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Review Article

Phytochemical complexity and antimicrobial resistance modulation of *Zanthoxylum limonella* essential oil in oral health: A membrane-targeted review

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Abstract

Purpose: *To summarize current evidence based on knowledge of the phytochemical composition, membrane-targeted antimicrobial mechanisms, and potential oral health applications of Zanthoxylum limonella essential oil, with particular investigation into its biological activities against antimicrobial resistance (AMR).*

Methods: *A literature search was carried out using PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar. The search mainly focused on peer-reviewed original research articles and review articles that were published between 2000 and 2025; where appropriate, relevant grey literature concerning the antimicrobial properties of Z. limonella was also incorporated.*

Results: *Essential oil extracted from the fruit of Z. limonella primarily consists of lipophilic monoterpenes, including limonene, sabinene, and terpinen-4-ol, which exhibit notable antibacterial activity. This activity extends to methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum β-lactamase (ESBL)-producing Escherichia coli. Terpenoid compounds from Z. limonella disrupt bacterial phospholipid bilayers, increase membrane fluidity, dissipate the proton motive force (PMF), and impair ATP synthesis. Findings from a randomized crossover clinical trial reviewed in this article demonstrate that a 0.5 % alcohol-free mouthwash containing Z. limonella essential oil reduced both plaque and gingival indices. These results suggest potential biofilm-modulating effects in addition to direct bactericidal activity.*

Conclusion: *This review has demonstrated that essential oil from Z. limonella is a membrane-active phytochemical system which has considerable antimicrobial activity and potential for modifying oral biofilms. Before it can be developed translationally as an adjuvant oral antimicrobial agent, biochemical validation and standardized clinical trials are necessary.*

Keywords: *Antimicrobial resistance, Essential oil, Membrane disruption, Oral biofilm, Phytochemical synergy, Terpenoids, Zanthoxylum limonella*

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INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most critical threats to global public health, with multidrug-resistant (MDR) bacteria increasingly implicated in both systemic and oral infections [1,2]. Conventional antibiotics have particular molecular targets, including cell wall synthesis, inhibition of protein synthesis, or inhibition of DNA gyrase, that are intrinsically

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vulnerable to single-point antibiotic resistance mutations, enzymatic degradation by β-lactamases, and overexpression of efflux pump systems [1,3]. These factors reduce therapeutic efficacy and urgently require alternative antimicrobial strategies that avoid antibiotic resistance pathways. Interestingly, membrane-active phytochemicals have been targeted as mechanistically distinct antimicrobial agents. Lipophilic terpenoids are dominantly found in many plant essential oils, serving as antibacterial agents primarily through physical disruption of the bacterial cell membrane-a target that is structurally essential for cellular viability and is theoretically less susceptible to classical resistance mechanisms [4, 5]. This biophysical mode of action represents a fundamental departure from the enzyme-binding pharmacology of conventional antibiotics.

Zanthoxylum limonella (Ma-khwaen), a *Rutaceae* species with deep-rooted use in Thai and Southeast Asian traditional medicine, produces fruit essential oils dominated by limonene, sabinene, and terpinen-4-ol [6, 7]. Previous *in vitro* studies have reported antibacterial activity of *Z. limonella* essential oil against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [8]. Activity against resistant strains, including methicillin-resistant *S. aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing *E. coli*, has also been reported in an earlier study [9]. Additionally, a randomized crossover clinical trial demonstrated that a 0.5 % alcohol-free mouthwash formulation of *Z. limonella* essential oil considerably reduced plaque and gingival indices, with efficacy comparable to chlorhexidine, albeit without sustained suppression of *Streptococcus mutans* colony counts [10]. Despite several previous studies showing that *Z. limonella* exhibits antimicrobial activity, a comprehensive synthesis of the biochemical and membrane-level mechanisms underlying the activity of *Z. limonella* has not previously been published. This review summarizes current phytochemical, mechanistic, and clinical evidence to construct a membrane-targeted antimicrobial model for *Z. limonella*, with particular emphasis on its resistance-evasive properties.

