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ANTIBACTERIAL ACTIVITY OF CLOVE OIL AGAINST ORAL STREPTOCOCCUS MUTANS ISOLATES

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Abstract

Dental caries remains one of the most common diseases worldwide, and Streptococcus mutans is its best-known bacterial contributor. Clove oil, obtained from the flower buds of Syzygium aromaticum and rich in eugenol, has long been used in dentistry, which has prompted many laboratory studies of its antibacterial action. This review summarises the published literature on the activity of clove oil and eugenol against oral streptococci, the methods used to measure it, the proposed mechanisms, and issues of toxicity and formulation. It reports no new data. Reported activity is consistent, yet methods vary widely, clinical evidence is almost absent, and tissue tolerance limits the concentrations that can be used.

1. INTRODUCTION

Clove oil has a familiar smell in dental surgeries, and for good reason. Oil of cloves has been rubbed on aching teeth for centuries, and eugenol, its main constituent, is still found in temporary fillings, root canal sealers and some dressings [1, 2, 3]. Beyond its numbing effect, the oil is antibacterial, and a large number of laboratory reports have tested it against the organisms of the oral cavity. Dental caries, meanwhile, remains one of the most prevalent diseases worldwide, affecting people of every age and income level, and untreated decay in permanent teeth is among the most frequent of all health conditions [4, 5]. The match between a traditional dental remedy and a common oral disease is evident, and it explains the continuing interest in

whether clove oil can help to control cariogenic bacteria.

This review summarises the published evidence on the activity of clove oil against *Streptococcus mutans*, the species most closely associated with the initiation of caries. It reports no new experimental data. The aim is to set out what the studies measured, how they measured it, which mechanisms have been proposed, and how far the findings can be applied to oral health products [1, 6]. Attention is paid to the chemistry of the oil, because eugenol dominates its composition and often accounts for most of the activity, and to the testing methods, since essential oils behave awkwardly in standard assays [7, 8, 9]. The final sections consider safety to oral tissues, practical formulation questions and the main gaps in knowledge, which are considerable [10, 11, 12].

It helps to be clear about what 'antibacterial' means in this setting. In the laboratory, an agent may prevent growth, kill cells outright, interfere with attachment to a surface or reduce acid production. These are different outcomes with different relevance to caries. A preparation that kills planktonic bacteria in broth need not prevent a biofilm from forming on enamel, and one that reduces acid output might not kill anything [6, 13]. The oral literature is not always careful to say which of these was measured. Abstracts often compress these distinctions, so a reader should check the methods before accepting that an oil kills a species.

2. STREPTOCOCCUS MUTANS AND DENTAL CARIES

Streptococcus mutans is a Gram-positive, facultatively anaerobic coccus that lives on tooth surfaces as part of the dental plaque community. Early work established its link with caries in humans and animals, and it became the prototype cariogenic organism [14, 15]. Three properties explain its role. It ferments dietary sugars quickly to lactic acid, it tolerates the low pH that results (it is aciduric), and it builds sticky extracellular glucans from sucrose using glucosyltransferases, which anchor it and other bacteria firmly to the tooth [13, 15, 16]. The glucan matrix traps acid against the enamel and shapes the structure of plaque. Reviews of its biology emphasise that these traits, rather than simple numbers of cells, make it an effective agent of damage [16, 17]. Human studies also link higher salivary counts of mutans streptococci with greater caries risk, although the association is moderate at the level of the individual and counts alone predict decay poorly [4, 15].

Today the disease is usually viewed as ecological. Instead of a classic infection by a single pathogen, caries results from a shift in the plaque community driven by frequent sugar exposure and repeated acid episodes, which favours acid-producing and acid-tolerant species over those compatible with healthy enamel [18, 19]. *S. mutans* is an important member of this group but not the only one, and other streptococci, lactobacilli and *Actinomyces* species contribute, particularly at later stages [19, 20]. The mineral balance of the tooth surface moves between demineralisation and remineralisation, and decay appears when the first outweighs the second for long enough [4, 21]. The ecological view has consequences for antimicrobials, because killing one species may simply change the balance and not restore health [18, 22].

