**Microemulsification of essential oils for the development of antimicrobial and mosquito repellent functional coatings for textiles**

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**Abstract**

**Aims:** To develop an essential oil (EO)-loaded textile coating using an environmentally friendly microemulsion technique to achieve both antimicrobial and mosquito repellent functionalities.

**Methods and Results:** Minimum inhibitory concentrations and fractional inhibitory concentrations of litsea, lemon and rosemary EOs were determined against *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Trichophyton rubrum*. A 1:2 mixture of litsea and lemon EOs inhibited all the microorganisms tested and was incorporated into a chitosan-sodium alginate assembly by a microemulsification process. The EO-loaded microemulsions were applied to cotton and polyester fabrics using a soak-pad-dry method. The textile challenge tests demonstrated 7–8 log_{10} reductions of *S. epidermidis*, *S. aureus* and *E. coli* after 24 h and *T. rubrum* after 48 h. *Aedes aegypti* mosquito repellency was also assessed which demonstrated 71.43% repellency compared to 52.94% by neat EO-impregnated cotton.

**Conclusions:** Textiles treated with the litsea and lemon EO microemulsion showed strong antimicrobial activity against the skin associated microorganisms *E. coli*, *S. aureus*, *S. epidermidis* and *T. rubrum* and potential mosquito repellent properties.

**Significance and Impact of the Study:** EOs could be useful for the development of natural, environmentally friendly functional textiles to protect textiles and users from microbial contamination in addition to possessing other beneficial properties such as mosquito repellency.

**Introduction**

Consumer demand for functional textiles has increased in recent years. Antimicrobial textiles are of particular interest due to an increase in awareness of deleterious effects of microorganisms on textiles (Riaz and Ashraf 2020). The moisture content and nutrient availability in textiles offer a favorable environment for the growth of bacteria and fungi, leading to the formation of odor and deterioration of the textiles, in addition to potentially contributing to the spread of pathogenic microorganisms (Sterndorff et al. 2020; Sanders et al. 2021). Antimicrobial textile finishes offer a variety of potential applications including prevention of odor (Morais et al. 2016) or as
treatment or prophylaxis for skin infections, such as tinea pedis, caused by the dermatophyte *Trichophyton rubrum* (Gupta and Versteeg 2019), and atopic dermatitis, which is associated with *Staphylococcus aureus* colonization (Srour et al 2019).

Several currently available textile finishes are potentially toxic to humans and the environment. For example, triclosan accumulates within the aquatic environment, where it is toxic to aquatic organisms; triclosan is also potentially toxic to humans, with a number of studies demonstrating cell cytotoxicity and endocrine disrupting properties and may induce multidrug resistance in bacteria (Zheng et al 2019). This has resulted in the need for effective antimicrobial finishes that are safe, biodegradable, environmentally non-toxic and limit the development of microbial resistance.

Natural products could offer an environmental-friendly and biodegradable alternative to currently employed biocides for antimicrobial textile finishes. Essential oils (EOs) are aromatic natural products, typically extracted from plant matter by distillation (Georgiev et al. 2019). EOs have been a subject of interest as alternative antimicrobial agents, with a body of research suggesting that they convey broad spectrum antimicrobial activity. For example, cinnamon bark inhibited *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* with minimum inhibitory concentrations (MICs) ranging 0.015–0.125%, and all tests species except *P. aeruginosa* were inhibited by cinnamon leaf, clove, lemongrass, rosewood and thyme EOs with MICs ranging 0.125–1.0% (Elcocks et al. 2020).

