

REVIEW

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Biofilm battles: confronting stubborn resistance in dental healthcare by harnessing the antimicrobial potential of South African medicinal plants

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Abstract

Background Dental biofilms are complex microbial communities that play a pivotal role in the onset and progression of oral diseases such as dental caries and periodontal infections. Their inherent structural resilience and enhanced resistance to conventional antimicrobial agents pose significant challenges to effective treatment. This persistent nature of biofilms necessitates the exploration of innovative therapeutic strategies beyond traditional antibiotics and antiseptics. South Africa's rich ethnobotanical heritage offers a valuable resource of medicinal plants historically used to treat infections, presenting potential alternatives for managing biofilm-related oral diseases.

Methods A systematic search was performed across multiple scientific databases, including PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar, covering publications from 2000 to 2024. The following keywords and combinations were used.

Results This review investigates the role of South African medicinal plants in mitigating biofilm-associated infections within oral healthcare. Drawing from ethnobotanical traditions and current pharmacological research, the study highlights the therapeutic significance of indigenous flora such as *Warburgia salutaris*, *Combretum molle*, and *Aloe ferox*, among others. These plants exhibit broad-spectrum antimicrobial, anti-inflammatory, and anti-biofilm activities, targeting key mechanisms such as microbial adhesion, quorum sensing, and EPS matrix disruption. These activities are mediated through disruption of microbial adhesion, interference with quorum sensing signaling pathways, and degradation of the EPS matrix, which is essential for biofilm stability. Additionally, anti-inflammatory properties contribute to the reduction of host tissue damage. However, pharmacodynamic variability across extracts and the lack of standardized extraction protocols limit reproducibility and clinical translation. Furthermore, concerns about the sustainability of harvesting endangered species highlight the need for conservation efforts.

Conclusion South African medicinal plants represent a promising natural arsenal for combating resilient oral biofilms and their associated infections. To fully realize their therapeutic potential, future research must focus on standardizing extraction methods, elucidating molecular mechanisms, and conducting rigorous clinical evaluations. Sustainable

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harvesting and benefit-sharing frameworks are essential to preserve indigenous biodiversity. Integrating these botanical resources into modern oral healthcare could significantly advance the management of biofilm-mediated diseases and address the growing threat of antimicrobial resistance globally.

Keywords Anti-biofilm activity, Ethnobotany, Extracellular polymeric substance, Oral biofilms, Phytochemicals, South African medicinal plants, Traditional medicine

Introduction

Biofilm-associated infections represent a persistent and growing concern in modern dental healthcare, with oral biofilms playing a central role in the development of conditions such as dental caries, gingivitis, and periodontitis. These complex microbial communities, embedded within a self-produced extracellular matrix, are notoriously resistant to conventional antimicrobial agents and host immune defences. Compounding this challenge is the global rise in antimicrobial resistance (AMR), which significantly limits the efficacy of standard treatments against biofilm-forming pathogens in the oral cavity [1, 2]. In response to these clinical hurdles, interest in plant-based therapeutics has surged, particularly those derived from traditional medicinal systems. South Africa, recognized as one of the world's biodiversity hotspots, is home to a vast repertoire of medicinal flora with longstanding ethnobotanical relevance. An estimated 80% of the South African population relies on traditional plant remedies for primary healthcare, including the treatment of oral infections [3, 4]. Recent pharmacological studies have validated many of these traditional practices, revealing that several indigenous species possess potent antibacterial and antibiofilm properties, offering a promising alternative to synthetic antimicrobials.

For instance, *Melianthus comosus* and *Pelargonium sidoides* have demonstrated minimum inhibitory concentrations (MICs) as low as 0.19 mg/ml against resistant strains such as *Staphylococcus aureus* and *Klebsiella pneumoniae*, highlighting their broad-spectrum antimicrobial efficacy [5]. Likewise, *Lippia javanica* has shown remarkable activity against multidrug-resistant *K. pneumoniae*, with MIC values around 0.78 mg/ml, and its ethyl acetate extracts have been shown to significantly reduce biofilm formation and disrupt biofilm architecture [6]. Additionally, *Vetiveria zizanioides* has achieved up to 75% inhibition of *S. aureus* biofilms, reinforcing the therapeutic potential of native plant extracts in managing oral microbial colonization [7]. These antimicrobial effects are primarily attributed to the rich phytochemical content of these plants, including diterpenes, flavonoids, and other secondary metabolites. Advanced chemical profiling has begun to identify specific compounds responsible for these bioactivities, suggesting potential for future drug development [6, 7]. Despite this promise, challenges remain regarding extract standardization, formulation stability, and clinical validation, which are

critical for transitioning these natural products into regulated therapeutic applications.

Given the urgent need for new strategies to counteract biofilm-related resistance and the wealth of untapped botanical resources in South Africa, this review explores the antimicrobial and antibiofilm potential of South African medicinal plants through various mechanisms as shown in Fig. 1. It highlights their traditional use, pharmacological validation, and possible integration into contemporary dental care frameworks aimed at overcoming the limitations of current antimicrobial therapies.

Methodology

This study employed a narrative literature review approach to critically explore the role of South African medicinal plants in combating biofilm-associated resistance in dental healthcare. The review integrated interdisciplinary evidence from microbiology, ethnobotany, dentistry, and pharmacology to provide a comprehensive synthesis of existing knowledge. A systematic search was performed across multiple scientific databases, including PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar, covering publications from 2000 to 2024. The following keywords Anti-biofilm activity; Ethnobotany; Extracellular polymeric substance; Oral biofilms; Phytochemicals; South African medicinal plants; Traditional medicine.

Search Strategy and Keyword Selection: The search strategy employed a combination of Medical Subject Headings (MeSH) terms and relevant keywords connected by Boolean operators (AND, OR). The search was structured into three primary concepts: Plant Origin (e.g., "South African medicinal plants", "ethnomedicine"), Biological Target (e.g., "oral biofilm", "Candida albicans", "antibiofilm"), and Specific Activity (e.g., "antimicrobial", "synergism", "phytochemicals"). The final comprehensive query was structured as 3 combinations to ensure both sensitivity and specificity.