Acid is the proximate cause of damage, so the behaviour of plaque pH after sugar intake is central. After a sugary meal, plaque pH can drop below the critical value at which enamel starts to dissolve, which is often given as about 5.5 but varies with the calcium and phosphate levels of saliva and plaque fluid, and it takes tens of minutes to recover [4, 21]. With frequent exposure, the community adapts: acid-tolerant species grow, produce more acid, and the pH stays low for longer [18, 19]. This is the ecological drift described earlier. A useful antibacterial would therefore act where it counts, reducing acid output or aciduricity of the plaque community during the minutes after eating, which cannot be learned from simple broth experiments that measure growth only [19, 22].

Control of plaque has traditionally relied on mechanical cleaning, fluoride, dietary sugar control, and in some cases antiseptic mouthrinses. Chlorhexidine is the reference antiplaque agent and reduces mutans

streptococci counts, but it stains teeth, can alter taste, and has shown limited ability to prevent caries in children and adolescents in systematic reviews [11, 23, 24]. This gap is part of the reason natural antimicrobials attract attention. A plant product that was safe, pleasant and effective against cariogenic organisms would be a useful adjunct, particularly in places where access to dental care and fluoride is limited [5, 6]. Whether clove oil can fill that role is the question that the later sections address.

3. COMPOSITION OF CLOVE OIL

Clove oil is obtained by steam or hydrodistillation of the dried flower buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry, formerly *Eugenia caryophyllata*, a tree of the Myrtaceae family native to the Maluku islands of Indonesia and now cultivated in many tropical countries [1, 2]. Bud oil is a pale yellow liquid with a strong spicy odour. Its composition has been determined repeatedly by gas chromatography, and the consistent finding is that eugenol makes up the large majority, with eugenyl acetate, β-caryophyllene and α-humulene as the main minor components [1, 2, 3]. Leaf and stem oils differ in proportions, and they are also marketed as 'clove oil', so sourcing must be stated whenever results are compared [1, 25].

Eugenol (4-allyl-2-methoxyphenol) is a phenylpropanoid with a phenolic hydroxyl group and an allyl side chain. The hydroxyl group lets it donate hydrogen and interact with proteins and membranes, while the lipophilic ring and side chain favour partitioning into lipid [3, 7]. Antioxidant, analgesic, anti-inflammatory and antimicrobial properties have all been attributed to it, and its dual nature explains much of its pharmacology, including the cytotoxicity discussed later [3, 26]. Because eugenol is usually dominant, the oil and the pure compound often give similar results in antibacterial tests, but this should not be assumed. Minor constituents can contribute, antagonise or modify activity, and the real balance has been examined only occasionally [9, 25, 27]. Eugenol is also chemically reactive, and oxidation products formed on storage may differ from the parent compound in both activity and toxicity, a point that is seldom checked [3, 25].

The traditional and dental uses of clove are well documented. In many cultures the whole bud or the oil has been applied to a painful tooth, and the spice is chewed to freshen the breath [1, 2]. Dentists use eugenol in combination with zinc oxide as a temporary restorative and sedative dressing, and in root-canal sealers, where its mild anaesthetic and antibacterial effect and slow release from the set cement are valued [3, 28]. These uses involve small amounts under controlled conditions, which is very different from daily rinsing of the whole mouth. Apparent safety in one setting should not be carried over to another without evidence, a point that matters again in the section on safety [10, 28].

The composition of an individual batch is not constant. Geographic origin, harvest stage, drying, storage and the details of distillation all alter the proportions, and essential oils oxidise and lose volatile components on storage, especially when exposed to air, heat and light [2, 25]. For antibacterial studies this means that a certificate of analysis or a chromatographic profile should accompany the oil. Many reports instead rely on the label of a commercial supplier, and a few do not state the botanical source at all [1, 7]. Authentic material also needs to be separated from adulterated products, which are not unknown in the essential oil trade [2, 25].