EOs could potentially be incorporated into textiles to create antimicrobial fabrics, however EO compounds are often volatile, and sensitive to light and oxygen. The successful application of EOs onto textiles requires a formulation that protects the EOs from volatilization and degradation and controls its release rate to prevent unacceptable deterioration of the final product (Volić et al. 2020). Microencapsulation of EOs may preserve the functional and physicochemical properties of the oil and allow for greater durability of the final product; Sayed et al. (2017) applied a nanoemulsion encapsulating neem EO on cotton fabric and reported a 71.73 and 65–69% reduction of *S. aureus* and *E. coli* after four washes. Biopolymers including chitosan and alginites are an attractive option for drug delivery systems due to their favorable biodegradable, biocompatible and mucoadhesive properties (Gómez-Guillén and Montero 2021). Previous research demonstrated the successful encapsulation of EOs within biopolymers and their use within antimicrobial textile finishes; cotton treated with alginate and gelatin-encapsulated lime EO was antimicrobial against *Klebsiella pneumoniae*, *E. coli*, *Staphylococcus epidermidis* and *S. aureus* according to the disc diffusion method (Julaeha et al. 2021).

Textile finishes based upon encapsulated EOs have also been shown to possess mosquito repellent activity. For example, Grancaric et al. (2020) reported 100% repellency of *Aedes aegypti* mosquitoes by cotton fabric treated with microencapsulated immortelle EO. Mosquitoes are important vectors for several diseases, for example the female *A. aegypti* mosquitoes are vectors for the transmission of yellow fever, dengue fever and Zika fever viruses. Current commercially available mosquito repellants such as N,N-diethyl-3-methylbenzamide (DEET) have shown potentially serious adverse skin reactions (Azeem et al. 2019), providing a rationale for the development of novel mosquito repellents.

Therefore, the aim of this study is to develop a functional antimicrobial cum mosquito-repellent coating for textiles based on EO-loaded microemulsions using a novel green polyelectrolyte micro-assembly technique. It is envisioned that the EO-loaded microemulsion self-assembly prepared in this study will provide a tool for controlled (sustained) delivery of a combination of EOs with synergistic antimicrobial activity against the skin associated pathogens including *S. aureus*, *P. aeruginosa*, *S. epidermidis*, *E. coli* and *T. rubrum* as well as potential mosquito repellency effects. These provide unique advancements in science of the application of EOs over the existing knowledge in literature.

Materials and Methods

Microorganisms

*Staphylococcus aureus* NCTC 8327, *P. aeruginosa* NCIMB 8626 and *E. coli* NCTC 8003, methicillin resistant *S. aureus* (MRSA) NCTC 12479, and *E. coli* and *S. epidermidis* clinical isolates (Leicester Royal Infirmary, Leicester, UK) were cultured aerobically at 37°C using brain heart infusion (BHI) media (Sigma Aldrich, Gillingham, UK).

*Trichophyton rubrum* ATCC 28188 was cultured aerobically at 30°C with Sabouraud dextrose (SD) media (Oxoid, Basingstoke, UK).

Essential oils

Bergamot (*Citrus bergamia*), citronella (*Cymbopogon nardus*), lemon (*Citrus limon*), litsea (*Litsea cubeba*), bitter orange (*Citrus aurantium var amara*), sweet orange (*C. aurantium var sinensis*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), rosewood (*Aniba rosaeodora*) and wild thyme (*Thymus serpyllum*) EOs were obtained from Penny Price Aromatherapy Ltd (Hinckley, UK).
Antimicrobial activity of EOs

Screening for antimicrobial activity

*Trichophyton rubrum* total spore suspensions were prepared by scraping spores from a pre-cultured agar plate with 1 ml of 0.01% polysorbate 80 solution (Fisher Scientific, Loughborough, UK). The resulting suspension was filtered through five layers of muslin cloth and washed thrice (2000 g, 5 min) in sterile distilled water. The total spore count was adjusted to 10⁷ colony forming units (CFU) per ml using a hemocytometer. A method adapted from Fisher and Phillips (2006) was used. Aliquots (50 µl) of *T. rubrum* spore suspension (10⁷ CFU per ml) or overnight cultures of *S. aureus*, *P. aeruginosa*, *E. coli*, and *S. epidermidis* (10⁸ CFU per ml) were spread on BHI agar plates before filter paper discs (20 mm, Whatmann, Maidstone, UK) were placed on the surface and impregnated with 25 µl EO. Plates were incubated at 37°C for 18 h (bacteria) or 30°C for 7 days for *T. rubrum* before zones of inhibition (ZoIs) were measured. Controls were plates without oil impregnated filter discs.