Inclusion criteria

- Peer-reviewed articles, books, and reviews published in English.
- Studies focusing on oral biofilms, microbial resistance, and plant-based antimicrobials.
- Research on South African medicinal plants with documented antimicrobial or antibiofilm activity.

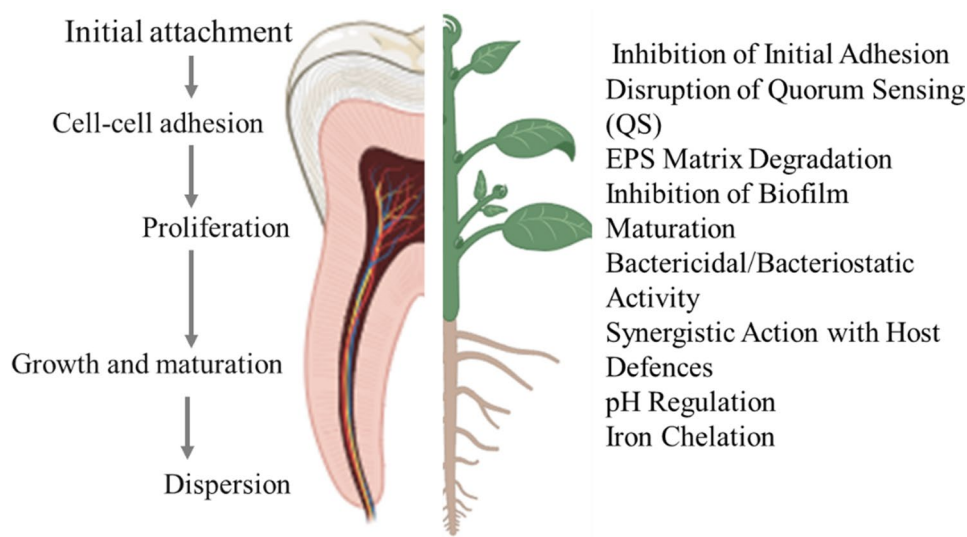


Fig. 1 Mechanisms of action of South African medicinal plant extracts against the oral biofilm lifecycle. This schematic illustrates the multi-target effects of the identified phytochemicals, including inhibition of initial microbial adhesion, interference with quorum sensing (QS) signalling, disruption of the extracellular polymeric substance (EPS) matrix, and subsequent reduction of virulence factors. (Created by MC Manganyi)

- Articles addressing the phytochemistry, ethnopharmacology, or traditional oral health uses of medicinal plants.

Exclusion criteria

- Publications focused on synthetic drugs without any mention of plant-derived compounds.
- Editorials, opinion pieces, and commercial product reviews without scientific backing.

Definition and significance of biofilms in dental healthcare

Biofilms are complex, structured assemblies of microbial populations that adhere irreversibly to living tissues or inert surfaces. These communities are encased in a matrix of self-produced extracellular polymeric substances (EPS), which provides both cohesion and structural support [8, 9]. The ability to form biofilms represents a key evolutionary adaptation of prokaryotic organisms, enabling planktonic bacterial cells to transition into sessile forms by anchoring themselves onto a wide array of substrates ranging from biological materials such as oral tissues to synthetic surfaces like plastic and metal, as well as static water environments [10].

In the context of oral health, dental plaque commonly referred to as a biofilm comprises a three-dimensional, heterogeneous community of microorganisms, including pathogenic species such as *Streptococcus* spp [11]. The defining feature of biofilms lies in their structural complexity. Microbial cells within these biofilms are not randomly clustered; instead, they are spatially arranged within the EPS matrix, which serves as a scaffold that preserves the integrity of the colony while promoting

intercellular communication and metabolic cooperation [12]. The EPS matrix itself consists of a diverse composition of biomolecules, including polysaccharides, extracellular DNA, proteins, and lipids. This matrix facilitates adhesion to oral surfaces, supports nutrient acquisition, and provides a shield against environmental stressors such as antimicrobial agents and host immune responses [9]. Importantly, the formation of biofilms in the oral cavity is not merely a mechanical process but also a biological one that involves intricate microbial organization and regulation. These biofilms are often implicated in the etiology of various oral pathologies, including dental caries and periodontal diseases. However, they also contribute to the maintenance of ecological balance in the oral microbiome, playing a dual role in both health and disease.

Epidemiological data suggest that biofilm-associated infections account for more than 60% of all human infectious diseases, emphasizing their significance in clinical settings [8]. In dentistry, biofilms are commonly found on tooth surfaces, dental prostheses, and restorative materials. Their presence has implications for both preventive and therapeutic dental strategies. Additionally, the physicochemical properties of dental biomaterials, including their surface roughness, hydrophobicity, and electrochemical attributes, are critical factors influencing the degree and pattern of biofilm colonization [10]. As such, understanding the interaction between oral biofilms and biomaterials is essential for optimizing dental treatment outcomes and preventing secondary infections.

In conclusion, regulating the formation and development of biofilms within the oral environment is crucial for the prevention of biofilm-associated dental diseases.

Effective biofilm management strategies not only safeguard oral health but also contribute to the broader goal of reducing the incidence of chronic infections that stem from untreated or mismanaged microbial colonization.

Understanding biofilm formation in the oral cavity

Role of biofilms in oral diseases

Biofilms play a critical role in the etiology of the most prevalent oral diseases, notably dental caries and periodontal disorders. While pathogenic biofilms are of primary concern, it is essential to recognize that certain biofilm communities contribute positively to oral health. These health-associated biofilms commonly include bacterial genera such as *Neisseria*, *Streptococcus*, *Actinomyces*, *Veillonella*, and *Granulicatella*, which are integral to maintaining microbial homeostasis within the oral cavity [13]. A substantial portion of contemporary biofilm research has relied on model organisms known for their biofilm-forming capabilities. These include *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, and *Vibrio cholerae* [13]. In the oral environment, biofilms are highly structured, multispecies microbial communities in which the resident microorganisms exhibit both spatial and metabolic specialization. These microbial consortia are implicated in both caries and periodontal pathologies, where their cooperative behaviours and stress responses contribute to disease progression [9].

