The Therapeutic Potential of Topically Applied Essential Oils in Preventing or Treating Early *Borrelia burgdorferi* Infection: A Review

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ABSTRACT

Lyme disease, caused by *Borrelia burgdorferi*, presents significant diagnostic challenges, often leading to delayed treatment and decreased therapeutic response to conventional antibiotics. This review aims to evaluate the potential of plant essential oils, known for their bacteriostatic, bactericidal, and anti-quorum sensing properties, as prophylactic, adjunct, or complementary treatments during the early stages of *B. burgdorferi* infection. The authors explore how these essential oils can target adaptive mechanisms and interactions of *B. burgdorferi*, including complement regulator-acquiring surface proteins (CRASPs), immune modulation, motility, chemotaxis, biofilm formation, efflux-pump mechanisms, and cyst formation. The authors identify current research gaps and propose frameworks to substantiate the clinical efficacy of essential oils for Lyme disease treatment. This review indicates that essential oils have multifaceted therapeutic potential and could provide a viable option for early intervention in Lyme disease. Further research is necessary to confirm their clinical applicability and safety.

Introduction

Lyme disease is a growing epidemic in the United States, with revised CDC reporting guidelines estimating a rate of infection previously theorized as 11.2 cases per 100 000 in 2017 to 2019 to 68.3 cases per 100 000 [1]. Additional reporting frameworks estimate the number of people diagnosed and treated for Lyme disease at 476 000 per year [2]. It originates as a bacterial infection caused by bacteria of the genus *Borrelia*, notably the *Borrelia burgdorferi* s.1. complex [3] and sometimes *Borrelia mayonii* spirochetes [4]. The bacteria are primarily carried by *Ixodes* spp. and there is some evidence that non-vector transmission occurs [3].

Clinically, Lyme disease falls along a gradient but is practically discussed as having three distinct phases [5]. Early localized phase (< 30 days) occurs from the first few days to one month after infection and is typically characterized by a bulls-eye rash and flu-like symptoms. Although there is overlap in the stages, after 30 days, a second, early disseminated phase follows (< 3 months), which is characterized by the more systemic symptoms (e.g., joint pain, headaches, and cardiac symptoms). Finally, disease persisting for more than 3 months is considered a late disseminated phase. Within the context of this paper, the early localized infection will refer to the first phase of this conceptual framework.

Early diagnoses are hampered by several known factors. Diagnostic tests do not include early antigen targets and complications of cross-reactive epitopes influencing the results, as noted by Branda et al. [6]. There are complications in the *Borrelia* genome that may make species-specific identification unattainable. Because of these diagnostic complexities, there may be subsequent delays in diagnosis that make it difficult for patients to receive antibiotics during the early phase.

Although there are standard diagnostic approaches established according to the CDC [7], there are instances where a prophylactic dose of doxycycline can be used if certain key criteria are

met. Those criteria include that a patient's exposure occurred in an area with high incidence of Lyme disease and that an engorged *Ixodes* tick was removed within 72 hours [8]. Variations in diagnostic and clinical approaches are common among physicians who specialize in Lyme disease that may not be accounted for in this overview.

A Lyme disease diagnosis is also complicated by a wide range of clinical manifestations such as the following: variance or absence of erythema migrans; lymphocytoma; acrodermatitis chronica atrophicans (ACA); urticaria purpura; fever; lymphadenopathy; balance disorders; dizziness; hotophobia; joint arthralgia; myalgia; muscle weakness; myositis; nervous system, cardiovascular, and ocular symptoms–all of which can manifest in the presence of negative serological tests [9].

In addition, there is a delay of hours during which the tick attaches to the host's skin and the tick saliva promotes the transmission of Borrelia, and then hours for the spirochete to migrate to the prepared site in the skin; this is the optimal point of therapeutic intervention [10]. All these elements point to early treatment as an important strategy.

This review will evaluate the feasibility of using full-spectrum plant essential oils (FSEOs) as a complementary or alternative therapy, immediately preceding and after *B. burgdorferi* transmission during the early localized phase of *B. burgdorferi* infection. The paper will explore the bacteriostatic, bactericidal, and anti-quorum sensing mechanisms of select FSEOs, consider their toxicity and pharmacokinetics, evaluate their potential for prophylactic use, and recommend further research and clinical strategies.

The authors conducted searches in PubMed, Consensus, Web of Science, Google Scholar, and Research Rabbit, using a targeted approach to capture all relevant studies. The initial search focused on articles providing mechanistic evidence of how plant volatile oils influence cellular processes employed by *B. burgdorferi* such as cellular mechanisms as complement regulator-acquiring surface proteins (CRASPs), lipoprotein modulation of the immune system, motility and chemotaxis, biofilm, efflux-pump mechanism, cyst formations, and host interactions: outer surface protein polymorphisms and dendritic cell inhibition. The review focused on these mechanisms in bacterial species other than *Borrelia* spp. as well, using terms related to plant volatile oils and bacterial adaptive processes. The authors expanded the search to include *in vitro* and *in vivo* impacts of topically applied essential oils on bacterial infections.

Search strategy

This review was based on a comprehensive search of English-language literature focused on the antimicrobial and mechanistic effects of essential oils on *Borrelia burgdorferi* and similar bacterial pathogens. Databases searched included PubMed, Web of Science, Scopus, Google Scholar, EBSCOhost, and Europe PubMed Central, as well as research tools Research Rabbit and Consensus. Search terms used included "essential oils AND Borrelia burgdorferi" and "volatile oils AND Borrelia burgdorferi", as well as variations relevant to bacterial adaptive mechanisms such as biofilm formation, efflux pumps, CRASPs, and host immune evasion strategies. The search encompassed *in vitro* and *in vivo* studies and was supplemented by manual citation screening of key references and forward citations. Pertinent grey literature, including regulatory reports and conference abstracts, was reviewed. Articles were excluded if there was uncertainty about the identity or purity of the essential oil(s) studied or if the oils were combined with other substances, making it difficult to attribute observed effects to specific essential oils.