METHODS

Literature search strategy

A comprehensive literature search was conducted in January and March 2025 using different repositories and search engines (PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar). Primary search terms included: "*Zanthoxylum limonella*", "Ma-khwaen", "essential oil", "antimicrobial", "membrane disruption", "oral biofilm" and "terpenoids". Boolean operators (AND and OR) were used to combine the search terms as appropriate.

The reference lists of the included articles were also hand-searched to identify additional relevant publications.

Inclusion and exclusion criteria

Studies were included studies of the following criteria: (1) reported the phytochemical properties, antimicrobial activity, or mechanism of action of *Z.* limonella or related Zanthoxylum species; (2) investigated membrane-active terpenoids and their biochemical effects on bacterial membranes; (3) evaluated plant-derived essential-oil mouthwash formulations in clinical or in vitro oral health models; and (4) provided mechanistic evidence regarding the effects of terpenoid compounds on the proton motive force, reactive oxygen species (ROS) generation, or ATP synthesis.

Study types and time frame

Both primary research studies (*in vitro* antibacterial assays, randomized clinical trials, pharmacognostic studies) and secondary literature reviews were included. Studies published between 2000 and 2025 were considered.

Seminal mechanistic studies published before 2000 were included where they provided foundational evidence for membrane-disruption mechanisms. No language restriction was applied to original data sources; however, all interpreted findings were evaluated from English-language reports.

Conceptual framework

Evidence was synthesized according to a hierarchical mechanistic model which includes phytochemical composition and chemical variability, physicochemical basis for membrane interaction, membrane disruption cascade and energy metabolism collapse, secondary oxidative stress amplification, resistance-evasive properties, and clinical and oral biofilm-modulating effects.

This framework ensured logical progression from bench-level to clinical evidence.

RESULTS

Phytochemical composition and chemical variability of *Zanthoxylum limonella*

Major chemical constituents

The fruit essential oil of *Z. limonella* is chemically dominated by monoterpenes, principally limonene, sabinene, and terpinen-4-ol [6, 7]. Gas chromatography-mass spectrometry (GC-MS) analysis of *Z. limonella* essential oil identified limonene (57.94%) and α-phellandrene (15.54%) as the major constituents, while sabinene (0.22%) and terpinen-4-ol (0.27%) were shown only in minor amounts [11]. Beyond volatile monoterpenes, phytochemical investigations also document the presence of alkaloids dictamnine, lignan (-)-asarinin, and coumarins within *Z. limonella* [7]. These non-volatile secondary metabolites may contribute to broader biological effects, including anti-inflammatory and anti-biofilm activities.

From a physicochemical standpoint, the predominance of low-molecular-weight, highly lipophilic terpenoids confers strong membrane-partitioning capacity, which constitutes the proposed primary basis for the antimicrobial mechanism. Table 1 provides a summary of major phytochemical constituents of *Z. limonella* and their proposed biological roles.

Phytochemical synergy and cooperative bioactivity

A previous *in vitro* study reported that crude *Z. limonella* essential oil exhibited greater antibacterial activity than isolated sabinene alone [9], suggesting that the antibacterial activity may arise from cooperative interactions among multiple terpenoid and non-terpenoid constituents—a phenomenon recognized as phytochemical synergy [13, 21]. Proposed mechanisms of synergistic interaction include: additive membrane destabilization through multi-compound lipid bilayer insertion, enhanced bilayer penetration arising from mixed hydrophobic interactions between structurally distinct terpenoids and combined perturbation of membrane-bound proteins by major and minor constituents collectively. This cooperative model is particularly relevant to explaining rapid bactericidal kinetics and sustained activity against MDR strains, as a multi-component membrane attack imposes greater pharmacological complexity than resistance mechanisms typically overcome (Figure 1).