The development of dental caries, in particular, is driven by frequent exposure of the oral biofilm to fermentable carbohydrates. This leads to acid production by acidogenic and aciduric species, creating a localized acidic environment that facilitates the demineralization of enamel and dentin [13, 14]. Dental caries is recognized as a multifactorial and dynamic disease, influenced by dietary habits, inadequate oral hygiene, limited public awareness of the biofilm-mediated nature of caries, and the high prevalence of cariogenic bacteria such as *Streptococcus mutans* [15, 16]. Notably, *S. mutans* expresses a collagen-binding protein that enhances its adhesion and virulence [17]. As a primary colonizer within dental biofilms, *Streptococcus* spp., particularly *S. mutans*, are known for their ability to efficiently metabolize carbohydrate substrates, contributing to pH reduction and subsequent enamel erosion [14]. The repeated intake of sugar-rich foods further promotes the dominance of acid-tolerant bacteria, including *Actinomyces naeslundii*, which synergistically contribute to the cariogenic process [18].

The pathogenesis of oral diseases is driven by complex, polymicrobial biofilms where the fungal component, predominantly *Candida albicans*, plays a crucial, synergistic role alongside primary bacterial pathogens. While

bacterial species like *Streptococcus mutans* and key periodontopathogens are central to the biofilm structure, *C. albicans* acts as a significant co-colonizer, particularly in immune-compromised or high-sugar environments, substantially enhancing the overall virulence of the community [19]. The formation of these inter-kingdom, mixed-species biofilms allow for metabolic cooperation, increased biomass accumulation, and enhanced resistance to host defences and antimicrobials [20, 21]. Specifically, in conditions like severe Early Childhood Caries (ECC), *C. albicans* and *S. mutans* form a synergistic alliance where *S. mutans*-derived exoenzymes (glucosyltransferases) bind to the fungal surface, increasing the production of extracellular polysaccharides (EPS) that structurally stabilize the biofilm. This combined activity amplifies acid production and promotes the development of highly acidic microenvironments, which accelerates enamel demineralization [22, 23]. Therefore, any successful therapeutic strategy must address this inter-kingdom dynamic to effectively manage the increased pathogenicity and resilience of oral biofilm-related infections [20]. In the context of periodontal disease, there is a notable transition in the biofilm composition from commensal to pathogenic species. This shift favours proteolytic, anaerobic bacteria that incite host inflammatory responses and degrade the connective tissues supporting the teeth [10]. Beyond localized effects, chronic biofilm formation has been linked to systemic health implications, including persistent inflammatory and autoimmune conditions [2].

Resistance to antimicrobial agents

The escalating challenge of antimicrobial resistance (AMR) presents significant public health concerns, particularly in managing bacterial infections. Current global estimates attribute approximately 1.2 million deaths annually to infections linked with AMR, highlighting the urgent need for effective interventions [1]. Addressing this issue requires a comprehensive understanding of resistance development and the identification of antimicrobial resistance gene (ARG) reservoirs within the human microbiome.

Microorganisms embedded in biofilms exhibit markedly increased resistance to antimicrobial agents compared to their free-floating planktonic counterparts. This resistance arises from restricted diffusion of antimicrobial agents through the dense EPS matrix, protective microenvironments with variable pH and oxygen levels, and dormant or persistent cells less susceptible to antibiotics [1].

Recent studies highlight the oral cavity as a key reservoir for antimicrobial resistance, with distinct microbial niches contributing to the diversity and persistence of resistant strains. Oral biofilms, which can harbour over 700 distinct bacterial species, contain numerous

antibiotic resistance determinants, making the oral microbiota an ideal model for exploring AMR mechanisms and evolution [24]. Comparative studies reveal a higher abundance and broader spectrum of resistance genes in periodontitis patients than in healthy individuals [1].

Furthermore, biofilms promote horizontal gene transfer, facilitating dissemination of resistance genes among microbial populations and complicating treatment [25]. Novel antimicrobial agents such as chitosan, a natural cationic polysaccharide with antibacterial and antifungal properties, offer promising alternatives or adjuncts in biofilm infection management [1].

Implications for dental materials and devices

Biofilm formation on dental materials and prosthetic devices (implants, orthodontic components, dentures) is a major concern. Surface roughness and hydrophobicity critically influence microbial adhesion and biofilm development [10]. Accumulated biofilms on intraoral devices are associated with peri-implantitis and secondary caries, threatening treatment longevity. Developing biomaterials with intrinsic antimicrobial properties and using localized antimicrobial delivery via biodegradable carriers during implant placement show promise to reduce these risks [10]. Moreover, biofilm-associated infections affect medical devices broadly, with microbial colonization from patient skin, healthcare workers, environment, or inadequately sterilized equipment posing infection risks [2]. Early detection and alternative therapeutic

approaches beyond conventional device-dependent methods are needed for sustainable management [25].