Volatility and poor water solubility cause practical difficulties from the start. Clove oil is hydrophobic, it evaporates from open wells and plates, and it can be lost to plastic surfaces. These properties mean that the nominal concentration in a test may differ from the effective one, and they complicate comparisons between papers [7, 8, 29]. A sealed, solvent-controlled design is therefore preferable to open-well testing. A short gas chromatography profile is inexpensive and settles most doubts. Sealing plates with film, or including a vapour-phase control, shows how much of any inhibition is due to the vapour, which for a volatile oil is not trivial [7, 9].

4. ANTIBACTERIAL ACTIVITY AND MECHANISMS

The reported findings on clove oil and its constituents are brought together in table 1, and the number of entries in each category is shown in fig. 1. A bioassay-guided study of *S. aromaticum* isolated several compounds with growth-inhibitory activity against oral pathogens, among them *S. mutans* and the periodontal pathogen *Porphyromonas gingivalis*, with eugenol among the active principles [30]. Comparative screening of commercial essential oils against *S. mutans* found clove among those with notable activity [31]. Reviews of the oil's biological properties report inhibition of a wide range of Gram-positive and Gram-negative bacteria and fungi [1, 32]. The activity against *S. mutans* is therefore consistent with the broad antibacterial profile of the oil [7, 25].

Quantitative results are harder to summarise. Minimum inhibitory concentrations reported for clove oil and eugenol against oral streptococci span a wide range, from fractions of a milligram to a few milligrams per millilitre, depending on the strain, medium, solvent and inoculum [7, 9, 33]. Direct comparison between laboratories is not appropriate. Within a single study, however, the ranking is informative: clove oil was commonly at or near the top among essential oils tested [9, 31]. The minimum bactericidal concentration is usually close to the inhibitory concentration, which suggests that the effect is bactericidal rather than purely bacteriostatic at these levels, though few papers report time-kill curves [29, 34]. Giving results in micrograms per millilitre, with the molar equivalent for eugenol, would help, since the oil is a mixture and the comparison with the pure compound is otherwise unclear [7, 9].

The accepted mechanism for phenolic components of essential oils is disruption of the cytoplasmic membrane. Eugenol and related aromatics were shown to damage cell membranes of Gram-positive and Gram-negative bacteria, increasing permeability and causing leakage of intracellular material such as potassium ions and ATP [34, 35]. Reviews of food-related studies make the same point, adding inhibition of membrane-bound enzymes, collapse of the proton motive force and effects on respiration [7, 29]. For *S. mutans*, whose acid tolerance relies on a proton-translocating membrane ATPase, a membrane-active agent is a plausible challenge [16, 35]. Electron microscopy studies show deformed cells, though most were carried out on other species and extrapolated here [29, 34].

Table 1: Published findings on clove oil and eugenol relevant to Streptococcus mutans and oral care

Material or topic	Reported finding	Ref.
Clove oil composition	Eugenol is the dominant constituent, with eugenyl acetate and caryophyllene as minor components.	[1, 2]
Syzygium aromaticum bioassay-guided	Compounds including eugenol inhibited growth of oral pathogens, S. mutans among them.	[30]
Commercial essential oils S. mutans	Clove oil was among the oils showing notable activity.	[31]
Oil and main compounds oral bacteria	Synergy with antibiotics reported in checkerboard tests.	[27]
Eugenol rats and S. mutans	Reduced acid production, adherence and glucosyltransferase activity, and lowered caries scores.	[36]
Aromatic compounds bacterial cells	Membrane damage and leakage of cell contents.	[34, 35]
Diffusion and dilution assays	Diffusion zones poorly suit volatile hydrophobic oils; solvent controls are essential.	[8, 9]
Method standards natural products	Activity depends on method, solvent and inoculum; agreed criteria for activity are lacking.	[12]
Eugenol human pulp and fibroblasts	Inhibited respiration and cell division at concentrations used clinically.	[37]
Clove oil skin cells	Cell viability reduced at concentrations overlapping antibacterial ones.	[10]

Virulence traits have also been investigated, and this is arguably where the findings are most relevant to caries. In an often-cited study, eugenol reduced acid production, adherence to surfaces and glucosyltransferase activity in *S. mutans* at concentrations below

those that killed the cells, and it lowered caries scores in rats fed a cariogenic diet [36]. Inhibition of glucan synthesis would limit the extracellular matrix that gives plaque its protective structure [13, 17, 38]. Sub-lethal effects of this kind fit the ecological view of caries, in which controlling virulence may be more useful than eradicating a species [18, 22]. Confirmation in other laboratories, with defined clove preparations, would strengthen the case considerably [6, 36].