Table 1: Major phytochemical constituents of *Zanthoxylum limonella* and their proposed biological roles

Compound	Chemical class	Reported activity	Relevance to antimicrobial mechanism	Ref.
Limonene	Monoterpene	Antibacterial	Membrane permeabilization and depolarization, with leakage of intracellular constituents.	[6, 9, 12]
Sabinene	Monoterpene	Antibacterial, antioxidant	Membrane partitioning; synergistic destabilization with limonene; demonstrated minimum inhibitory concentration (MIC) as isolated compound	[9]
Terpinen-4-ol	Monoterpene alcohol	Broad-spectrum antibacterial, anti-biofilm	Membrane permeabilization; disruption of proton motive force (PMF); documented activity against MRSA and oral pathogens	[6, 13]
Dictamnine	Alkaloid	Antibacterial, cytotoxic	Hypothesised membrane protein inhibition; intercalation into lipid bilayer microdomains	[7, 14, 15]
(-)-Asarinin	Polyphenolic lignan	antioxidant, antimicrobial	Modulation of oxidative membrane damage; hypothesised extracellular polymeric substance (EPS) interference in biofilm	[7, 16, 17]
Coumarins	Benzopyranone	antibacterial, anti-biofilm, and anti-quorum-sensing	Inhibition of quorum-sensing signaling; possible interference with glucosyltransferase activity relevant to oral biofilm	[7, 18-20]

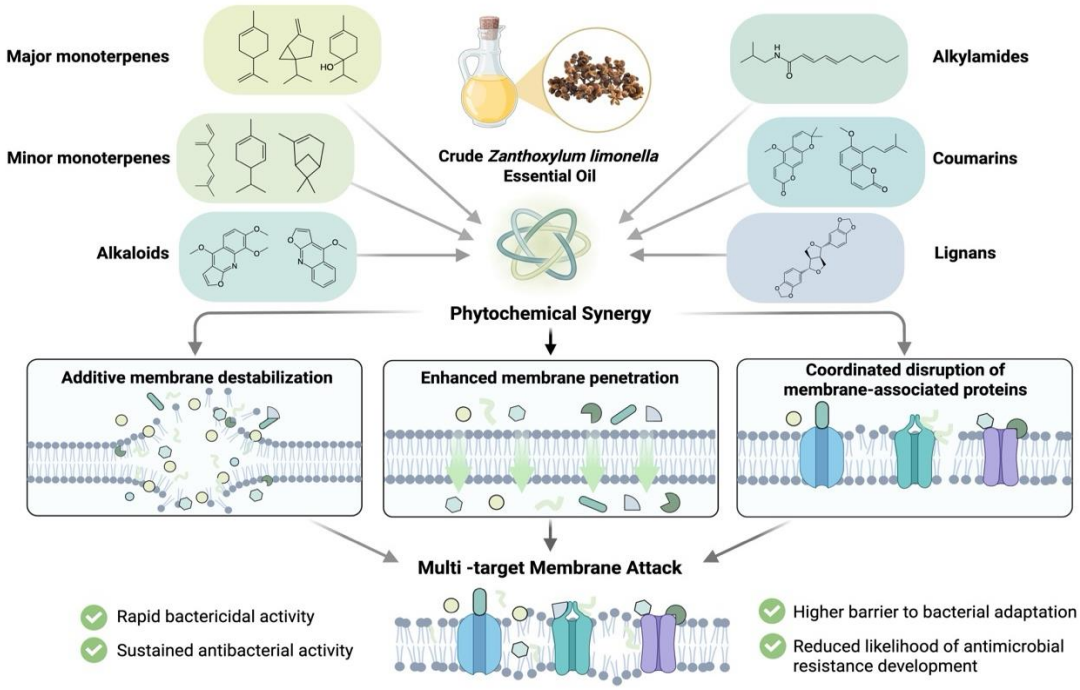


Figure 1: Proposed model of phytochemical synergy associated with *Zanthoxylum limonella*

Reported monoterpenes and other phytochemical classes may cooperatively promote membrane destabilization, enhance membrane penetration, and disrupt membrane-associated functions, resulting in multi-target antibacterial effects. This conceptual model was constructed by the authors based on published evidence concerning the phytochemical composition, antibacterial activity, and synergistic interactions involving *Z. limonella* essential oil [6, 7, 9, 10, 13]. Direct synergistic interactions among the individual constituents and their effects on resistance development require further experimental validation.