Systemic health implications

Growing evidence links oral biofilms to systemic diseases including cardiovascular disease, diabetes, chronic kidney and pulmonary diseases, cancers (pancreatic, colon, prostate), preterm birth, erectile dysfunction, Alzheimer's disease, and rheumatoid arthritis [26, 27]. *Streptococcus mutans*, implicated in various systemic conditions, is a primary pathogen in chronic biofilm-mediated periodontitis affecting nearly half the population. Untreated, it leads to irreversible tissue destruction and tooth loss, disrupting oral function [11, 13]. Translocation of biofilm pathogens into the bloodstream triggers systemic inflammation, aggravating existing health issues. For example, poor oral hygiene elevates risk for latent rheumatic heart disease progression, with antibiotic prophylaxis reducing this risk [28]. Antibiotic premedication is also advised before invasive dental procedures in high-risk patients to prevent infective endocarditis [29]. Oral pathogens may contribute to neuroinflammation and neurodegenerative disease via the inflammatory and pathogen hypotheses, crossing the blood-brain barrier [30]. These findings underline the systemic importance of oral biofilms and the need for integrated dental-medical care to maintain oral microbial balance and overall health [26]. Finally, public health authorities must prioritize biofilm-specific risk assessments and regulations to protect human and environmental health [25]. Biofilms on teeth contribute to major oral health problems such as dental caries, gingivitis, periodontitis, and antimicrobial resistance, as illustrated in Fig. 2, which outlines the key complications and progression of biofilm-related infections in the oral cavity.

Strategies for biofilm management

Mechanical disruption

Mechanical removal remains the foundation of effective oral hygiene, with toothbrushing serving as the primary method for disrupting dental biofilms [11, 31]. This approach significantly reduces microbial accumulation, thereby mitigating the risk of oral and systemic complications [12]. Additional tools, including dental floss, interdental brushes, sticks, and water irrigators, contribute to more thorough plaque control. Despite the variation in cleaning devices, oral hygiene guidance for patients with dental implants is largely informed by protocols established for natural dentition. Educating patients on proper oral hygiene practices is vital for long-term success. Innovations in both manual and powered toothbrush designs continue to enhance biofilm removal and promote oral health [31, 32].

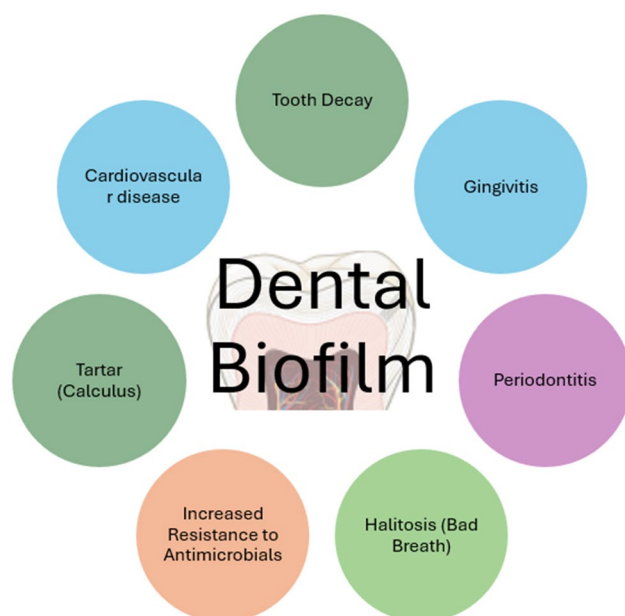


Fig. 2 Problems associated with biofilm formation of teeth. This diagram illustrates the progression from initial plaque formation to the development of dental caries (tooth decay) and various forms of periodontal diseases (gingivitis and periodontitis)

Chemical control

Chemical agents, particularly antimicrobial mouth rinses, serve as effective adjuncts to mechanical cleaning by targeting residual biofilm microorganisms [11]. Chlorhexidine (CHX), widely used in various dental disciplines, offers strong antimicrobial effects, especially when mechanical access is limited. Mouthrinses containing CHX at concentrations between 0.12% and 0.2% are most effective, while higher concentrations may increase side effects unnecessarily. CHX is especially useful in managing conditions such as peri-implant mucositis, where it helps reduce inflammation when used alongside mechanical therapy [33, 34]. These chemical interventions complement mechanical techniques by disrupting the extracellular biofilm matrix and decreasing microbial viability.

Innovative therapeutic approaches

Probiotics, particularly lactic acid bacteria (LAB), are gaining attention as promising agents for disrupting oral biofilms. These microorganisms inhibit pathogen adhesion, biofilm formation, and microbial overgrowth by interfering with bacterial signalling and aggregation processes, offering a potential alternative to conventional antibiotics [35].

Advancements in biofilm control now include the design of anti-biofilm dental materials, the therapeutic use of probiotics to modulate the oral microbiota, and the application of quorum sensing inhibitors to interrupt bacterial communication. Although probiotics are well established for gastrointestinal health, recent studies suggest their efficacy in managing oral conditions such as dental caries, mucositis, and halitosis [17]. These approaches aim to either prevent biofilm establishment or increase their vulnerability to treatment.

Challenges in biofilm research

The unique characteristics of biofilms and the unparalleled effects they have on oral health among other health risks—make them very difficult to study under laboratory conditions. Several challenges are faced when dealing with biofilms, and they fall under the following four categories. Firstly, challenges when analysing biofilms in laboratories arise. Culturing naturally occurring biofilms in a laboratory can alter microbial behaviour due to a different environment. Laboratory cultures are rich in nutrients, in contrast to the scarcity of nutrients in natural settings. There is also reduced pressure regarding adherence and predation, which modifies the behaviour of the biofilms [36]. It can therefore be concluded that in laboratories, biofilms cannot be accurately represented. Secondly, diagnosing biofilms is challenging due to their complexity. Recognising biofilm infections clinically and accurately remains difficult because of nonspecific signs

and symptoms. Universally accepted protocols for diagnosing biofilm infections are still lacking, resulting in varied approaches across different settings. Furthermore, the diversity of diagnostic methods—often expensive, time-consuming, and requiring specialised expertise—is a barrier to standardisation [37]. This shows that biofilm diagnosis is not universally accessible, primarily due to complexity and ongoing research.