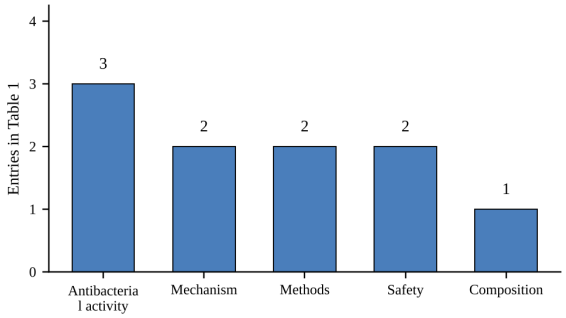


Fig. 1: Number of entries in table 1 by topic

Combinations deserve a mention. Moon and colleagues reported synergistic effects between clove oil or its main compounds and conventional antibiotics against oral bacteria, including streptococci, using checkerboard methods [27]. Such synergy is of limited relevance for caries, where antibiotics are not used, but it indicates that eugenol can weaken bacterial defences enough to lower the dose of another agent [27, 29]. A more practical combination for dentistry might be clove oil with fluoride or with a lower concentration of chlorhexidine, but published data on such mixtures against *S. mutans* are scarce [11, 23]. Interactions with the other ingredients of mouthwashes and pastes, including surfactants and alcohol, are untested for the most part [7, 29].

Chlorhexidine offers a useful yardstick, because it is the agent against which new oral antimicrobials are usually compared. It is a cationic bisbiguanide that binds to the negatively charged bacterial surface and disturbs the membrane, and it adsorbs to oral surfaces and is released slowly, giving a lasting effect called substantivity [11, 23]. Phenolic essential oil components work differently, being neutral and lipophilic, and little is known about how long they remain in the mouth after rinsing [7, 29]. In practical terms, a clove-based rinse would have to match both the potency and the persistence of chlorhexidine, or offer other benefits such as better acceptability and no staining, to justify clinical development [23, 24].

Susceptibility varies between bacterial groups, and the generalisations in the literature are only partly reliable. Gram-negative bacteria are often described as less sensitive to essential oils than Gram-positive ones because of their outer membrane, yet many studies, including those with eugenol, found little difference, and the pattern depends on the oil and the test conditions [7, 9, 29]. Streptococci have no outer membrane but have a thick peptidoglycan layer and a polysaccharide capsule or glucan coat that may slow penetration [13, 16]. The extracellular glucans formed by *S. mutans* in the presence of sucrose could, in principle, shield cells from lipophilic agents. Testing in sucrose-containing media is therefore more realistic than in plain broth [17, 25].

Biofilm studies are fewer than those on planktonic cells. Cells in plaque biofilms are much less susceptible to antimicrobials than the same cells in suspension, partly because of the matrix and partly because of altered physiology, and data from broth tests overstate effects in plaque [17, 20, 38]. Studies that grow *S. mutans* biofilms on hydroxyapatite discs or in flow cells, and that measure cell viability and matrix structure after short exposures similar to a rinse, would better represent the intended use. The few biofilm results with essential oils suggest that higher concentrations are needed than for planktonic cells [6, 22]. Time-kill experiments in the presence of sucrose are especially lacking. Studies that report the oil and eugenol side by side at equal molar concentrations would also settle how much the minor components add.

5. TESTING METHODS AND THEIR PROBLEMS

Two families of method dominate. Diffusion tests, in which the oil is applied to a paper disc or a well in seeded agar and the zone of inhibition is measured, are simple and cheap, and they dominate older and smaller studies [8, 9]. Dilution tests, in which serial dilutions

are made in broth and growth is assessed visually or by absorbance, yield a minimum inhibitory concentration and are the basis of standard procedures [33, 39]. For hydrophobic and volatile materials diffusion tests are poor. The oil spreads unevenly in agar, evaporates, and the zone size depends on diffusion rate more than on potency, so a larger zone does not mean greater activity [8, 12]. Zone diameters should not be used to rank one oil against another unless the conditions are identical.