Chemical variability and standardization considerations

Geographic origin significantly influences both the volatile oil content and the relative proportions of major constituents in *Z. limonella* [6, 11]. This batch-to-batch variability has direct biochemical implications, including altered membrane interaction potency, variable antibacterial activity, and differences in oxidative stability. For translational development, standardized extraction conditions and GC-MS chemical profiling are important for ensuring batch-to-batch consistency of *Z. limonella* essential oil, whereas controlled storage conditions are required to minimize compositional changes caused by temperature, light, and oxygen exposure [22, 23].

Membrane-driven antimicrobial mechanisms

Physicochemical basis for membrane interaction

The dominant lipophilic monoterpenes of *Z. limonella* essential oil possess physicochemical properties—low molecular weight, high log P values, and structural amphiphilicity—that favor rapid diffusion across bacterial cell envelopes and preferential insertion into phospholipid bilayers [4, 5]. This membrane partitioning is the central biophysical event underpinning the proposed antimicrobial mechanism. Unlike antibiotic classes that require binding to specific protein targets (e.g., penicillin-binding proteins, ribosomal subunits), terpenoid action is predominantly non-specific and concentration-dependent [1, 3, 24, 25]. Bactericidal activity is therefore governed primarily by membrane architecture rather than enzyme-specific binding kinetics.

Membrane destabilization and energy metabolism collapse

An *in vitro* study demonstrated that *Z. limonella* essential oil achieves rapid bactericidal activity against both *S. aureus* and *E. coli* within approximately 9 min for susceptible *S. aureus* and *E. coli* and within approximately 90 min for MRSA and ESBL-producing E. coli at 2× MBC [26]. Such rapid kill kinetics are mechanistically consistent with physical membrane disruption

rather than inhibition of intracellular enzymatic pathways, which characteristically produce slower growth inhibition profiles.

Biochemically, insertion of lipophilic terpenoids into the lipid bilayer is proposed to generate a sequential cascade involving increased membrane fluidity and disruption of lipid packing order [24, 27, 28], followed by increased membrane permeability and leakage of intracellular ions, particularly potassium (K^+) [24, 28]. The resulting dissipation of transmembrane electrochemical gradients, including the membrane potential (Δψ) and pH gradient (ΔpH), leads to collapse of the proton motive force (PMF) [27, 28]. Because the PMF provides the driving force for ATP synthesis by the membrane-bound F_0F_1-ATP synthase complex, its dissipation reduces proton-driven ATP generation and contributes to depletion of the intracellular ATP pool [28]. The consequent loss of electrochemical gradients required for bacterial respiration and nutrient transport is proposed to cause rapid cellular energy failure (Figure 2).

Lipophilic monoterpenes, including limonene, sabinene, and terpinen-4-ol, are proposed to partition into the bacterial phospholipid bilayer, perturb lipid organization, and increase membrane permeability. This membrane destabilization promotes leakage of intracellular ions, particularly K^+, and dissipation of the membrane potential (Δψ) and transmembrane pH gradient (ΔpH), resulting in collapse of the proton motive force (PMF). Disruption of the PMF may reduce ATP production by limiting the proton gradient required for F_0F_1-ATP synthase activity. As ATP availability declines, energy-dependent processes, including respiration and nutrient transport, may also be affected. Together, these effects could contribute to the rapid bactericidal activity reported for *Z. limonella* essential oil. The proposed pathway is based on evidence from studies of membrane-active terpenoids and essential oils [5, 24, 25, 27, 28] together with the antibacterial activity reported for *Z. limonella* [9]. However, the complete pathway has not yet been experimentally confirmed in *Z. limonella*. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Pi, inorganic phosphate; PMF, proton motive force; K^+, potassium ion; H^+, proton; Δψ, membrane potential; ΔpH, transmembrane pH gradient.

Secondary oxidative stress amplification

Membrane disruption by terpenoids may also affect the bacterial electron transport chain and promote the formation of reactive oxygen species (ROS) [24, 25]. Increased ROS can damage membrane lipids, proteins, and nucleic acids, adding to the cellular injury caused by membrane disruption. However, the contribution of oxidative stress to the antibacterial activity of *Z. limonella* essential oil has not been directly established. Further studies are therefore needed to determine whether ROS generation occurs alongside PMF disruption and contributes to bacterial killing.