Thirdly, the environmental and health impacts of biofilms present another major challenge due to their widespread nature. Microorganisms associated with biofilms are responsible for approximately 65% of all microbial infections. These organisms evade host immune defences and antimicrobial agents, thereby increasing morbidity, mortality, and healthcare costs. Environmentally, biofilms can colonise nearly all surfaces exposed to natural fluids, impacting both ecological and industrial systems [38]. Lastly, biofilms slow down research progress and hinder effective treatment. Their contribution to chronic infections and their resistance to antibiotics is largely due to the protective matrix that shields them from immune responses and antimicrobial agents. Furthermore, biofilms resist infiltration even by invasive devices such as catheters and implants. Emerging strategies such as omics-based analysis show promise for faster and more specific biofilm identification, although they require significant expertise and meticulous data interpretation [37]. Thus, biofilm research and therapeutic innovation remain in a gradual state of advancement.

Challenges of antibiotic-resistant dental infections

Bacterial biofilms and antibiotic resistance

Bacterial biofilms are significantly more resistant to antibiotics than their planktonic counterparts—typically 10 to 1000 times more resistant. As a result, the required minimum inhibitory concentration (MIC) for effective treatment is often 100 to 800 times greater. Even when exposed to high antibiotic concentrations, individual cells can survive and regenerate into more resistant biofilm communities [39]. The biofilm's three-dimensional structure allows microorganisms to attach firmly to surfaces and embed themselves within an extracellular polysaccharide matrix (EPS), forming a protective barrier against external threats. Nutrient channels within the biofilm structure maintain bacterial growth and maturation, promoting sustained colonization. Furthermore, bacterial cells are coated with a sticky membrane, and specialized efflux pumps actively expel antibiotics. Biofilms can also alter gene expression to enhance antibiotic resistance and increase survival rates [39, 40].

Immune evasion by bacterial biofilms

Biofilms evade immune responses using their extracellular matrix (ECM), which acts as a physical barrier, preventing immune cells from accessing embedded bacteria. Their architecture helps them avoid both innate and adaptive immune defences. Some biofilms produce toxic metabolites that neutralize the antimicrobial action of neutrophil extracellular traps (NETs), while others interfere with the complement system. Additionally, biofilms can modulate the T-cell response, leading to chronic infections and prolonged health issues [41].

Fungal biofilms and antifungal resistance

Fungal biofilms exhibit resistance mechanisms similar to those of bacteria. The ECM hinders the penetration of antifungal agents. Efflux pumps are often overexpressed, consistently removing antifungals from the biofilm environment [42]. Genetic mutations in biofilm-associated fungal cells can alter drug targets, reducing antifungal efficacy. Persister cells—metabolically dormant variants—further contribute to resistance and can later reactivate to restore biofilm formation. Proteins involved in filamentation and developmental transitions of biofilms also play critical roles in resistance [42].

Immune evasion by fungal biofilms

Fungal biofilms can form on medical devices, endothelial surfaces, and epithelial linings. The ECM serves as a protective shield against neutrophils and monocytes, enhancing immune evasion. Like bacterial biofilms, fungal biofilms can produce enzymes and toxins that neutralize NETs and evade complement-mediated destruction. They may also suppress T-cell responses, contributing to persistent and chronic infections [41, 43].

South African medicinal plants: a rich resource

South Africa, home to approximately 10% of the world's plant species, has a rich tradition of using medicinal plants rooted in indigenous knowledge. While the region holds immense botanical potential, only a limited number of these species have been thoroughly studied or commercialized [44]. Nonetheless, recent growth in research and innovation reflects a renewed scientific interest in the therapeutic applications of native flora, reinforcing their importance in the broader healthcare context. South Africa is a global hub renowned for its rich repository of medicinal plants and is recognized as a biodiversity hotspot, particularly for its diverse flora. As documented by Cornelius and van Wyk, approximately 4,120 taxa with medicinal or ritual applications have been recorded, with 1,239 newly identified taxa added since 2002, demonstrating ongoing discoveries within the field of ethnobotany [45]. These plants play a pivotal role in both traditional and contemporary healthcare systems,

emphasizing their cultural, economic, and ecological significance [46]. Traditional medicine remains the primary source of healthcare for nearly 70% of the South African population, illustrating its deep-rooted cultural relevance [47]. It is estimated that around 19,500 tons of medicinal plants are harvested annually to meet the demands of traditional practitioners and local communities, significantly supporting rural economies through harvesting, processing, and commercialization [48]. Many of these plants are transformed into natural health products for domestic consumption and international markets [48]. According to Fajinmi et al., this sector has become an important income-generating avenue for rural populations [46]. Szuman et al. report that South Africa exports approximately 6,000 tons of medicinal plants annually, reflecting both global demand and economic potential [47]. Recent studies have shed light on the extensive use of medicinal flora across the country, emphasizing their therapeutic applications and the urgent need for sustainable utilization and conservation strategies [46, 49, 50].

Medicinal plants in South Africa also have a rich history of use in oral healthcare, particularly due to their potent antimicrobial properties. They have been integral to traditional medicine, providing natural alternatives to combat bacterial infections in the face of rising antibiotic resistance. Historically, indigenous species have been employed to manage oral diseases such as gingivitis, dental caries, and mouth ulcers, highlighting their therapeutic significance [3, 51]. Continued scientific investigation offers promising prospects for the development of phytotherapeutic agents in modern dentistry and public health [52, 53]. This historical context is deeply rooted in indigenous knowledge systems. Traditional practices among South African communities have incorporated plants into daily oral hygiene routines using plant-based rinses, chewing sticks, and pastes for toothaches, gum diseases, and mouth sores [53, 54]. Knowledge of medicinal plants used to treat oral ailments remains embedded in the heritage of communities such as the Zulu, Xhosa, Pedi, and Venda. Plants such as *Warburgia salutaris*, *Sutherlandia frutescens*, and *Combretum molle* are commonly employed for their antimicrobial, analgesic, and anti-inflammatory properties [52]. Traditionally, these plants are prepared as decoctions, infusions, or chewed raw, offering accessible and affordable remedies in rural and peri-urban areas.