Mechanism of Infection

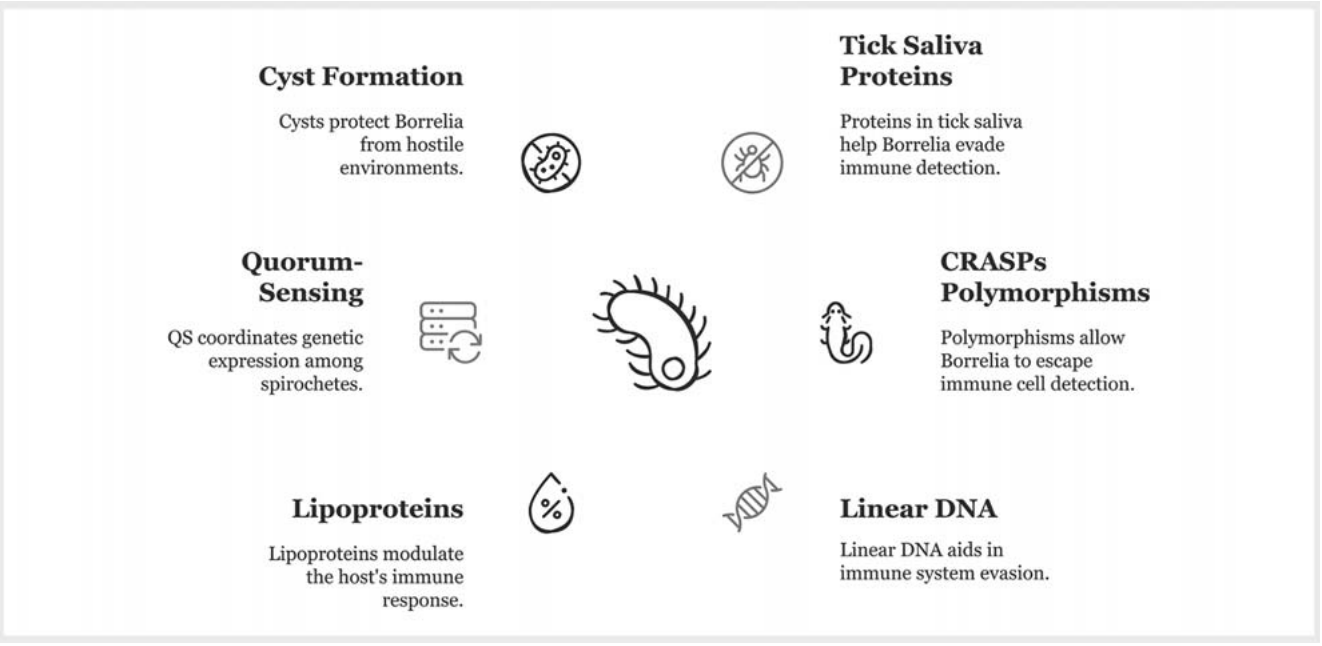
Borrelia spp. have several mechanisms for infecting a human host. During the initial transmission of the spirochete to the human host, *Borrelia* exploits the *Ixodes* tick carrier by relying on protective components in the tick's saliva, such as the salivary tick protein 15 (Salp15), to stay hidden from human immune detection (Ramamoorthi et al., 2005). Once established inside the host, the spirochete's host-specific outer-surface CRASPs can allow it to escape detection by immune cells and succeed at complement evasion [11, 12]. Additional elements that allow *Borrelia* species to evade mammalian immune systems include the adaptive benefits of its linear DNA (0.9-Mbp chromosomes). As *B. burgdorferi* disseminates throughout the host, some of the spirochete's lipoproteins modulate the host's immune system by binding complement activators, destroying neutrophils, and activating B and T cells [13]. Because of these features, the spirochetes have been described as being "in a state of rapid evolution" [14, 15]. Those alterations affect the amount of time for an individual to mount an immune response. Consequently, many people unknowingly harbor a *B. burgdorferi* infection [16].

Spirochetes also utilize quorum-sensing (QS) signaling to coordinate genetic expression among the surrounding population of spirochetes [17]. Some genetic expressions enable them to shroud themselves in biofilm to protect their community from hostile environments within the body [18]. Additionally, when the spirochete enters hostile environments (or is under stress from antibiotics) the spirochete may lose motility and form membrane-covered cysts [19]. Once conditions become favorable, genetically viable spirochetes reemerge from the cystic form [20]. With these evasive capabilities (▶ **Fig. 1**), the infection may become dormant and resist antibiotic intervention only to return to full function when antibiotic therapy has ended.

Because of *Borrelia*'s mode of transmission and protective mechanisms, the spirochete is the most vulnerable during the process of host-to-host transmission and before entering systemic circulation. There is a key window of time during which the spirochetal load is below the level necessary for biofilm formation to occur. During this window, exposure to antibacterial and anti-quorum-sensing FSEOs may have the greatest impact on preventing Lyme disease.

Essential Oils as Therapeutic Option

A group of volatile plant secondary metabolites, FSEOs consist of terpenes, sesquiterpenes, and aromatic phenolic compounds, which protect against herbivores or attract pollinators. Many are responsible for the sensory characteristic fragrance in plants. The predominant extraction methods for commercial FSEOs products are steam distillation, cold pressing, or supercritical fluid extrac-



▶ **Fig. 1** Mechanisms of *Borrelia* spp. infection.

tion with CO_2. Additionally, solvent extractions with alcohols or hexanes have been used.

Due to the scant body of literature on this topic directly assessing the effects of volatile metabolites on *B. burgdorferi*, the search strategy expanded to identify studies assessing the effects of plant volatile oils on the adaptive mechanisms utilized by other gram-negative bacteria and spirochetes.

The multifaceted therapeutic potential of FSEOs comprises several mechanisms: synergistic applications with antibiotics, bacteriostatic properties, immunomodulatory effects, impacts on quorum sensing, biofilm formation, and influence on flagella function (▶ **Table 1**).