Broth dilution is better, but standard documents were written for water-soluble drugs and need adaptation for essential oils [33, 39]. A solvent or emulsifier is needed, commonly dimethyl sulfoxide, ethanol or a polysorbate such as Tween 80, and each can affect bacterial growth or the dispersion of the oil. A solvent control at the highest concentration used is essential, and the final solvent concentration should be reported [8, 9]. Turbidity from an emulsified oil interferes with absorbance readings, so a colorimetric growth indicator or plating for viable counts is often needed, and a method designed for essential oils uses a colour change to read growth in microplates [33, 40]. Streptococci also grow poorly in some standard media, so supplemented broth is used, and its protein content can bind phenolic compounds and shift the MIC upwards [7, 39].

Several decisions affect results and are not always reported. The inoculum size, which for susceptibility testing is about 5×10^5 colony-forming units per millilitre in standard methods, determines how much active compound is needed per cell [33, 39]. Incubation conditions matter for *S. mutans*, which grows best under reduced oxygen or with added carbon dioxide, and the time of reading matters because volatile components are lost with long incubation [8, 9]. The reference strain and, preferably, a set of clinical isolates should be tested, together with a positive control such as chlorhexidine for comparison [23, 31]. Replication, the number of independent experiments and the way MICs are recorded (the first clear well or a percentage of growth) should all be stated.

Time-kill experiments answer a different question from the MIC. Instead of recording growth after a fixed incubation, they sample a culture at intervals after exposure and count survivors by plating, which shows how quickly an agent acts and whether killing is sustained or followed by regrowth [8, 33]. For a mouthrinse the first minute matters most, so short exposure times, followed by dilution or neutralisation of the agent before plating, are the relevant design. Without a neutraliser, carry-over of eugenol onto the recovery plate can suppress growth and exaggerate killing [8, 39]. A fall of three logarithmic units in viable count (99.9% killing) is the usual bactericidal convention [33]. Few clove oil papers use this approach, so claims of killing are seldom backed by kinetic data.

Proof-of-concept in vitro work for natural products has been criticised for loose standards. Kalemba and Kunicka, reviewing the antimicrobial data on essential oils, drew attention to differences in method, solvent and inoculum between laboratories, which make results hard to compare or to interpret without agreed criteria for what counts as active [12]. The practical corollary is that only inhibitory concentrations that could realistically be achieved at the site of action deserve to be pursued. Applied to clove oil in the mouth, where a mouthrinse might deliver concentrations that are tolerable to mucosa only within a narrow range, this caution is a useful one [10, 12]. Many of the reported MICs lie close to concentrations that are toxic to human cells, which narrows the practical window [10, 26].

Strain-to-strain variation is a further source of uncertainty. Clinical isolates of *S. mutans* differ in genome content, acid tolerance, biofilm-forming ability and glucosyltransferase activity, and these traits may well affect their susceptibility to an agent that acts on the membrane or on matrix synthesis [16, 31]. Papers that report results for a single reference strain give no sense of this spread. Replicate testing of a panel of isolates, ideally from patients with different caries experience, would allow the distribution of MIC values to be seen and help in judging whether the reported activity is robust [33, 39]. Such panels are rare in the clove oil literature.

Quality of reporting could be improved by a few simple steps. A recognised checklist of the details to be given, including oil source and composition, solvent, inoculum, medium, controls, replicates and the endpoint definition, would let readers compare studies. Authors should also use standard terms: MIC, minimum bactericidal concentration and zone of inhibition mean different things and should not be used interchangeably [8, 33]. Cell viability should be confirmed by plating if the claim is of killing. Even a short table in the supplement, listing the source and batch of the oil, the solvent, the inoculum and the incubation conditions, would take little space and would make later comparison between studies possible.