Resistance-evasive properties

Many established mechanisms of antibiotic resistance, such as β-lactamase production, modification of penicillin-binding proteins, and increased efflux activity, affect specific antibiotics or their molecular targets. Membrane-active terpenoids differ in that their antimicrobial effects are associated with disruption of the bacterial lipid bilayer, which is essential for cell viability [25, 29]. Bactericidal activity of *Z. limonella* essential oil against MRSA and ESBL-producing *E. coli* has been reported in one *in vitro* study [9].

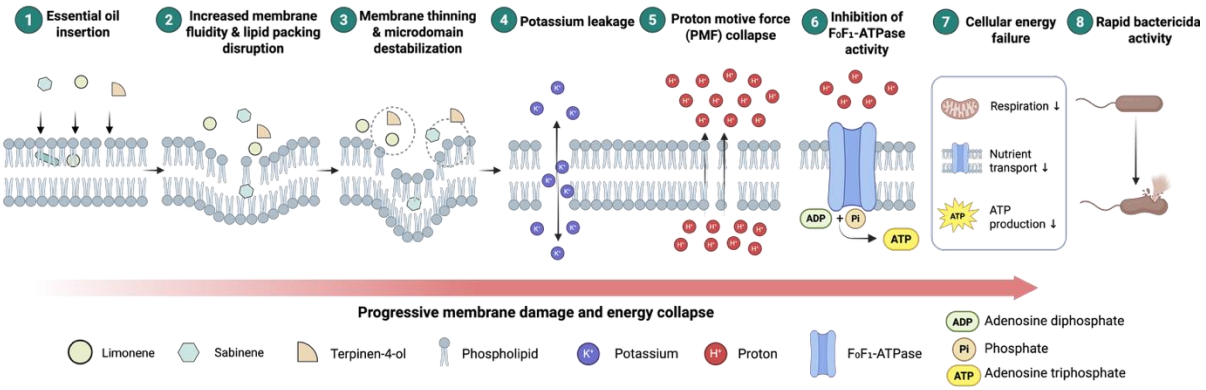


Figure 2: Proposed bacterial membrane-disruption mechanism of *Zanthoxylum limonella* essential oil

This finding provides preliminary evidence of activity against resistant bacteria, but it does not establish that *Z. limonella* can prevent or overcome the development of antimicrobial resistance. Serial-passage studies are needed to determine whether repeated exposure leads to reduced susceptibility.

Antimicrobial and oral health evidence

A randomized crossover clinical trial demonstrated that an alcohol-free 0.5 % *Z. limonella* mouthwash significantly reduced the plaque index (PI) and gingival index (GI) relative to baseline, with efficacy comparable to chlorhexidine, while *S. mutans* colony-forming unit (CFU) suppression was limited and not sustained [10]. This dissociation between plaque reduction and bactericidal killing suggests that *Z. limonella* may modulate biofilm structural integrity independently of direct bactericidal activity. Proposed biofilm-modulating mechanisms include: disruption of extracellular polymeric substance (EPS) integrity, alteration of membrane-associated glucosyltransferase activity relevant to sucrose-dependent *S. mutans* adhesion and modulation of cell-cell adhesion and quorum sensing signaling pathways. Even without complete bacterial eradication, these biofilm-modulating effects may account for clinically observed reductions in plaque accumulation and gingival inflammation [10, 30]. Table 2 summarizes the current evidence for antimicrobial and oral health-related effects of *Z. limonella*, and Figure 3 presents the proposed translational biofilm-modulation model. Membrane-active terpenoids are proposed to penetrate the extracellular polymeric substance (EPS) matrix, disrupt bacterial membranes, and alter biofilm architecture based on published mechanistic evidence [30]. In a randomized crossover clinical trial, a 0.5 % alcohol-free *Z. limonella* essential-oil mouthwash reduced plaque index (PI) and gingival index (GI), whereas the reduction in *Streptococcus mutans* colony counts was limited and not sustained [10]. The clinical findings therefore suggest biofilm modulation rather than complete bacterial eradication. This author-constructed conceptual model integrates mechanistic and clinical evidence from the cited studies [10, 30]. Direct experimental validation of the complete proposed pathway is still required.