Adjunct use with antibiotics

Using innate defense responses, *B. burgdorferi* not only avoids host immune system detection but also deploys several biological mechanisms that can withstand antibiotic treatment [16]. Antibiotics are typically considered a first response. *B. burgdorferi* uses an efflux-pump mechanism to excrete them [18]. Antibiotics also induce conditions that trigger morphological shifts, and since the spirochetes have an affinity for tissue, they only stay in the blood stream temporarily [14, 21]. As their numbers increase, they migrate to areas around the joints with limited blood flow where antibiotics do not readily penetrate. These points all highlight the limitations of using a single-target antibiotic therapy for this complex issue.

Antibiotics, topically administered as a prophylactic treatment immediately after tick removal or in the very early stages of infection, have had some success in animal models using a transdermal delivery method with a combination of 4% azithromycin in Lipoderm cream applied at the site of the tick bite and areas distal to the site as long as the application occurred within three days of tick removal [22]. A couple of challenges with this approach are that many who are infected with *Borrelia* never find a tick bite and those who do must get a prescription from a physician willing to prescribe prophylactic antibiotics without a clear diagnosis (N. Fishman, personal communication, May 28, 2015). The hesitancy to prescribe antibiotics without a clear diagnosis is appropriate given the concerns associated with antibiotic resistance and overuse (Ventola, 2015).

Increasingly the use of FSEOs as an alternative or complement to standard antibiotics is being explored using *in vitro* and *in vivo* research based on their antibacterial properties [23–26]. Because of the extraordinary chemical complexity of plant FSEOs, there is significant evidence showing antibacterial activity and an incomplete picture of their mechanisms of action.

Recent literature provides some additional *in vitro* data on the intersections between FSEOs and antibiotics to treat gram-negative bacteria. In one case, synergistic antibacterial effect was found combining colistin and eugenol to overcome colistin-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [27], another when combining thymol with chloramphenicol against *Acinetobacter baumannii*, thymol with gentamicin against *Staphylococcus aureus*, and thymol with streptomycin against *S. aureus* and *S. aureus* [28]. Asadi et al. [29] reported results for the antibacterial and anti-biofilm properties of cefixime combined with carvacrol against the gram-negative bacteria, *Escherichia coli*, the mechanism for the effect appearing to be the downregulation of the *luxS* and *pfs* genes following treatment.

In their review of *in vitro* studies, Langeveld et al. [30] reported synergy between FSEOs, as well as isolated components of essential oils (IEOs) and numerous antibiotics, to treat a wide range of bacteria. However, discrepancies became known in this review resulting from either the use of different bacterial strains or the im-

▶ **Table 1** *In vitro* data on select essential oils and their mechanistic impact on innate immune response and toxicity data.

Essential Oil	Form Used Isolate/ Whole	Mechanistic Impact on Borrelia	Mechanistic Impact on Immune response	Toxicity Data	Citation
Garlic (*Allium sativum*)	Full Spectrum Oil	Most potent against early disseminated phase	–	–	[62]
Oregano (*Origanum vulgare*)	Carvacrol	High activity against late disseminated phase	–	Carcinogenic properties reported in animal trials	[36, 40, 149]
Cinnamon bark (*Cinnamomum verum*)	cinnamaldehyde	Greater activity against persistent infection than daptomycin	–	Cytotoxic and genotoxic in high doses	[41, 150]
Clove bud (*Syzygium aromaticum*)	Eugenol	High activity against anti-quorum-sensing effects	–	Eugenol and safrole displaying carcinogenicity	[36, 149, 151]
Thyme (*Thymus vulgaris*)	Thymol	Bactericidal activity	–	–	[36]
Cistus (*Cistus creticus*)	epi-manoyloxide	Bacteriostatic activity of phenols and labdane-type diterpenes, antispirochateal	–	–	[37, 39]
Tea tree (*Melaleuca alternifolia*)	Terpinen-4-ol	–	Immunoinhibitory effects; speculated endocrine disruption	–	[53–55, 152]

pact of unknown and less concentrated essential oil constituents found in the specific full-spectrum essential oil. They noted that the use of IEOs instead of FSEOs has two major benefits. Using IEOs improves reproducibility by limiting batch-to-batch variability in FSEO composition. But when it comes to activity, such as the inhibition of efflux pumps, the components not directly antimicrobial may play important roles.

Wound healing

The use of FSEOs in wound healing provides evidence of both antibacterial activities and impact on skin dynamics. In their systematic review of FSEOs for healing and/or preventing infection of surgical wounds, Nascimento et al. [31] found that, in animal and *in vitro* studies, *Lavandula angustifolia* FSEO used topically displayed antimicrobial activity and improved the rate of wound contraction. They pointed out that the *in vivo* studies focused on episiotomies, which supported the use of *L. angustifolia* FSEO in the healing of surgical wounds.

An analysis by Zhang et al. [32] studied over 30 components in *Cinnamomum burmannii* FSEO, with borneol having the highest concentration and lower levels of caryophyllene, α-terpineol, bornyl acetate, linalyl propanoate, bornyl ester, and eucalyptol. The authors noted that wound healing was associated with the presence of all components, and network pharmacology data identified potential mechanisms of action via cell proliferation and migration, inflammatory reduction, and immune responses, which was supported by their *in vivo* and *in vitro* results.

Gorain et al. [33] reviewed several animal studies using advanced drug-delivery systems to improve the impact of herbal medicine on wound healing. *L. angustifolia* FSEO combined with a nanofiber platform using a mouse model did not show any cytotoxicity but did provide antibacterial activity against *S. aureus* and *K. pneumonia*.

Vasile et al. [34] developed a wound-dressing material of silver-impregnated nanoparticles combined with FSEOs of mandarin orange (*Citrus reticulata*), clove (*Syzygium aromaticum*), and niaouli (*Melaleuca quinquenervia*) to prevent wound infection with *in vitro* studies showing improved inhibition of *E. coli, C. albicans*, and *S. aureus* attachment while reducing biofilm formation. Data from *in vitro* and *in vivo* studies by Balaure et al. [35] using zinc peroxide nanoparticles incorporated with 1% *Citrus x sinensis* essential oil as a wound dressing showed prevention of bacterial growth and accelerated wound healing with no reported cytotoxicity.