6. SAFETY AND ORAL CARE USE

Eugenol is both therapeutically useful and a recognised irritant. In dentistry, zinc oxide–eugenol has long been used as a sedative dressing and sealer, and eugenol has analgesic and anti-inflammatory actions on dental pulp, yet cell culture studies show dose-dependent cytotoxicity [28, 37]. Hume found that eugenol inhibited respiration and cell division in human pulp cells, mouse fibroblasts and liver cells at concentrations that could be reached in clinical use [37]. Markowitz and colleagues reviewed the biological properties of eugenol and zinc oxide–eugenol, noting that low concentrations were anti-inflammatory and high concentrations toxic [28]. In keratinocytes and fibroblasts, clove oil and its main components reduced viability at concentrations that overlapped those reported to be antibacterial [10].

The mechanism of cytotoxicity is partly redox in nature. Eugenol and related compounds can act as antioxidants at low concentrations but promote formation of reactive species and cell injury at higher ones, and the phenoxyl radicals formed are linked to the toxicity seen in cell lines [26]. Contact allergy and mucosal irritation have also been described, and undiluted oil applied to gums can produce burning and tissue damage, even though it is sold for toothache [3, 28]. Accidental ingestion of concentrated oil, especially by children, can cause serious toxicity, so product labelling and packaging matter [3, 10]. These findings are the chief reason that low concentrations in carefully formulated products are the only reasonable approach.

Formulation is the practical bridge between laboratory finding and oral product. Mouthrinses, toothpastes, gels, varnishes and slow-release devices have all been proposed for essential oils, and each deals differently with the volatility, taste and tissue tolerance of the oil [7, 29]. Encapsulation in cyclodextrins, liposomes or polymer particles can protect the oil, reduce its odour and moderate release, although its use with clove oil in the mouth is largely at the proof-of-concept stage [6, 29]. Mouthrinse contact time is short, perhaps thirty seconds to a minute, so the oil must act quickly or adhere to surfaces. Clinical studies with a validated clove-based product measuring mutans streptococci counts or caries increment have not, to this reviewer's knowledge, been reported in a form that supports claims [6, 11].

How long an active agent stays in contact with plaque is as important as its concentration. A mouthrinse is diluted by saliva within seconds, swallowed or expectorated within a minute, and cleared from the mouth quickly, so effective concentrations at the tooth surface may be a small fraction of the nominal ones [11, 29]. Volatile compounds are lost still faster. Formulations that adhere to the enamel pellicle, such as gels, varnishes and bioadhesive systems, might provide longer contact, and slow-release approaches have been tried with other natural products [6, 22]. For clove oil, exposure studies in the mouth are lacking, so calculations of whether laboratory concentrations are attainable remain theoretical, and the narrow safety margin makes the question more pressing [10, 12].

A comparison with established products clarifies expectations. Chlorhexidine mouthrinse has decades of trials showing reduced plaque and gingivitis, and its side effects are known [11, 23]. Essential-oil mouthrinses based on thymol, menthol and eucalyptol have also been tested clinically, and the practice standard for any new plant-based rinse would be a trial of this kind,

with plaque and gingival indices as the main outcomes. Clove oil has not reached that stage in the literature considered here. Head-to-head trials in which a plant product is compared with an accepted reference rinse, and not only with placebo, would say more about its relative merit.

Taste and acceptability are practical matters that are rarely tested. Clove oil has a strong flavour and numbing effect that some users like and others dislike, and a rinse that people will not use cannot reduce caries. Sweeteners must be non-fermentable, and ethanol is best avoided in products for children [11, 24]. Questions of this kind are unglamorous, but they decide whether laboratory findings ever reach the bathroom shelf. Colour, flavour and aftertaste are worth recording in any early product study, as are reports of numbness or burning, since these shape adherence more than a small difference in colony counts would.

7. LIMITATIONS AND FUTURE DIRECTIONS

The case for clove oil against *S. mutans* therefore rests mostly on in vitro evidence, supported by one animal study of eugenol [30, 31, 36]. Its limits are plain. Test methods differ and are often unsuitable for hydrophobic oils, the composition of the oil is poorly documented, biofilm models are rare, and tests rely on reference strains [6, 8, 33]. There are no randomised clinical studies of caries outcomes that can be cited, and the margin between antibacterial and cytotoxic concentrations is narrow [10, 12]. A fair summary is that clove oil is a plausible adjunct against cariogenic bacteria, with a believable mechanism, but remains unproven as a preventive agent [1, 6].