DISCUSSION

Research gaps and future directions

Although current evidence supports membrane-associated antimicrobial activity for *Z. limonella* essential oil, several critical biochemical and translational research gaps remain unaddressed. The mechanistic model proposed in this review—encompassing PMF dissipation, ATP synthesis collapse, and secondary ROS generation—is inferred from analogy with well-characterized terpenoids such as thymol, carvacrol, and tea tree oil [24, 25, 27]. Direct experimental confirmation for *Z. limonella* specifically is lacking and represents the primary research priority.

Direct membrane integrity studies should assess membrane depolarization using $DiSC_3(5)$ (3,3′-dipropylthiadicarbocyanine iodide), a membrane-potential-sensitive fluorescent probe, together with potassium-ion efflux measurements [28] and transmission electron microscopy (TEM) or atomic force microscopy (AFM) to evaluate ultrastructural membrane damage [33, 34]. Mechanistic investigations of energy metabolism should include intracellular ATP quantification, oxygen-consumption analysis, and measurement of the activity of the membrane-bound F_0F_1-ATP synthase complex following exposure to *Z. limonella* essential oil. F_0F_1-ATP synthase comprises the proton-translocating F_0 sector and the catalytic F_1 sector responsible for ATP synthesis. These experiments would help determine whether proton motive force (PMF) dissipation and impaired ATP production contribute to bactericidal activity [28]. Intracellular reactive oxygen species (ROS) should also be quantified using an appropriate fluorescent probe, such as 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) [35], together with measurements of lipid peroxidation and antioxidant enzyme activities, including superoxide dismutase and catalase, to characterize the potential oxidative-stress component.

The discrepancy between plaque reduction and limited bactericidal suppression of *S. mutans* highlights the need for targeted oral biofilm studies [10]. These studies should include crystal violet assays for biofilm biomass, confocal laser scanning microscopy (CLSM) for three-dimensional biofilm architecture, EPS carbohydrate quantification, and glucosyltransferase activity assays relevant to sucrose-dependent biofilm formation. Given the documented activity against MRSA and ESBL-producing strains [9], serial passage minimum inhibitory concentration (MIC) experiments are warranted to evaluate resistance development potential, membrane lipid composition remodeling, and efflux pump gene expression changes following repeated sublethal exposure.

Table 2: Summary of antimicrobial and oral health-related evidence for *Zanthoxylum limonella*

Study (author, year)	Study model / organism	Main findings	Oral health relevance	Ref.	Evidence level
Tangjitjaroenkun *et al*., 2012	*In vitro*: susceptible *S. aureus* and *E. coli*, MRSA, and ESBL-producing *E. coli*	Complete bacterial killing was achieved at 2× MBC within approximately 9 min for susceptible strains and 90 min for MDR strains. The crude essential oil showed greater antibacterial activity than isolated sabinene, suggesting possible additive or synergistic effects among its constituents.	Provides mechanistic evidence of rapid antibacterial activity; direct oral health relevance was not evaluated.	[26]	*In vitro*
Wongkattiya *et al*., 2018	*In vitro* ; comparative GC-MS and broth microdilution	Broad-spectrum antibacterial activity confirmed; limonene and phellandrene dominated *Z. limonella*; whereas sabinene and terpinen-4-ol were the major constituents of *Z. rhetsa*.	Chemical variability data critical for essential-oil mouthwash standardization	[11]	*In vitro* / pharmacognostic
Vachirarojpisan *et al*., 2021	Randomized crossover clinical trial; dental biofilm, gingivitis; *S. mutans*	0.5 % alcohol-free mouthwash significantly reduced plaque index and gingival index vs baseline; comparable to chlorhexidine; *S. mutans* CFU suppression limited and not sustained	Direct clinical evidence for oral biofilm modulation; EPS disruption is hypothesised but was not directly evaluated in the clinical study.	[10]	Randomized crossover clinical trial
Supabphol & Tangjitjaroenkun, 2014	Literature review; multiple *in vitro* models	Comprehensive phytochemical review: alkaloids, amides, lignans, coumarins, and terpenoids reported; anti-inflammatory, antimicrobial, and cytotoxic activities documented	Establishes multi-compound pharmacological basis; alkaloids and coumarins may contribute to anti-inflammatory effects in gingivitis management	[7]	Narrative review
Charoensup *et al*., 2016	Pharmacognostic study; fruits and seeds from multiple Thai regions	Pooled data from 15 Thai sources were used to establish volatile oil content and GC-MS-based quality-control specifications for standardization.	Volatile oil variability must be controlled in clinical mouthwash production	[6]	Pharmacognostic / *in vitro*
Nanasombat, 2023	*In vitro* ; medicinal plant mouthrinse formulations; oral pathogens	Plant-derived mouthrinse formulations showed antioxidant, antimicrobial, and anti-oral cancer activities, whereas the mouthrinse formulations were evaluated only for antimicrobial activity against *Candida albicans* and *Lactobacillus casei*	Validates plant-derived oral care products as a clinical category; provides comparative context for *Z. limonella* formulation	[31]	*In vitro*
Suwandi *et al*., 2025	*In vitro* ; *S. sanguinis*, *P. gingivalis* biofilms	*Hibiscus sabdariffa* mouthwash extract reduced 24-h biofilms of oral pathogens relevant to gingivitis and periodontitis	Supports plant-derived mouthwash efficacy against key oral pathogens; comparator context for *Z. limonella* clinical studies	[32]	*In vitro*