Bacteriostatic properties

Research by Goc et al., [36] evaluated the *in vitro* effect of multiple, volatile-oil-containing plant extracts against typical motile, knob/round-shaped persisters, and biofilm-like aggregates of *Borrelia* spp., finding that FSEOs *Pimenta racemosa* (bay leaf oil) and *Cinnamomum cassia* (cassia oil) had the highest efficacy at a lower minimal inhibitory concentration (MIC) for two different species: *B. burgdorferi* and *Borrelia garinii*. Using spectrofluorometric measurements, they identified several FSEOs with bacteriostatic and bactericidal activity against *B. burgdorferi* compared to a control of the combination of antibiotics (daptomycin+cefoperazon+dox-

ycycline) at 0.03 mg/mL. Of the 30 FSEOs evaluated, 22 demonstrated bactericidal activity. Minimal bactericidal concentrations (MBC) of 90% eradication after 72 hours were observed at concentrations ranging from 0.15 to 0.25% and MIC ranging from 0.0005% to 0.0075% for FSEOs *Origanum vulgare, S. aromaticum, P. racemosa, Betula lenta, C. cassia, Matricaria chamomilla*, and *Thymus vulgaris*.

Cistus creticus essential oil has also been used successfully as a bacteriostatic agent *in vitro* with *B. burgdorferi* [37]. Using Bbss N40 isolate culture of *B. burgdorferi*, the authors highlight the antibacterial effects of select IEO phenols (eugenol, carvacrol, and thymol) and labdane-type diterpenes (manoyl-oxide and epi-manoyl-oxide) with the greatest activity against *B. burgdorferi*. They tested various preparations of *C. creticus* FSEO against *B. burgdorferi* with amoxicillin serving as a positive control. The growth mediums treated with amoxicillin always showed complete inhibition of *B. burgdorferi* after four days in the medium. Of the *C. criticus FSEO* preparations tested, the steam-distilled volatile fraction was the most potent and the outcomes were dose-dependent. Although bacteriostatic effects were seen at concentrations as low as 0.0015% w/v in the medium, the 0.05% concentration led to immediate and complete inhibition of the *B. burgdorferi*. The IEOs carvacrol, manoyl-oxide, and epi-manoyl-oxide were the most abundant constituents in the volatile oils sample that they used.

In general, the labdane diterpene containing lipophilic fractions exhibited the greatest antimicrobial activity. However, no active principles were elucidated to explain the antibacterial effect of FSEO *C. creticus* against *B. burgdorferi* growth. Chary-Valckenaere et al. noted that due to their high tissue absorption potential, IEO manoyloxides, in particular, are able to penetrate skin tissue at high concentrations and increase the effectiveness of essential oil treatment during the spirochetes' dormant state [38]. This also suggests penetration enhancement via nanotechnology may be a useful strategy for increasing the effectiveness of both IEOs and FSEOs

Subsequent *in vitro* research by Rauwald et al. [39] built on these initial findings and successfully isolated and identified specific active constituents responsible for the observed effects. The monoterpene carvacrol and four major labdane-type diterpenes–manoyloxide, 3-acetoxy-manoyloxide, 3-hydroxy-manoyloxide, and epi-manoyloxide–were the IEOs identified. Among these, epi-manoyloxide exhibited the strongest antispirochaetal effect, equivalent to amoxicillin.

In their 2017 *in vitro* study, Feng et al. [40] found 1% FSEOs *C. cassia, S aromaticum*, and *O vulgare*, as well as its active component IEO carvacrol, most active against the growth of *B. burgdorferi* during the stationary phase compared to daptomycin at 40 µM [40]. In their 2018 paper [41], Feng et al. used *in vitro* cultural methods to assess if FSEOs might target persistent *B. burgdorferi* infections by screening 35 whole-plant essential oil extracts identifying 10 FSEOs *Allium sativum, Pimenta dioica, Cuminum cyminum, Cymbopogon martinii, Commiphora myrrha, Hedychium spicatum, Amyris balsamifera, T. vulgaris, Litsea cubeba*, and *Eucalyptus citriodora*, as well as IEO cinnamaldehyde, the active component of *C. cassia* bark, with greater activity during the latent, persistent phase of *B. burgdorferi* grown in culture. Although these represent later-stage activity against the infection, they identify the ability of FSEOs to limit *B. burgdorferi* growth.

Although several FSEOs have been identified as having powerful antibacterial effects, only a few have been linked to having efficacy against other spirochaetal bacteria. As explored in a short review, the FSEO of *S. aromaticum* has been extensively researched [42] and contains the primary constituent eugenol. Chaieb et al. [42] found that IEO eugenol was the most active component and that it displayed antibacterial activity against five strains of *S. epidermidis*. The steam-distilled volatile oil of this commonly found spice has been well known as an antiseptic [43]. The primary concentrations of the phytochemical constituents of the volatile oil fraction include eugenol 73.5–96.9%, *β*-caryophyllene 0.6–12.4%, eugenyl acetate 0.5–10.7%, *α*-caryophyllene 0.4–1.4%, isoeugenol 0.1–0.2%, and methyleugenol 0–0.2%. The bacteriostatic properties of *S. aromaticum* are well established in research on bacteria common to periodontal disease, inhibiting the spirochete *Treponema vincentii* with a minimum inhibitory concentration of 0.06 µg/mL (0.006%) [44,45]. This specific research, however, used synthetic eugenols, which may create uncertainty about the minimum inhibitory concentrations (MIC) identified when applying a full-spectrum extract.

Immunomodulatory effects

While little is known about their immunostimulatory potential, *in vitro* and *in vivo* evidence for FSEOs from *Eucalyptus globulus, S. aromaticum*, and *Melaleuca alternifolia* [46,47] suggests they can enhance phagocytic activity, which is critical for clearing infections [48]. The predominant IEOs for each are 1,8-cineole, eugenol, and terpinen-4-ol, respectively [49]. Additional data on the use of aromatherapy massage on humans using *L. angustifolia* FSEO provides evidence from phagocytosis stimulatory effects [50]. Linalool and linalyl acetate are predominant terpenes in *L. angustifolia* FSEO. Another study [51] applied aromatherapy massage with 2% *L. angustifolia* FSEO on pregnant women with the intervention group immediately displaying significantly elevated salivary IgA and reduced cortisol levels. This appeared to be a long-term alteration of immune function.