Part of the difficulty is the way the field is organised. Most clove oil studies are small, single-centre laboratory experiments, often done as part of a wider screen of plant materials, and positive results are more likely to be published than negative ones [6, 12]. Reviews of natural products in caries research have repeatedly drawn attention to the limited knowledge, the gap between screening and development, and the shortage of studies that go beyond planktonic assays [6, 22]. A coordinated programme using the same standardised oil, methods and strains across laboratories would be more valuable than further isolated screens, and it would give the dental community a clearer view of what clove oil can and cannot do [8, 12, 33].

The next steps are fairly clear. Standardised oil or purified eugenol should be tested by validated broth methods, with solvent controls, against clinical isolates as well as reference strains [8, 33, 39]. Biofilm and ecological models, in which a multispecies community is exposed to realistic rinse-like treatments, would show whether clove oil can shift plaque towards health and not just kill bacteria [20, 22, 38]. Safety studies on oral mucosa should accompany efficacy work from the start, and formulation research should aim for the lowest effective concentration [10, 29]. Only then would a small, well-controlled clinical trial be justified, with plaque, salivary mutans counts and early caries lesions as outcomes [11, 24].

8. CONCLUSION

The laboratory literature agrees that clove oil, mainly through eugenol, inhibits *Streptococcus mutans* and other oral bacteria, and that it may also curb acid production, adherence and glucan synthesis at sub-lethal concentrations [30, 31, 36]. The most plausible mechanism is damage to the bacterial membrane, with additional effects on virulence-related enzymes [34, 35]. This profile fits modern thinking about caries as an ecological disease in which controlling the behaviour of the plaque community matters as much as killing a particular organism [18, 22]. For dental practice, the long familiarity of eugenol gives the oil a head start in acceptability, if not in evidence [3, 28]. Overall, the oil is credible as a source of lead compounds.

The same literature shows how far the evidence is from practice. Methods are inconsistent and often unsuited to volatile oils, biofilm data are scarce, the oil's composition is rarely documented, and there are no clinical trials [6, 8, 33]. Eugenol is toxic to human cells at concentrations not far above the antibacterial range [10, 37]. Clove oil is therefore best described as a promising

natural antimicrobial awaiting standardised testing, safe formulation and clinical evaluation, not as an established anticaries agent [11, 12]. Collaborative studies using one characterised oil, validated broth methods and biofilm models would be the most efficient way forward [8, 22, 33]. Patients should be advised not to apply undiluted oil to the gums.

9. ABBREVIATIONS

ATCC-American Type Culture Collection, ATP-Adenosine triphosphate, ATPase-Adenosine triphosphatase, MIC-Minimum inhibitory concentration, UA159-Streptococcus mutans reference strain UA159, CFU-Colony-forming units, Tween 80-Polysorbate 80

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11. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

