anaerobic oral pathogens than the essential oil did [50]. Prior work has shown that S. sclarea can restrict methicillin-resistant Staphylococcus epidermidis when administered with oxacillin, likely due to diterpenes that suppress penicillin-binding protein (PBP) expression [51].

The ethyl acetate extract of M. aquatica resulted in only mild inhibition of facultative anaerobes, in line with EO studies noting weak effects against E. coli and S. aureus and almost no action against C. albicans [52]. Evidence suggests that the antimicrobial profile of M. longifolia is influenced not merely by elevated monoterpene hydrocarbons but by a proportional mixture of these hydrocarbons with oxygenated monoterpenes [53].

When M. longifolia ethyl acetate extract was compared with its aqueous counterpart, the ethyl acetate preparation produced a slightly stronger bactericidal response toward S. aureus [54], matching the pattern observed in this study. Interestingly, spherical organisms like S. aureus typically display less visible structural injury at MIC levels compared with rod-shaped bacteria such as E. coli. Although M. longifolia ethyl acetate extract inhibited S. mutans, it did not eradicate it, whereas the hydroalcoholic preparation evaluated by Kermanshah *et al*. achieved an MBC of 0.1 mg/ml [55]. Overall, the M. longifolia extract produced more pronounced inhibition than M. aquatica, in agreement with Mimica-Dukić *et al*. [52].

Prior antimicrobial assessments of the ethyl acetate extract of S. spinosa are absent. The inhibitory activity noted here may be associated with terpenoid constituents such as thymol and carvacrol.

The ethyl acetate fraction of P. cretica suppressed the proliferation of S. oralis as well as several obligate anaerobes. This pattern aligns with earlier research employing a P. cretica EO, which reported comparatively elevated MIC values for S. aureus and E. coli. The tendency observed here likely relates, at least in part, to the α-pinene content of the extract—shown to influence these microbes—rather than to caryophyllene [56].

When examining how the harvesting stage affects EO potency, it was found that S. parnassica and S. thymbra gathered at full bloom yielded the lowest MIC values toward the foodborne organisms Salmonella enterica and Listeria monocytogenes [57]. Importantly, activity seemed linked not to sheer amounts of carvacrol and thymol, but to their proportion, with an optimal balance around 3:2 (carvacrol:thymol). The ethyl acetate extract of S. thymbra displayed stronger suppression of Gram-positive species such as S. aureus and E. faecalis than of the Gram-negative E. coli. This does not align precisely with the essential oils tested by Giweli *et al*. [58], where MIC values were slightly lower for S. aureus than for E. coli.

The ethyl acetate extract of T. longicaulis produced notable inhibitory activity against Gram-positive bacteria, including S. aureus and S. mutans. Yet this aromatic species also affected Gram-negative strains, consistent with an EO study by De Martino *et al*. [59] analyzing plants from two distinct regions. Interestingly, the essential oil richest in thymol and carvacrol—approximately in a 2:3 ratio—showed weaker overall inhibition. Encapsulation approaches may enhance extract performance, as suggested by the

superior activity of a methanolic extract compared with a dichloromethane extract [60].

Although only a few investigations have assessed the antibiofilm effects of these plants, the available data are informative [30, 61, 62]. Components such as salvipisone and aethiopinone from S. sclarea reduced biofilm mass produced by S. aureus and S. epidermidis [61]. Biofilm formation by methicillin-resistant S. aureus also declined in response to a rosemary ethanolic extract [62]. Remarkably, rosemary EO incorporated into toothpaste—in concentrations ten times lower than CHX—exhibited stronger inhibition of S. mutans biofilm development [30].

Variation in MIC values is routine in microdilution testing following Clinical and Laboratory Standards Institute (CLSI) procedures. EUCAST recognizes that two-fold differences fall within acceptable limits [European Committee on Antimicrobial Susceptibility Testing. MIC distributions and epidemiological cut-off value (ECOFF) setting, EUCAST SOP 10.2, 2021]. Such fluctuations often depend on the culture conditions and timing of inoculum preparation. MICs for many antimicrobials are therefore expressed as ranges. In our analysis, the MIC of DMSO shifted only by a factor of two in a small number of organisms, and this background inhibition was considered when interpreting MIC values for each plant extract.

Our investigation sought to confirm whether the extracts reduced biofilm formation using the crystal violet staining protocol, consistent with prior studies. The MTT assay, which can quantify metabolic activity within established biofilms, represents a promising direction for evaluating the antimicrobial effects of these preparations. We used mono-species biofilms to create a standardized baseline for S. mutans, ensuring that any observed inhibition stemmed from the extracts themselves rather than competitive interactions typical of multi-species communities.

Collectively, the results highlight the strong inhibitory properties of the Mediterranean botanicals examined, especially against the obligate anaerobes relevant to the oral cavity. These outcomes point to the possibility of developing natural antibiofilm and antimicrobial formulations from these herbs. Proposed mechanisms include interference with quorum-sensing pathways, disturbance of extracellular polymeric substance (EPS) synthesis, and prevention of bacterial attachment [63–65]. Further work should determine whether such effects occur at sub-MIC levels and assess potential influences on acid formation and tolerance in cariogenic species. Because phytochemical composition varies with factors such as harvest timing, geographic conditions, and extraction procedures, these parameters must be carefully considered. Rising tolerance to CHX further motivates the exploration of new antimicrobial and antibiofilm options such as the plant extracts evaluated here. Future investigations may also examine their long-term effectiveness and resistance patterns relative to conventional antiseptics.

Notably, the ethyl acetate extracts from Rosmarinus officinalis and Origanum vulgare showed substantial antimicrobial effects across all tested oral pathogens. In addition, the Lavandula stoechas extract displayed strong potential for limiting S. mutans biofilm formation. A deliberate combination of these extracts could form the basis of new antibacterial preparations designed to help manage biofilm-driven oral conditions, including periodontitis and dental caries.

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