Several *in vitro* studies of *M. alternifolia* FSEO have shown immunoinhibitory effects on immune cells [52–55]. These effects can be beneficial in the context of Lyme disease, where excessive inflammation and immune response can contribute to tissue damage and chronic symptoms. By modulating the immune response, tea tree oil may help reduce the inflammatory damage caused by *B. burgdorferi*.

In vitro research by Serafino et al. [46] shows eucalyptus FSEO treatment drastically increases phagocytic activity in human monocyte-derived macrophages (MDMs) as both pretreatment before lipopolysaccharide (LPS) challenge and treatment in comparison to LPS exposure studies. Yadav and Chandra [56] investigated the phagocytic activity of IEO 1,8-cineole, the major constituent of *E. globulus* oil, on lung alveolar macrophages, supporting Serafino et al.'s findings. A study from Juergens et al. [57] using cultured human lymphocytes and monocytes pretreated with 1,8-cineole resulted in reduced inflammatory responses in conditions such as type 2 diabetes, inflammatory bowel disease, and atherosclerosis [48].

Giovannini et al. [58] demonstrated a significant increase in phagocytic activity and a reduction in intracellular bacterial replication for *L. angustifolia* FSEO treatment of human monocyte-derived macrophages (MDMs) infected with *S. aureus*, which suggests that *L. angustifolia* FSEO could stimulate innate immune responses to bacteria. This points to early intervention strategies.

Effects on Quorum Sensing

A vital intraspecies communication system for bacterial species involves control of quorum-sensing genetic expression [59]. The QS system in gram-negative bacteria relies on autoinducers (AIs) in the form of acylated homoserine lactones (AHLs) [60–62]. These small signal molecules activate transcription of QS gene expression, which can be considered a complex response to changes in cell population density [60] exhibiting control over a variety of processes, including biofilm formation, sporulation, and virulence factors, as well as the production of secondary metabolites, pigment, and antibiotics [63–68]. From expression studies of the LuxS gene, Skotarczak suggested the data were incomplete about whether this mechanism was at play in *B. burgdorferi* [14]. Camele et al. [69] noted that multiple FSEOs display anti-quorum-sensing ability, but an explicit link was not made to AI-2 as the mechanism for the effect.

Although currently there is no research to directly demonstrate that FSEOs interfere with the AHL binding in *B. burgdorferi*, there is *in vitro* evidence related to other gram-negative bacteria. Clove FSEO specifically showed significant disruption of swarming motility in the gram-negative bacteria *C. violaceum* and *P. aeruginosa*, respectively [62]. The impact of the *S. aromaticum* oil was dependent on its concentration, with the highest level that they used, 20 µl, showing the greatest effect.

Essential oil anti-quorum-sensing effects *in vitro* against gram-negative bacteria *E. coli* and *Chromobacterium violaceum* were also established for FSEOs *Rosa damascena* (rose) and *Rosmarinus officianalis* (rosemary) [70]. Also showing significant quorum-sensing inhibitory effects were FSEOs *Cinnamomum verum, L. angustifolia*, and *Mentha x piperita* [62]. *In vitro* research from [71] tested *E. coli* and *C. violaceum* along with 29 individual phytochemical compounds including IEOs isoeugenol, carvacrol, thymol, citral, and linalool, each showing significant inhibition. Researchers did not compare full-spectrum extracts of essential-oil-containing plants.

Yap et al. [25] used biosensors and bioluminescence assays to screen for quorum-quenching activities, identifying FSEOs *R. damascena, Pelargonium graveolens, L. angustifolia, S. aromaticum*, and *R. officinalis* oils as candidate QS inhibitors in strains in several bacteria [25, 62]. Next-generation natural or synthetic therapeutics being tested to overcome antibiotic resistance are finding some promising results using molecules as QS antagonists called quorum-sensing inhibitors [72, 73].

Biofilm disruption

B. burgdorferi is also capable of actively producing biofilms. This biofilm confers resistance to antibiotics and leads to inflammatory damage. Interference contrast and atomic force microscopy data also indicates potential host tissue rearrangement during biofilm growth; the exact mechanism is still an area of active research [74].

Evidence from non-*Borrelia* species on the influence of specific IEOs on these same systems include eugenol down-regulating AI-2 production and disrupting biofilm formation and virulence gene expression in *K. pneumoniae* [75]. IEOs carvacrol and eugenol also inhibited pectobacteria QS genes, biofilm formation, and production of cell-wall-degrading enzymes, which led to reduced virulence (Joshi et al., 2016). Also, *Cinnamomum camphora* leaf FSEO exhibited strong antibacterial and QS inhibitory activities, significantly reducing biofilm formation and virulence gene expression in pathogens *P. aeruginosa* and *C. violaceum* [76].

Effects on flagella function

The *B. burgdorferi* spirochete is also very motile, having periplasmic flagella protected under its outer membrane [77]. These flagella help the spirochete to travel from the inoculation site, through the blood, to joints and other tissue where it can avoid immune system detection [78]. The spirochetes' movement and evasion techniques are coordinated and enhanced by their use of chemotaxis [18], with N-acetylglucosamine and glucosamine, chitosan dimers, glucose, and L-glutamate among its chemical attractants [79]. N-acetylglucosamine is present in elevated amounts in adipose tissue [80], serving as a potential systemic attractant of motile *B. burgdorferi*. Similarly, glucosamine is most concentrated in the liver [81].

With no data on *Borrelia* flagella function, Kovács et al. [82] present results showing that *S. aromaticum* FSEO altered the expression of virulence genes that were part of *Campylobacter jejuni* flagella synthesis, including PEB1, PEB4, 35 LPS, and serine protease. Their findings indicated that, in addition to its major component, eugenol IEO, at least four other clove FSEO constituents possessed flagella modulating effects on *C. jejuni*, suggesting a role for FSEOs as a more therapeutically active approach than IEOs.