12. CONFLICT OF INTERESTS

Declared none

13. REFERENCES

1. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, *et al.* The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): a short review. Phytother Res 2007;21:501–6.
2. Cortés-Rojas DF, de Souza CRF, Oliveira WP. Clove (Syzygium aromaticum): a precious spice. Asian Pac J Trop Biomed 2014;4:90–6.
3. Kamatou GP, Vermaak I, Viljoen AM. Eugenol: from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. Molecules 2012;17:6953–81.
4. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007;369:51–9.
5. Peres MA, Macpherson LMD, Weyant RJ, Daly B, Venturelli R, Mathur MR, *et al.* Oral diseases: a global public health challenge. Lancet 2019;394:249–60.
6. Jeon JG, Rosalen PL, Falsetta ML, Koo H. Natural products in caries research: current (limited) knowledge, challenges and future perspective. Caries Res 2011;45:243–63.
7. Burt S. Essential oils: their antibacterial properties and potential applications in foods: a review. Int J Food Microbiol 2004;94:223–53.
8. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: a review of the literature. J Ethnopharmacol 1988;23:127–49.
9. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999;86:985–90.
10. Prashar A, Locke IC, Evans CS. Cytotoxicity of clove (Syzygium aromaticum) oil and its major components to human skin cells. Cell Prolif 2006;39:241–8.
11. James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, *et al.* Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev 2017;3:CD008676.
12. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem 2003;10:813–29.
13. Bowen WH, Koo H. Biology of Streptococcus mutans-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res 2011;45:69–86.
14. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986;50:353–80.
15. Hamada S, Slade HD. Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiol Rev 1980;44:331–84.
16. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, *et al.* The biology of Streptococcus mutans. Microbiol Spectr 2019;7:GPP3-0051-2018.
17. Koo H, Falsetta ML, Klein MI. The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm. J Dent Res 2013;92:1065–73.
18. Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology 2003;149:279–94.
19. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res 2011;90:294–303.
20. Marsh PD. Dental plaque as a biofilm and a microbial community: implications for health and disease. BMC Oral Health 2006;6(Suppl 1):S14.

21. Featherstone JDB. The continuum of dental caries: evidence for a dynamic disease process. J Dent Res 2004;83(Spec Iss C):C39–42.

22. Marsh PD, Head DA, Devine DA. Ecological approaches to oral biofilms: control without killing. Caries Res 2015;49(Suppl 1):46–54.

23. Autio-Gold J. The role of chlorhexidine in caries prevention. Oper Dent 2008;33:710–6.

24. Walsh T, Oliveira-Neto JM, Moore D. Chlorhexidine treatment for the prevention of dental caries in children and adolescents. Cochrane Database Syst Rev 2015;4:CD008457.

25. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils: a review. Food Chem Toxicol 2008;46:446–75.

26. Fujisawa S, Atsumi T, Kadoma Y, Sakagami H. Antioxidant and prooxidant action of eugenol-related compounds and their cytotoxicity. Toxicology 2002;177:39–54.

27. Moon SE, Kim HY, Cha JD. Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria. Arch Oral Biol 2011;56:907–16.

28. Markowitz K, Moynihan M, Liu M, Kim S. Biologic properties of eugenol and zinc oxide-eugenol. Oral Surg Oral Med Oral Pathol 1992;73:729–37.

29. Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol 2012;3:12.

30. Cai L, Wu CD. Compounds from Syzygium aromaticum possessing growth inhibitory activity against oral pathogens. J Nat Prod 1996;59:987–90.

31. Chaudhari LKD, Jawale BA, Sharma S, Sharma H, Kumar CDM, Kulkarni PA. Antimicrobial activity of commercially available essential oils against Streptococcus mutans. J Contemp Dent Pract 2012;13:71–4.

32. Nzeako BC, Al-Kharousi ZSN, Al-Mahrooqui Z. Antimicrobial activities of clove and thyme extracts. Sultan Qaboos Univ Med J 2006;6:33–9.

33. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother 2001;48(Suppl 1):5–16.

34. Devi KP, Nisha SA, Sakthivel R, Pandian SK. Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. J Ethnopharmacol 2010;130:107–15.

35. Gill AO, Holley RA. Disruption of Escherichia coli, Listeria monocytogenes and Lactobacillus sakei cellular membranes by plant oil aromatics. Int J Food Microbiol 2006;108:1–9.

36. Xu JS, Li Y, Cao X, Cui Y. The effect of eugenol on the cariogenic properties of Streptococcus mutans and dental caries development in rats. Exp Ther Med 2013;5:1667–70.

37. Hume WR. Effect of eugenol on respiration and division in human pulp, mouse fibroblasts, and liver cells in vitro. J Dent Res 1984;63:1262–5.

38. Koo H, Xiao J, Klein MI, Jeon JG. Exopolysaccharides produced by Streptococcus mutans glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. J Bacteriol 2010;192:3024–32.

39. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotechnol 2008;7:1797–806.

40. Mann CM, Markham JL. A new method for determining the minimum inhibitory concentration of essential oils. J Appl Microbiol 1998;84:538–44.