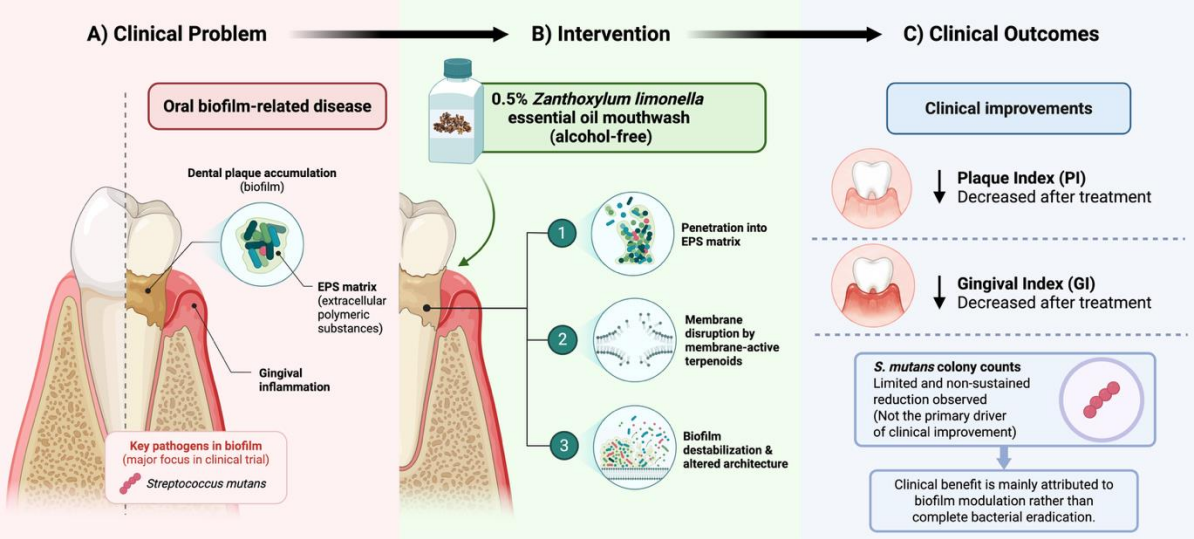


Figure 3: Proposed translational model of ***Zanthoxylum limonella*** essential-oil mouthwash for oral biofilm control

Such data would provide empirical support for the theoretically elevated resistance barrier of membrane-targeted antimicrobial agents.

Translational and clinical implications for oral health

The reviewed evidence indicates that *Z. limonella* contains diverse phytochemicals, including monoterpenes, alkaloids, aromatic amides, lignans, and coumarins [6, 7]. The composition of the fruit essential oil varies among studies, with limonene frequently reported as a major constituent, whereas the relative abundance of sabinene and terpinen-4-ol differs among samples [7, 11]. Variation in geographic origin, extraction conditions, and storage may alter the relative abundance of these constituents and consequently influence antibacterial activity and reproducibility.