Also, p-cymene IEO is the precursor of carvacrol IEO and is a monoterpene with a benzene ring without any functional groups on its side chains [83]. Treatment with p-cymene resulted in decreased cellular motility because the proton motive force is required for flagellar movement [84]. Carvacrol also inhibited the synthesis of another microbial protein, flagellin, and gave rise to cells without flagella that subsequently exhibited decreased motility [85]. These two constituents are part of the same metabolic pathway. Understanding how to maintain both as part of a full-spectrum extract would be vital to optimize the therapeutic effect.

Marini et al. [86] reported that *L. monocytogenes* flagella incubated with *Cannabis sativa* FSEO displayed aggregate flagella and that there were fewer flagella present. The following were predominant terpenes in the cannabis chemovar: α-pinene, β-pinene, myrcene, terpinolene, camphor, β-caryophyllene, caryophyllene-oxide, and α-humulene. Their concentration range was 3.27 to 19.20% of total FSEOs. An additional investigation of *L. monocytogenes* response to thyme FSEOs suggests that the synthesis of flagellar structures was impaired by thyme FSEO treatment [87].

Pharmacokinetics

Skin permeability

As part of their review of the literature, Kohlert and al. [88] translated earlier research published in German scientific journals, noting that monoterpenoid compounds in *E. globulus* FSEO that contained a- and b-pinene, camphor, 3-carene, and limonene were readily absorbed after dermal application (citing [89–91] in humans and [89] in mice). For the animal study, C_{max} was measured 10 min after application [89]. They also found that the level of absorption was proportional with the size of treated skin area and that skin did not prevent diffusion of essential oil compounds [89, 90]. Topical application in humans saw an increase in plasma levels [91], with distribution occurring in 3 to 4 minutes and maximum plasma levels being achieved within 10 minutes. Elimination half-life occurred within 60 ± 65 min [92]. An *in vitro* study using cinnamaldehyde [93], as well as animal studies using pulegone [94] and eugenol [95], described formation of glutathione S-transferase enzyme conjugation, used to facilitate lipophilic xenobiotics excretion and/or metabolism [96].

Apparently, the results from most of the early essential-oil pharmacokinetic studies showed FSEOs are eliminated with a biphasic profile [92, 97–99], which suggests that they move from the blood into other tissues, including fatty tissue. In their human study, Jäger et al. [100] detected peak plasma concentrations of two *Lavandula* spp. oil constituents (linalool and linalyl acetate) 20 minutes after topical application, falling to zero after 90 minutes. In another human study of topical application, Sadgrove et al. [101] found maximum plasma concentrations of 100 $ng \cdot ml^{-1}$ linalool and 121 $ng \cdot ml^{-1}$ linalyl acetate. In line with previous data, linalool accumulates in organs and fat at much higher concentrations than in plasma [102].

In a human study, Friedl et al. [103] showed that inhalation not only resulted in 1,8-cineole appearing in the plasma earlier but also at much higher concentrations than through topical application. Sarkic and Stappen [104] suggested this was due to accumulation in fatty tissue after dermal application. After a single dose, FSEOs are metabolized and released from adipose tissue with the compounds remaining for some time [88]. Animal studies support these findings [105], although they are difficult to translate to humans. The results from the genetic analysis by Gerber et al. [106] comparing murine *in vivo* skin models with human skin found differences not only in skin genes identified but also great variance in genes common to both species, with human skin exhibiting greater activity than mouse skin in skin morphogenesis and growth.

In an *in vivo* study, Wang and Tso [107] measured percutaneous absorption of 5-methoxypsoralen (5-MOP) from *Citrus bergamia* oil with peak absorption occurring within the first 2.0 hours, with C_{max} of 250 $ng \cdot ml^{-1}$ 4–6 h after application. The human serum levels of 5-MOP was significantly higher for up to 16 hours, which points to an effective dosing strategy of topical application twice a day to maintain specific serum levels for 24 h coverage.

The study by Schuster et al. [91] of a single monoterpenoid showed a rise of a-pinene concentrations 6–10 hours after dermal application. The large degree of variation in time may be accounted for because measuring low concentrations prevented statistically precise calculation of pharmacokinetic data.

Immunocytochemical staining data from human trials provide evidence that the following cytochrome P450 enzymes, CYP1A1, CYP2B6, CYP2E1, and CYP1B1, are predominantly expressed in keratin-forming cells [108], while CYP3A5 expression mainly occurs in the basal layer and inner vascular-layer endothelial cells. In their review, Zehetner et al. [109] reported that FSEO isolates showed both inducing and/or inhibiting activity on metabolizing CYP enzymes. The findings were generated from a variety of assays, which limits understanding of clinical relevance. Also, since the bulk of the studies are *in vitro*, translating the findings into *in vivo* results is limited. The selection of CYP enzymes tested were based on the application of commonly prescribed drugs with more data on inhibition rather than on induction as a primary outcome. They also do not account for FSEOs made up of multiple IEO components.

Dermal penetration enhancement

From their review of the literature, Jian et al. [110] noted the data strongly supported FSEOs' ability to improve the delivery of different drugs across the skin. In their own study, FSEOs from *Angelica archangelica, C. cassia*, and *S. aromaticum* significantly enhanced skin penetration of ibuprofen and lowered the skin toxicity of the drug. Enhanced penetration applying FSEOs in a topical drug formulation acts mainly on the lipid structure between the cells of the outermost layer of the epidermis to increase the fluidity [111]. The size and degree of long-chain alkyl functionality has the greatest influence on the degree of the effect, so that menthol with ring structures impacts the fluidity less than nerol with a long-chain alkyl side group [112]. Additionally, full-spectrum essential oils increase permeation enhancement compared to essential oil isolates, as well as displaying lower toxicity in animal [113] and *in vitro* studies [114, 115]. Jäger et al. [100] showed that almost all dermally applied linalool dissolved in ethanol evaporated; however, mixed with a carrier oil, it was largely absorbed.