Minimum inhibitory concentrations

MICs were determined using a method adapted from the International Standards Office (2006) broth microdilution method. Doubling dilutions of EOs (final concentrations 0·1–40 µl ml⁻¹) were prepared for litsea, lemon and rosemary EO in BHI or SD broth supplemented with 10% dimethyl sulfoxide (DMSO; Fisher Scientific). Aliquots (100 µl) of the EO suspension were mixed with an equal volume of *T. rubrum* spore suspension (10⁷ CFU per ml) or overnight cultures of *S. aureus*, *P. aeruginosa*, *E. coli* or *S. epidermidis* (10⁸ CFU per ml) in 96 well plates. A control of 10% v/v DMSO only was included.

For bacteria, the MIC was determined by measuring optical density (595 nm) before and after 24 h incubation at 37°C using a SpectraMax Plus 384 microplate reader with SoftMax Pro version 6.4 software (Molecular Devices, San Jose, CA). For *T. rubrum*, the MIC was determined by visually inspecting wells for growth after incubation at 30°C for 7 days.

Fractional inhibitory concentrations

A method adapted from Owen et al. (2017) was used. Serial dilutions of rosemary, lemon and litsea EOs were prepared in BHI broth supplemented with 10% DMSO (bacteria) or 10% polysorbate 80 (*T. rubrum* spores) before inoculation with the microbial test species. Double and triple combinations of EOs were prepared in 96 well plates to yield a matrix of varying concentrations of each EO (0–4·5 µl ml⁻¹ litsea EO, 0–20 µl ml⁻¹ lemon EO and 0–45 µl ml⁻¹ rosemary EO) and mixed by pipetting. The MICs of each combination was determined as described above and used to calculate the fractional inhibitory concentration (FIC) and FIC index (FICI) according to Eqn 1:

\[
\text{FICI} = \frac{\text{MIC}_{\text{EO1}} + \text{MIC}_{\text{EO2}}}{\text{MIC}_{\text{EO1}} \text{ alone}} + \frac{\text{MIC}_{\text{EO2}} \text{ in combination}}{\text{MIC}_{\text{EO2}} \text{ alone}}
\]

where FICI ≤ 0·5 indicates a synergistic effect, whilst 0·5 ≤ FICI ≤ 4 indicates no interaction and FICI > 4 indicates an antagonistic effect (Odds 2003).

Gas chromatography-mass spectroscopy of Litsea and lemon EOs

The major components of litsea and lemon EOs were determined using a Bruker (Billerica, MA) 450-RG gas chromatograph (GC) equipped with a Rxi-5ms (Restek, Bellefonte, PA) column (30 m × 0·25 mm i.d. × 0·25 µm film thickness) and coupled with a 300-MS SQ signal electron impact mass spectrometer (MS). EOs were diluted 1 : 100 in n-hexane (Fisher Scientific), filtered through a 0·45 µm polyethylene (PET) filter (Sigma-Aldrich) and 1 µl was injected into the GC (injection temperature 280°C; split ratio 1 : 100) using a Bruker (Billerica, MA) CP8400 autosampler. Helium was used as a carrier gas at 1·5 ml min⁻¹. Oven temperature was held at 60°C for 5 min, followed by a 4°C min⁻¹ ramp to 220°C before an 11°C min⁻¹ ramp to 250°C, held for 15 min. MS was conducted in positive mode with a source temperature of 230°C, ionizing energy of ~70 eV, CID gas pressure of 1·5 mTorr and a detector voltage of 1000 V. Mass spectra were acquired over a mass range of 50–350 m/z. EO components citral and limonene were identified by comparing with retention time and mass of analytical grade standard reference compounds (citral (99%), (R)-limonene and (S)-limonene; Sigma Aldrich). Calibration curves were prepared for citral and limonene between 0·78 and 200 mmol l⁻¹ (R² = 0·99, data not shown).