The antimicrobial effects of *Z. limonella* essential oil are proposed to arise primarily from membrane-targeted activity. Lipophilic terpenoids may partition into bacterial phospholipid bilayers, disrupt lipid packing, increase membrane permeability, promote ion leakage, dissipate the proton motive force, and impair ATP synthesis [5, 24, 25, 27]. Nevertheless, the complete sequence of these proposed events has not yet been directly demonstrated for *Z. limonella*, and targeted mechanistic validation remains necessary.

Because membrane disruption is less dependent on a single protein target than the mechanisms of many conventional antibiotics, terpenoid-rich essential oils may theoretically present a higher barrier to resistance development [29, 36]. However, only one *in vitro* study has reported bactericidal activity of *Z. limonella* essential oil against MRSA and ESBL-producing *E. coli* [9]. This finding supports potential activity against selected resistant strains but is insufficient to establish a resistance-evasive mechanism. Serial-passage resistance studies are therefore required before conclusions regarding resistance development can be made.

From a translational perspective, the oral cavity represents a complex biofilm-driven ecosystem in which mechanical plaque control may be insufficient to prevent gingival inflammation. Chlorhexidine gluconate remains a widely used chemical adjunct but is associated with adverse effects such as tooth staining, altered taste perception [37].

A randomized crossover clinical trial reported that a 0.5 % alcohol-free *Z. limonella* mouthwash decreased both plaque index and gingival index, resulting in similar efficacy to chlorhexidine, with participants reporting satisfaction with its taste and smell [10]. However, inhibition of *S. mutans* colony counts was limited and not sustained. Therefore, the clinical improvement may reflect modulation of biofilm architecture or gingival inflammation rather than complete bacterial elimination. These findings on the effect of *Z. limonella* support further clinical investigation of *Z. limonella* mouthwash. However, the available evidence is insufficient to draw conclusions about its clinical efficacy or its effect on the development of antimicrobial resistance.

Several research gaps must be addressed before clinical translation. Geographic variability in essential-oil composition necessitates

standardized extraction conditions, GC-MS chemical profiling, batch-to-batch quality control, and controlled storage [6, 11]. Future phase II/III clinical trials should include larger sample sizes, longer follow-up periods, safety monitoring, and oral microbiome profiling. Direct membrane-integrity assays, energy-metabolism measurements, and serial-passage experiments are also required to validate the proposed antimicrobial and resistance-related mechanisms.

CONCLUSION

Current evidence suggests that *Z. limonella* essential oil may have antibacterial activity related to its effects on the bacterial membrane. In particular, its major monoterpenes, including limonene, sabinene, and terpinen-4-ol, may disrupt membrane integrity and affect membrane-associated cellular functions. Nevertheless, several processes involved in the proposed mechanism, particularly PMF disruption, changes in ATP production, and oxidative stress, have not been directly investigated in *Z. limonella*. Evidence regarding its activity against antibiotic-resistant bacteria is also limited and requires further investigation.
Current clinical evidence shows that a mouthwash containing *Z. limonella* essential oil can reduce plaque and gingival indices. However, the reduction in *S. mutans* counts was limited and transient, indicating that these clinical effects may not be explained by bacterial killing alone. Therefore, further studies should directly investigate the effects of *Z. limonella* essential oil on oral biofilms. Further development of *Z. limonella* for oral use will require consistent essential oil composition, standardized extraction, and formulation. Mechanistic studies, together with larger and longer clinical trials, are needed to clarify its antimicrobial effects, safety, and potential role as an adjunct to existing oral antimicrobial approaches. Given the growing problem of global AMR, *Z. limonella* essential oil warrants further mechanistic and clinical investigation to determine its potential as an adjunct to oral antimicrobial therapy.

DECLARATIONS

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Conflict of interest

No conflict of interest associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. PS conceived the study and supervised the review; DP and PK conducted the literature search and data extraction; TL designed the mechanistic framework, drafted the manuscript, and revised it critically. All authors read and approved the final manuscript for publication.

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