Warburgia salutaris is used by chewing its bark to relieve toothaches and mouth infections due to its strong antibacterial compounds [55]. Recent studies also demonstrate its potential in controlling periodontal disease, antibacterial and antifungal activity, and significant anti-inflammatory effects via modulation of cytokines IL-10 and IL-2 [55, 56]. Its drimane-type sesquiterpenes contribute to antimicrobial efficacy against oral pathogens

like *Streptococcus mutans*, reducing biofilm formation [56, 57]. Similarly, *Combretum molle* is widely used as a natural toothbrush with both mechanical and chemical oral hygiene benefits [58]. Kulawe et al. showed its extracts inhibit *Salmonella typhi* and *Bacillus subtilis* with an MIC of 1.5 mg/ml; its flavonoid and tannin content contribute to antibacterial effects [59, 60]. The extracts also enhance wound healing in oral ulcers or post-surgical recovery [61]. *Zanthoxylum capense* serves as a traditional mouth freshener and toothcare agent; it inhibits *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Streptococcus mutans*, largely due to its alkaloid-rich phytochemistry [62–64].

Aloe ferox is applied topically to ulcers and inflamed gums for healing and pain relief, with antimicrobial efficacy reported against *P. gingivalis* [65, 66]. *Psoralea pinnata* and *Otholobium fruticans* exhibited high activity against *S. mutans* (MIC 31.25 µg/ml), also inhibiting biofilm formation [67]. *Alchornea laxiflora*, *Ficus exasperata*, *Morinda lucida*, *Jatropha gossypifolia*, *Ocimum gratissimum*, and *Acalypha wilkesiana* extracts showed >50% biofilm inhibition against bacterial and fungal pathogens [68]. *Acacia karroo*, *Psidium guajava*, *Punica granatum*, *Eucomis autumnalis*, *Vernonia amygdalina*, and *Cuathula unculata* demonstrated potent antibacterial activity against *E. coli* and *S. aureus* [69]. Acetone extracts of *Searsia leptodictya*, *S. lancea*, *S. batophylla*, *S. pendulina*, *Bauhinia bowkeri*, and *B. galpinii* exhibited antibacterial and anti-biofilm activity. Specifically, *S. pendulina* achieved ≥ 98% eradication of *Bacillus cereus* mature biofilms, and *B. bowkeri* and *S. lancea* had MICs as low as 0.01 mg/ml [70].

Carpobrotus edulis, *Vachellia rehmanniana*, *V. xanthophloea*, *Kigelia africana*, *Elephantorrhiza elephantina*,

and *Ochna pretoriensis* were also investigated, showing strong anti-biofilm and antioxidant activities [71]. *Salvia africana-caerulea* demonstrated significant inhibition of biofilm formation, reinforcing its preventive potential in bacterial colonization [72]. Several South African medicinal plants have demonstrated remarkable anti-biofilm activity against oral pathogens, offering natural solutions to manage dental plaque and periodontal diseases. This highlights the therapeutic potential of South Africa’s rich botanical resources in combating biofilm-associated infections and promoting oral health, this is evident in various tribes in South Africa (Table 1).

Mechanisms of action of medicinal plants against biofilm

Medicinal plants have gained worldwide interest for their accessibility, cost-effectiveness, eco-friendliness, richness in bioactive compounds, and extensive biological benefits. For these reasons, they are positioned as viable alternatives or complements to traditional antibiotics in the global fight against multidrug-resistant (MDR) bacteria [73]. The mechanisms of action of medicinal plants against biofilm formation are multifaceted (Table 2), involving various bioactive compounds that disrupt microbial communication and biofilm integrity. These bioactive compounds interfere with bacterial processes by inhibiting biofilm formation and exerting potent antimicrobial effects. Their effectiveness lies in their ability to target multiple cellular components simultaneously, reducing the likelihood of resistance development. A well-established mechanism is the inhibition of biofilm formation, a critical bacterial survival strategy conferring high tolerance to antimicrobials. Phytochemicals such as flavonoids, alkaloids, and terpenoids impede bacterial adhesion and downregulate genes responsible

Table 1 Traditional medicinal plants used by South African tribes for oral health

Tribe	Medicinal plant	Plant part used	Traditional use for oral health	Other notes	Ref
Zulu	<i>Warburgia salutaris</i> (Pepper-bark tree)	Bark, leaves	Chewed for toothache, mouth sores, and gum infections	Known for antimicrobial and analgesic properties	[55]
Xhosa	<i>Sutherlandia frutescens</i> (Cancer bush)	Leaves, stems	Infusions for mouth ulcers and sore gums	Also used for general immune support	[44]
Tswana	<i>Helichrysum spp.</i> (Everlastings)	Leaves	Smoked or chewed to treat toothache and oral infections	Widely used by many tribes for multiple ailments	[44]
Pedi	<i>Acacia karroo</i> (Sweet thorn)	Bark, gum	Bark decoction used as mouthwash for gum disease	Contains tannins with astringent properties	[45]
Venda	<i>Combretum molle</i> (Velvet bushwillow)	Leaves, bark	Used as a toothbrush stick; decoctions for gum health	Also used for wound healing and skin infections	[58]
Ndebele	<i>Zanthoxylum capense</i> (Knobwood)	Bark, roots	Chewed for toothache relief and oral cleansing	Contains alkaloids with analgesic and antimicrobial effects	[62]
Sotho	<i>Leonotis leonurus</i> (Wild dagga)	Leaves, flowers	Infusions or decoctions for treating oral infections	Also used for respiratory ailments	[45]
Swati	<i>Aloe ferox</i> (Bitter aloe)	Leaf gel	Applied to inflamed gums and mouth ulcers	Known for strong anti-inflammatory and healing properties	[65]
San (Khoisan)	<i>Bulbine frutescens</i> (Burn jelly plant)	Leaf sap	Rubbed on gums to soothe inflammation and pain	Traditionally used for skin and oral care	[46]

Table 2 Mechanisms of action of medicinal plants against biofilm formation and persistence