Microemulsion preparation and characterization

Microemulsion preparation

Oil in water (o/w) microemulsions of litsea-lemon EO blend (1 : 2 ratio) were prepared by mixing the EO blend (30% v/v final concentration) with sodium alginate solution (Sigma Aldrich; 0·1% w/v final concentration) for 5 min before adding chitosan solution (Sigma Aldrich; 1·0% w/v final concentration) and homogenizing for 5 min at 8000 rev min⁻¹ (IKA Ultra-Turrax® disperser, Staufen, Germany). Calcium chloride solution (Fisher Scientific, Loughborough, UK) was added to achieve a final alginate concentration of 0·5% w/v for all formulations (FIC). The major components of litsea and lemon EOs were identified by comparing with retention time and mass of analytical grade standard reference compounds (citral (99%), (R)-limonene and (S)-limonene; Sigma Aldrich). Calibration curves were prepared for citral and limonene between 0·78 and 200 mmol l⁻¹ (R² = 0·99, data not shown).
Scientific; 0-1% final concentration) was added dropwise under further homogenization at 8000 rev min\(^{-1}\) for 1 h. All microemulsions were cured by storing them for 24 h at room temperature for elastic recovery, and at 40°C for the same period for consolidation of the crosslinked polyelectrolyte micro-assembly respectively, prior to further analysis. The pH was measured using a Mettler Toledo electrolyte micro-assembly respectively, prior to further analysis. The pH was measured using a Mettler Toledo pH Meter (target pH <5).

The percentage incorporation efficiency (%IE) of the EOs in the microemulsions was calculated from Eqn 2.

\[
\% \text{IE} = \frac{M_{\text{ME}}}{M_{\text{EO}}} \times 100\%
\]

where \(M_{\text{ME}}\) is the amount of the limonene and citral exhaustively extracted from the microemulsion and \(M_{\text{EO}}\) the initial amount of limonene and citral extracted in the neat EOs. \(M_{\text{ME}}\) and \(M_{\text{EO}}\) were determined from the area under the curve and peak height of the GC-MS spectra within the calibration standards of this study. All measurements were an average of six determinations.

**Microemulsion particle size**

Particle size and particle size distribution of the litsea-lemon EO microemulsion were determined by dynamic light scattering (DLS) using a NanoBrook Omni particle sizer (Brookhaven Instruments, Holtsville, NY). The microemulsion was diluted 1 : 100 in distilled water and measurements performed in triplicate five times at 25°C. Span value for the distribution of particle sizes (polydispersity index; PDI) was determined according to Eqn 3:

\[
\text{SPAN} = \frac{d(90) - d(10)}{d(50)}
\]

where \(d(90)\), \(d(50)\) and \(d(10)\) are the particle diameters at 90, 50 and 10% cumulative volume, respectively (Campelo et al. 2017).

**Microemulsion physical stability**

The flocculation/creaming stability of the microemulsion was assessed by storing 5 ml emulsion in measuring cylinders at room temperature for 5 weeks, with daily observation and recording of the volume of creaming. The Creaming Index (CI) was estimated according to Eqn 4:

\[
\% \text{Creaming Index} = \frac{H_c}{H_t} \times 100
\]

where \(H_c\) is the height of clear layer below the sample and \(H_t\) the total emulsion height.

**Microemulsion long-term physical stability**

The accelerated stability of the microemulsion was assessed by centrifuging at 3549 g for 2 h. The emulsions were observed every 5 min for separation of the internal phase and the CI under stress conditions was characterized. All samples were analysed in triplicate.