Chen et al. [116] summarized *in vitro* studies that showed terpenes enhance drug penetration through the skin, compared with synthetic enhancers. Based on the chemical structure and polarity, FSEOs were able to penetrate the skin and move into circulation. Using *in vitro* human skin studies, dermal application of several FSEOs lead to clinically appropriate blood concentrations of IEO components necessary to increase absorption of co-applied drugs [117–119].

Additional methods exist to improve the absorption rate of FSEO or IEOs. Sieniawska et al. [120] investigated the development of water-dilutable microemulsions (ME) for essential oils of *Cymbopogon nardus, M. piperita*, and *E. globulus* with the goal of improving their therapeutic effect while potentially limiting their toxicity. Essential oils solubilized with DMSO was the comparison treatment of Vero and HeLa cell lines in a cultural medium after 24 h incubation. They reported an increased antioxidant activity of 13.96%, 22.25%, and 45.60% for *E. globulus, M. piperita*, and *C. nardus* FSEO, respectively. A concern with this approach is that DMSO may have contributed to the clinical effect. Hutschenreuther et al. [37] verified that using DMSO alone for *in vitro* studies exhibited an inhibiting effect on *Borrelia* growth even in the

lowest concentration tested. This suggests that any solubilizing or penetrant additives should be tested for activity to fully gauge the effect of essential oils alone.

The use of MEs had been shown to enhance the antimicrobial activity of FSEOs against a range of microorganisms *in vitro* [121–124], as well as inhibiting their growth [110]. Surfactants have been found to promote cell lysis at low concentrations with the proposed mechanism suggesting they intercalate into the membrane, stimulating changes in permeability [125]. At high concentrations, cell lysis seems to occur because of membrane solubilization [126]. Andrade-Ochoa et al. [127] noted that FSEOs display a similar mechanism of action. Thus, the combination appears to increase their respective cytotoxic activities [120]. Hasanzadeh et al. [128] (2023) applied nanoliposomes embedded with essential oil cinnamaldehyde to pre-established biofilms of *L. monocytogenes* and *S. enteritidis*. Using an anti-biofilm assay, they reported that the cinnamaldehyde containing nanoliposome prevented biofilm formation to a greater degree than cinnamaldehyde alone, and its effects lasted longer than essential oil.

An additional benefit in using MEs as a carrier for FSEOs appears to be the reducing of their irritant properties [129]. However, even though MEs are created using GRAS ingredients, potential toxicity of surfactants was reported [130], especially since emulsifiers are used in high concentration [131].

Toxicity

De Groot and Schmidt [132] reported that, historically, 79 different essential oils have caused contact allergy or allergic contact dermatitis. However, Akmeri et al. [133] noted that many of the studies were based on humans displaying above-average allergic potential or on those suffering dermal diseases.

Some FSEO or IEOs may be characterized as secondary carcinogens requiring activation. Cuba [134] cautioned that *Salvia sclarea* and *M. quinquener* FSEOs may induce estrogen-dependent cancers without providing explicit evidence from topical applications. *Citrus sinensis, Citrus limonm*, and *L. cubeba* FSEOs contain flavins, cyanins, porphyrins, and hydrocarbons considered to be photosensitizing molecules [135].

The literature on FSEOs finds that they are cytotoxic, not apoptotic [136]. No evidence of mutagenic activity from FSEOs nor IEOs had been reported, although Bunse et al. [137] noted exceptions, summarizing several *in vitro* studies focused on bacterial and *Drosophila mutagenesis* assays, including FSEOs of *Artemisia dracunculus, Mentha spicata, A. graveolens, Pinus sylvestris, M. piperita*, and *Pimpinella anisum*, as well as IEOs trans-anethol, *β*-asarone, terpineol, trans-cinnamaldehyde, carvacrol, thymol, and S (+)-carvone. In their literature review, they also suggested that FSEOs may act to protect rather than harm, exhibiting *in vitro* antimutagenic properties. Almost all these experiments generated *in vitro* data and used concentrations that may not be reached in *in vivo* applications [137].

Regulation and Availability of Essential Oils

In the United States, plant essential oils are regulated by the Food and Drug Administration (FDA), and those regulations vary depending on the intention for the product [138]. If it is intended for topical application, it is generally regulated as a cosmetic and no pre-market approval is required. However, if there are heath claims being made about the product, it would be regulated as a drug. As a drug, a product would be evaluated by the Center for Drug Evaluation and Research for safety and efficacy before it could enter the consumer marketplace.

In the European Union, conversely, there are more stringent regulatory requirements for products to enter the marketplace as defined by the regulation Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), which has specific requirements for products to prove identity by providing analysis on constituents in the essential oil [139]. This is a best practice for clinicians and researchers that is not generally present for products purchased by most suppliers in the United States. There are also some proposed updates to REACH from the International Fragrance Association [140] that might further enhance the protections for consumers and the environment from potential hazards while also reducing costly and burdensome regulations on small producers.

Despite these regulatory differences, essential oils, particularly those highlighted in this paper, are generally easily attainable for consumers. The costs may vary depending on the cost to produce the specific essential oil; however, those EOs emphasized in this paper are lower- to moderately priced essential oils.

Discussion

The progression of Lyme's disease is described along a gradient with diagnosis complicated by delays in the actual diagnosis, variable clinical approaches, uncertainty about the amount of time for the human host to mount an immune response, and a range of clinical manifestations that all point to early stage intervention as a potent treatment strategy. The unique abilities of FSEOs to cross dermal layers, reach deep into tissue, penetrate biofilm, and disrupt cyst formation may make them a viable, commercially available primary step to protect a human host. Currently, after a tick bite, the CDC recommendation is to wait and see if symptoms develop and then see a physician for lab work and exam [8]. However, if FSEOs could provide an immediately accessible option for interfering with *B. burgdorferi* transmission before they can systemically colonize the human host, they may provide a viable option for helping to control this epidemic.