Mechanism	How it works	Examples of active compounds	Medicinal plant examples	References
Inhibition of Initial Adhesion	Prevents microbial cells from attaching to oral surfaces (teeth, gums)	Flavonoids, saponins, tannins	<i>Helichrysum spp.</i> , <i>Combretum molle</i>	[44, 58]
Disruption of Quorum Sensing (QS)	Interferes with microbial communication systems that regulate biofilm formation and virulence	Coumarins, alkaloids, phenolics	<i>Zanthoxylum capense</i> , <i>Warburgia salutaris</i>	[63, 57]
EPS Matrix Degradation	Breaks down extracellular polymeric substances (EPS) that hold biofilms together	Enzymes, polyphenols	<i>Acacia karroo</i> , <i>Aloe ferox</i>	[44, 45]
Inhibition of Biofilm Maturation	Prevents the transition from early biofilm to complex, structured communities	Terpenoids, quinones	<i>Leonotis leonurus</i> , <i>Sutherlandia frutescens</i>	[45, 52]
Bactericidal/Bacteriostatic Activity	Kills or inhibits planktonic and sessile (biofilm-embedded) bacteria	Essential oils, alkaloids	<i>Bulbine frutescens</i> , <i>Warburgia salutaris</i>	[46, 57]
Synergistic Action with Host Defences	Enhances innate immune responses to help clear biofilm	Immunostimulants	<i>Sutherlandia frutescens</i>	[45]
pH Regulation	Maintains oral pH to prevent acidogenic biofilm development	Organic acids, buffering agents	<i>Aloe ferox</i> , <i>Combretum molle</i>	[45]
Iron Chelation	Deprives bacteria of iron, essential for biofilm growth	Tannins, catechins	<i>Acacia karroo</i> , green tea	[45]

for extracellular polymeric substance (EPS) production [74]. These compounds target pathways including cyclic-di-GMP regulation, destabilizing the biofilm matrix and enhancing antibiotic penetration.

Recent findings reveal that some phytochemicals induce biofilm dispersal in mature biofilms, previously considered highly resistant to treatment. For instance, cinnamaldehyde and eugenol prevent biofilm formation and trigger dispersion of established biofilms by modulating nitric oxide signalling and increasing bacterial motility [75]. This biofilm-dispersing ability introduces novel therapeutic approaches for chronic infections where persistent biofilms are common. Quorum sensing (QS) disruption also remains a key target; QS inhibitors (QSIs) derived from plants such as *Garcinia kola* [76] and *Allium sativum* [77] block AHL-mediated communication in Gram-negative bacteria, suppressing virulence gene expression. Recent metabolomics analyses suggest that phytochemicals can reprogram bacterial metabolism transcriptomically, impacting QS and pathogenicity-associated metabolic fluxes.

Another important mechanism is disrupting cell membrane integrity and essential cellular processes, resulting in bactericidal effects. Many phenolic compounds compromise membrane permeability by integrating into the lipid bilayer, causing ion leakage, ATP depletion, and cell death [78]. Berberine interacts with intracellular targets such as ribosomes and DNA gyrase, inhibiting transcription and translation. Additionally, phytochemicals can bind β -lactamase active sites, acting as natural enzyme inhibitors that reverse resistance [79]. A recent study demonstrated that combining quercetin with ampicillin significantly reduced the minimum inhibitory concentration (MIC) against MRSA strains, suggesting a promising route to revive antibiotic efficacy [80]. Furthermore, compounds such as gallic acid generate reactive oxygen

species (ROS) and interfere with redox-sensing transcriptional regulators in pathogens, impairing adaptability and survival under stress [81].

At the molecular level, medicinal plants exert anti-biofilm effects by targeting regulatory mechanisms essential for biofilm development and maintenance. They down-regulate QS genes such as *luxS*, *lasI*, and *rhII*, which control microbial communication during biofilm formation. Plant-derived compounds also reduce expression of enzymes involved in EPS synthesis, weakening biofilm structural integrity. These phytochemicals inhibit adhesion molecules like fimbriae and surface proteins crucial for microbial attachment to host tissues. Moreover, some metabolites disrupt intracellular signalling pathways related to gene regulation, stress response, and virulence expression. Together, these actions hinder formation, maturation, and persistence of pathogenic biofilms in the oral cavity and other environments [82]. Table 2 details other mechanisms of medicinal plants against biofilms.

Technological advances have shifted towards nanotechnology to overcome phytochemical stability and bioavailability limitations. Encapsulation in liposomes, nanoparticles, or hydrogels improves targeted delivery, prolongs bioactivity, and reduces cytotoxicity. For example, in vitro studies showed that curcumin-loaded chitosan nanoparticles exhibit enhanced anti-biofilm activity against *Pseudomonas aeruginosa*, with potential applications in wound healing and dentistry [83]. Advanced techniques such as molecular docking, transcriptomics, and CRISPR-Cas9 gene editing now enhance understanding of plant-microbe interactions and identify novel antimicrobial targets. These mechanisms collectively offer a multifaceted arsenal against resistant pathogens. Continued exploration, leveraging emerging technologies and interdisciplinary research, is essential to fully harness

their therapeutic potential and integrate them into modern clinical practice.

Challenges and considerations with plants

A variety of medicinal plants are traditionally used in South African oral care, including *Carpobrotus edulis* (Sour fig), *Warburgia salutaris* (Pepper-bark tree), and *Siphonochilus aethiopicus* (Wild ginger) [84]. These plants have been extensively studied for their phytochemical and pharmacological properties. However, unsustainable harvesting and lack of proper conservation strategies have led to the decline and endangerment of several species, notably *Warburgia salutaris* and *Siphonochilus aethiopicus* [84]. A review by Álvarez-Vásquez et al. (2022) highlights key mechanisms by which phytochemicals relieve toothache, including antioxidant activity, modulation of transient receptor potential (TRP) channels, γ -aminobutyric acid (GABA) pathways, and anti-inflammatory effects through cyclooxygenase (COX) and lipoxygenase (LOX) inhibition [85]. Major phytochemical classes such as flavonoids, alkaloids, and terpenes exhibit diverse biological activities through multiple mechanisms [86, 87].