**Microemulsion chemical stability**

The concentration of citral within the microemulsion was assessed at zero and 28 days by shaking 1 ml emulsion in 5 ml of \(n\)-hexane (Fisher Scientific) for 5 min in a 50 ml separating funnel before GC-MS analysis as described above.

**Treatment of polyester and cotton fabric with the Litsea-lemon EO microemulsion**

Knitted bleached cotton and knitted polyester fabric samples were scoured with 2 g l\(^{-1}\) non-ionic surfactant Ultra-voxon PL (Ciba Specialty Chemicals, Basel, Switzerland) for 30 min at 60°C, followed by a hot and cold-water rinse to remove the surfactant. The fabric was sterilized at 160°C for 2 h before soaking for 15 min in the microemulsion. Soaked fabric samples were passed through a laboratory pad (Ernst Benz, Eysins, Switzerland) at 35 kg cm\(^{-2}\) pressure and 1 m min\(^{-1}\); re-soaked and padded again. All investigations were conducted using freshly treated fabric dried for 24 h at room temperature, unless otherwise specified.

**Distribution of major EO components on Litsea-lemon EO microemulsion treated fabric**

Microemulsion-treated polyester and cotton fabric samples (1, 2 and 16 cm\(^2\); 24 h post-treatment) were subject to solvent extraction in \(n\)-hexane; the citral and limonene content was then quantified using GC-MS as described above. Identical treated polyester and cotton fabric samples were also stored at 4°C for 7 days prior to determining the citral and limonene content.

**Antimicrobial activity of the litsea-lemon EO microemulsion**

**Screening for antimicrobial Activity**

The antimicrobial activity of the litsea-lemon EO microemulsion, the microemulsion components (10% litsea EO, 20% lemon EO, 1% chitosan solution, 1% sodium alginate) and major chemical components of litsea and lemon EO (citral, R-limonene and S-limonene) was assessed against S. aureus, S. epidermidis and E. coli using the disc diffusion screening method described above, however 6 mm filter paper discs were used.

**Time kill assay**

A neutralizing solution comprised of 10 g l\(^{-1}\) tryptone (Oxoid, Basingstoke, UK), 5 g l\(^{-1}\) sodium chloride (Fisher Scientific), 30 g l\(^{-1}\) saponin (Fisher Scientific), 1 g l\(^{-1}\) L-
histidine (Sigma Aldrich), 30 g l⁻¹ polysorbate 80 (Fisher Scientific), 3 g l⁻¹ asoletcin from soybean (Sigma Aldrich) and 5 g l⁻¹ sodium thiosulfate (Fisher Scientific) was used in time kill assays to prevent antimicrobial carryover during enumeration. The neutralizer was validated as non-toxic and effective using the BS EN 1276:2009 Annex C neutralizer toxicity and dilution-neutralization validation tests (British Standards Institute 2009). For time kill assays, the microemulsion was solubilized in an equal volume of DMSO and a 200 μl aliquot mixed with 9.7 ml sterile distilled water. The test solution was inoculated with \( S. aureus, S. epidermidis \) or \( E. coli \) (\( 10^7–10^8 \) CFU per ml) and incubated at 37°C (bacteria) or 30°C (\( T. rubrum \)). Aliquots (1 ml) of the test solution were taken at 0 and 5 min (bacteria) or 0, 5, 10, 20, 30, 40, 50 and 60 min (\( T. rubrum \)) and diluted in 9 ml neutralizer. The test mixture was left in contact with the neutralizer for 5 min before spread plating and enumeration. The inoculum of each microorganism was evaluated without the microemulsion as the respective negative control.