Direct evidence for FSEOs to treat *B. burgdorferi* consists of only three studies [36–37, 40–41], and they are all *in vitro*. Translating *in vitro* results to clinical outcomes can be difficult due to differences in complex biological systems. This paper represents very preliminary work to identify a readily available, safe, botanical option to address the gap in early phase and prophylactic treatments. *In vitro* and *in vivo* data and traditional evidence support the use of FSEOs for more widespread antimicrobial applications, although clinical trials on their antimicrobial efficacy have been limited to rinses for dental applications and topical treatments for infectious skin conditions. In particular, the research on FSEOs and IEOs has demonstrated anti-quorum-sensing activity, efflux-pump inhibition, and increased bacterial cell wall membrane permeability, which can lead to bacterial cell death [25, 70, 71].

Extrapolating on the research on antibacterial FSEOs and their mechanisms of action suggests that the following appear to have phytochemical constituent profiles that may be effective against *B. burgdorferi* (▶ **Table 1**). *S. aromaticum, T. vulgaris, C. creticus, L. angustifolia, C. verum, M. alternifolia*, and *R. officianalis* are among the FSEOs that may be clinically beneficial for treating *B. burgdorferi* infection. Many others have either direct substantial *in vitro* evidence of efficacy against spirochetes or other gram-negative bacteria, or they have primary constituents that have been identified as bacteriostatic, bactericidal, or anti-quorum sensing.

Although these properties may indicate that volatile oils could be useful throughout the various stages of *Borrelia* infection, preventing infection before it takes hold may be the most impactful first step. Essential oils can easily pass through the skin and individual cell membranes before they enter systemic circulation [119]. The pharmacokinetic data is largely *in vitro*. Studies in humans are required to suggest appropriate dosing levels. The FSEOs noted above contain a spectrum of IEOs with activity, and their combined effect represents an effective approach to counter many of the adaptive mechanisms presented by the organisms. Eucalyptus FSEO is distributed into human plasma within 3–4 min, while C_{max} is achieved within 10 min. This provides a fast-acting treatment. The biphasic distribution from blood plasma to adipose tissue may allow FSEOs to "chase" the *B. burgdorferi* infection into surrounding tissue. Additionally, since FSEOs act as enhancers of skin penetration, FSEOs may play a dual role as both active principle and penetrant enhancers, as opposed to using IEOs alone.

Several IEOs showed C_{max} of over 100 $ng \cdot ml^{-1}$. Synergistic interactions between IEOs would appear to enhance the overall effect based on penetrant enhancement, as well as activity against a wide range of bacteria, as noted by Langeveld et al. [30]. Standardization will be necessary since essential oil composition can vary based on extraction methods, plant genetics, and environmental factors, affecting reproducibility. Extraction processes will play an important role in optimizing specific FSEO ratios.

Cytotoxic effects have been observed at higher concentrations of certain FSEOs and their constituents that are unlikely to be reached with topical applications. Systemic toxicity may occur with prolonged use or absorption of certain components like eugenol and cinnamaldehyde. A more likely risk is the potential for contact dermatitis and allergic reactions, particularly with oils known to oxidize and form sensitizing compounds. Although nanoemulsion may limit this response.

One possible concern is that prophylactic use of FSEOs may mask or alter the symptoms and presenting clinical signs for early stage Lyme, thus making an already complicated diagnosis even more tenuous. If a substantial enough dose can safely be applied to the wound immediately after infection, this may not be a concern. However, it is a point that would need to be addressed in future evaluations of this therapeutic approach. Additionally, this strategy is limited since many people who contract Lyme disease do not recall having gotten a tick bite, with reports ranging from one-third of people [141] to one-half [142]. In these scenarios, it may be possible to design an intervention that could be applied topically immediately after high-risk exposure to an endemic area. Furthermore, a proactive application of topical essential oils prior to entering a high-risk endemic area may have a prophylactic effect on preventing infection. Either may be enough to reach a therapeutic dosage that provides protection. Another limitation of this particular paper is that it does not address the possibility of preventing the spread of the most common co-infections in both Europe and the U. S. caused by *Bartonella* spp., *Yersinia enterocolitica, Chlamydophila pneumoniae, Chlamydia trachomatis, Mycoplasma pneumoniae*, viral tick-borne encephalitis, *Anaplasma phagocytophilum*, and *Babesia* spp., the latter two largely absent in Europe [143–145].

There is evidence that the host's innate immune response may be sufficient to kill the *Borrelia* spirochete in the absence of tick salivary protein (Salp15), which intervenes in the immune response and facilitates infection. Salp15 binds to CD4 on host T cells, which is credited with much of the immunosuppressant activity of tick saliva and increased pathogenicity of *Borrelia* [146, 147]. It would be important to assess whether certain essential oils like *T. vulgaris* that have immunomodulatory effects that include reducing the production of T cells [148] would inadvertently support the action of Salp15 in increasing host susceptibility to infection at the wound site. Would the antimicrobial or immunomodulating effects be dominant?

Future Work

There is a significant gap in clinical data validating efficacy and safety in humans. More studies are required to show that topical application will result in a reduced *B. burgdorferi* load. Use of FSEOs would be a first choice, even with the more complex chemical signature, since higher activity is expected with this form. There are IEOs such as manoxloxides that might be added to enhance activity of a given FSEO. There is also a lack of comprehensive data on absorption rates and bioavailability in human subjects. This data might help improve the understanding of which FSEOs are the most viable candidates, as well as of what effects minor constituents have on clinical endpoints, pharmacokinetics, safety, and dosing. Dosing regimens will depend on additional pharmacokinetic, stability, and absorption kinetics and on a deeper understanding of the constituent ratios necessary for optimal treatment.

Thus, future research includes well-designed clinical trials to assess the efficacy of topically applied FSEOs in preventing or treating early stage Lyme disease. Safety profiling needs to be conducted, including skin irritation tests and long-term toxicity studies.

Contributors' Statement

Originated idea: K. Dolan drafting manuscript: K. Dolan, J. Courie, M. Tims figures, tables and references: J. Courie critical revision of manuscript: M. Tims

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Conflict of Interest

The authors declare that they have no conflict of interest.

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