Herbal medicine use varies regionally and culturally; some employ whole plants, others use plant combinations or plant-drug mixtures. Such combinations may enhance therapeutic effects but also increase the risk of side effects due to component interactions [85]. The global acceptance of herbal products is hindered by a lack of standardized formulations. Standardization involves establishing consistent norms, quantitative and qualitative parameters, and quality assurance protocols to ensure reproducibility and safety [88].

Despite analytical advances, complete isolation and characterization of all bioactive compounds in plant extracts remains challenging due to their complexity [89]. Furthermore, environmental and processing variables affect phytochemical composition, complicating quality control [90]. Standardization ensures uniform dosing and consistent therapeutic effects, vital for the scientific legitimacy of herbal medicines, which are inherently less stable and more variable than conventional drugs [91]. The World Health Organization (WHO) has issued guidelines for herbal drug standardization to harmonize quality control globally [92]. However, national regulations vary widely, influenced by cultural and scientific contexts. For example, the US “Dietary Supplement Health and Education Act of 1994” excludes herbal medicines from FDA approval [93], while France requires licenses for herbal sales [94]. India regulates herbal drug systems like Ayurveda and Unani through specific government bodies including the Department of AYUSH [95].

As Rojas et al. (2022) point out, safety and toxicological evaluations of herbal products are often not mandated

before market release in many countries, leading to unregulated manufacturing and quality standards. This gap exposes consumers to potentially unsafe or substandard products [96]. The need for stringent quality control and regulation is critical to protect public health as herbal medicine use expands worldwide [97].

Conservation strategies

Despite a robust bioconservation framework, South Africa continues to face significant challenges in ensuring the long-term sustainability of its medicinal plant resources [98]. National policies and local initiatives have encouraged sustainable harvesting, propagation, and cultivation practices, contributing positively to conservation efforts [99]. Nevertheless, unsustainable harvesting remains a critical threat to biodiversity, especially in ecologically sensitive and under-explored areas such as wetlands, which may harbour unique and undocumented medicinal species [100]. Addressing these challenges necessitates a multipronged strategy that combines traditional knowledge, scientific inquiry, community involvement, and rigorous policy enforcement [101]. The rich medicinal plant diversity of South Africa holds considerable promise for advancing healthcare, conserving biodiversity, and supporting rural livelihoods [102]. However, sustainable realization of this potential demands urgent attention to issues like overexploitation, limited research in marginalized ecosystems, and strengthened conservation policy implementation [103]. Investment in ethnobotanical research, improved cultivation technologies, and community-based conservation programs are vital to securing these botanical resources for future generations [104].

Future research directions

Despite compelling evidence supporting the antimicrobial and antibiofilm potential of South African medicinal plants, several research gaps remain that warrant strategic focus [105]. Future studies should prioritize standardizing and optimizing extraction protocols, as variations in solvent type, plant part, and harvesting conditions markedly affect bioactivity and reproducibility [106]. Establishing consistent phytochemical profiles is essential to translate traditional knowledge into reproducible, clinically relevant interventions [107]. Detailed mechanistic studies at molecular and cellular levels are required to elucidate how specific phytochemicals disrupt biofilm formation, interfere with quorum sensing, and overcome microbial resistance pathways. This includes transcriptomic, proteomic, and metabolomic analyses of gene expression, signalling, and microbial membrane interactions [108].

Synergistic testing of plant-derived compounds with conventional antibiotics or oral hygiene agents represents

another critical research direction. These integrative approaches may restore efficacy of outdated drugs, reduce dosages, minimize side effects, and slow resistance development [109]. Furthermore, in vivo validation of plant-based formulations through animal models and clinical trials is essential to assess safety, pharmacokinetics, and therapeutic efficacy [110]. Given the unique challenges of the biofilm environment, future investigations should explore targeted delivery systems such as nanoparticles, hydrogels, and bio-adhesive agents to improve phytochemical retention and bioavailability at infection sites [111]. Nanotechnology could enhance penetration into mature biofilms and enable controlled release of active compounds [112].

Lastly, multidisciplinary collaboration among microbiology, pharmacognosy, materials science, and dental medicine is vital to develop evidence-based, plant-derived interventions for modern dental healthcare [113]. Emphasis on conservation, sustainable harvesting, and benefit-sharing agreements will ensure ethical and long-term use of indigenous plant resources [114]. In summary, a coordinated, multidisciplinary approach is imperative to unlock the full therapeutic potential of South Africa's botanical heritage against oral biofilm infections and antimicrobial resistance [115].

Conclusion

In conclusion, the formidable challenge posed by dental biofilms in oral diseases necessitates the pursuit of novel and effective treatment strategies. South African medicinal plants, deeply rooted in traditional knowledge and supported by emerging scientific evidence, offer a valuable and largely untapped reservoir of bioactive compounds with potent antimicrobial and antibiofilm properties. Their multifaceted modes of action against biofilm formation and persistence position them as compelling candidates for adjunctive or alternative therapies in oral healthcare. However, realizing their full therapeutic potential requires addressing critical issues such as extract standardization, elucidation of molecular mechanisms, and sustainable harvesting practices. By fostering multidisciplinary research and clinical validation, South African indigenous flora can be strategically harnessed to enrich and diversify the arsenal against resilient oral biofilms, contributing meaningfully to global efforts in combating antimicrobial resistance and improving dental health outcomes.

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Author contributions

Kabelo Magoro contributed to the writing of the original draft of the manuscript. Nkululeko Mnisi was involved in both writing and critically reviewing the manuscript for intellectual content. Emma Musekene participated in the writing process and contributed to the reviewing and

editing of the manuscript. Madira Manganyi led the conceptualization of the study, contributed to the writing and critical revision of the manuscript, and was responsible for supervision and overall project administration.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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