**Qualitative antimicrobial efficacy test of litsea-lemon EO microemulsion-treated textile**

The antimicrobial and antifungal activity of microemulsion-treated polyester and cotton was determined against \( E. coli \) and \( S. epidermidis \) type and clinical isolates, \( S. aureus \), MRSA and \( T. rubrum \) using adapted BS EN ISO 20645:2004 method (British Standards Institution 2004). The antimicrobial activity was also determined against treated and untreated cotton and polyester following washing at 40°C in a standard domestic wash cycle (Indesit IWSD61251 Eco machine) using 24-08 g of ECE non-phosphate reference detergent (A) with 4-4 g of sodium perborate tetrahydrate (SDC Enterprises, Bradford, UK) and drying for 24 h at room temperature. Aliquots of 5 ml molten BHI or SD agar containing \( 10^5 \) CFU per ml \( E. coli \) and \( S. epidermidis \) type and clinical isolates, \( S. aureus \), MRSA or \( T. rubrum \) were overlayed onto 10 ml solid agar and allowed to set. Circular (25 mm) textile samples were pressed onto the surface of the agar and ZoIs were determined after incubation for 18–24 h at 37°C for bacteria and for 7 days at 30°C for fungi. Untreated textile was included as a control.

**Quantitative antimicrobial efficacy test of Litsea-lemon EO microemulsion-treated textile**

The antibacterial activity of microemulsion-treated polyester and cotton against \( E. coli, S. aureus \) and \( S. epidermidis \) was quantified using a method adapted from BS EN ISO 20743:2013 (British Standards Institution 2013) and antifungal activity determined against \( T. rubrum \) spores using a method adapted from BS ISO 13629-2:2014 (British Standards Institution 2014).

Treated fabric samples (0.40 ± 0.05 g) were inoculated with 0.1 ml of microbial culture (\( 10^7–10^8 \) CFU per ml). Immediately after inoculation, triplicate samples were vortexed in 10 ml neutralizer for 5 × 1 min cycles before spread plating on to BHI agar (bacteria) or SD agar (\( T. rubrum \) spores) for enumeration. A further three inoculated textile samples were incubated at 37°C for 18–24 h (bacteria) or 30°C for 48 h (\( T. rubrum \) spores) before neutralization and spread plating for enumeration. Control of untreated polyester and cotton were included. The percentage reduction (\( R \)) of viable microorganisms was calculated by comparing the number of surviving microorganisms on untreated and treated polycotton at 0 and 24 h or 48 h.

**Aedes aegypti mosquito repellency of Litsea-Levon EO microemulsion treated textile**

Female \( A. aegypti \) mosquitoes (\( n = 10; 12\)-h starved) were released through the base leg of an Olfactometer (Ross Lifescience, Pimpri-Chinchwad, India; airflow 0.20 ± 0.05 m s⁻¹; 0.40 ± 10 m s⁻¹) using aspirators and allowed to acclimatize in a holding port for 15 min without treatment (Rutledge et al. 2015). The following two treated fabrics were tested: microemulsion treated cotton samples and cotton samples impregnated with neat litsea-lemon EO blend (1 : 2). Untreated cotton was included as a control. After acclimatization, 100 cm² of the treated fabric was place in the test port of the olfactometer and 100 cm² untreated fabric place in the control port.

The Olfactometer control and test trapping ports were opened to allow the mosquitoes to migrate to either the test or control chamber. After 30 s, the base leg holding port was closed and the number of mosquitoes that migrated towards the control or test chamber was recorded every minute for 3 min. Physically injured mosquitoes and/or those incapable of flying or walking were not recorded in the results. Tests were conducted in triplicate.

The percentage of mosquitoes repelled from the treatment was calculated according to Eqn 5:

\[
\%\text{Repellency} = \frac{MC - MT}{MC + MT} \times 100
\]

where MC is the number of mosquitoes in the control port and MT the number of mosquitoes in the treatment port.

**Statistical analysis**

The significance of differences (\( P \leq 0.05 \)) between group means were determined by one-way analysis of variances (ANOVA) with Tukey’s post-hoc test using SPSS version 25.
Results

Antimicrobial activity of EOs

All EOs displayed antimicrobial activity against *S. aureus*, *S. epidermidis* and *T. rubrum* according to the disc diffusion method, with ZoIs ranging 22–90 mm (Table 1). *Escherichia coli* was inhibited by all tested EOs except citronella and peppermint EOs and all EOs displayed antimicrobial activity against *P. aeruginosa* with the exception of bitter orange, sweet orange, rosewood and wild thyme EOs. Bergamot, lemon, litsea and rosemary EOs inhibited all test species, with ZoIs ranging 20–90 mm; lemon, litsea and rosemary EOs generally had larger ZoIs than bergamot and were taken forward for further study (Table 1).

Litsea EO had the greatest antimicrobial activity against all test species, with MICs ranging 0.6 μl ml⁻¹ against *S. epidermidis* to 10 μl ml⁻¹ against *P. aeruginosa* (Table 2). Lemon EO exhibited the weakest antimicrobial activity with MICs ranging from 10 μl ml⁻¹ against *T. rubrum* to >40 μl ml⁻¹ against *P. aeruginosa*. *Pseudomonas aeruginosa* was the least susceptible microorganism with generally higher MICs than the remaining test species (Table 2).

All double combinations of lemon, litsea and rosemary EOs were synergistic against *S. aureus* (FICIs = 0.09–0.50) and *E. coli* (FICIs = 0.17–0.28) yet were indifferent against *S. epidermidis*, where FICIs ranged from 0.75 to 2.15 (Table 2). Only a combination of litsea and lemon (2.5 μl ml⁻¹ each) was synergistic against *P. aeruginosa*; litsea and rosemary were antagonistic (FICI = 4.06) while rosemary and lemon were indifferent (FICI = 0.75–2.03).

The triple combination showed synergism against *E. coli* (FICI = 0.50) yet was indifferent against *S. aureus*, *S. epidermidis* and *P. aeruginosa* (FICI = 0.66–2.03) and therefore did not further reduce the EO MICs compared to the double combinations. A double combination of litsea and lemon EO was synergistic against four out of five organisms tested, with the lowest FICI of 0.09 among the double combinations and so was taken forward for further investigation. The minimum concentrations of litsea and lemon EO to inhibit all test species in combination were 2.5 and 5.0 μl ml⁻¹ respectively, representing a litsea : lemon ratio of 1 : 2, which was tested hereafter.

GC-MS of litsea and lemon EOs

GC-MS analysis demonstrated that litsea EO produced 3 major peaks corresponding to limonene (retention time 152 min) and the E/Z isomers of citral with RTs of 17-70 and 18-84 min, (m/z): [M]⁺ 136-30 and 152-20. Limonene was also a major component of lemon EO, where a large peak was detected at an RT of 9.59 min. E/Z isomers of citral appeared as more minor components with RTs of 17-55 and 18-61 min.

Microemulsion characterization

The globule size of litsea-lemon EO microemulsion was 1.556 ± 0.142 μm and the span value was 0.500 ± 0.02 μm. The microemulsion was stable for 14 days, with an average CI of 12.67%, where after the CI increased to

| Zol (mm) of EOs against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Trichophyton rubrum* according to the disc diffusion method (n = 6 ± SD). |
|---|
| **Zol (mm)** | **Escherichia coli** | **Pseudomonas aeruginosa** | **Staphylococcus aureus** | **Staphylococcus epidermidis** | **Trichophyton rubrum** |
| Bergamot | 46.80 ± 10.10 | 20.00 ± 0.00 | 29.30 ± 2.20 | 47.33 ± 5.12 | 90.00 ± 0.00 |
| Citronella | 0.00 ± 0.00 | 20.30 ± 6.00 | 39.20 ± 2.20 | 90.00 ± 0.00 | 90.00 ± 0.00 |
| Lemon | 41.30 ± 4.00 | 21.30 ± 1.50 | 51.00 ± 7.60 | 27.00 ± 1.70 | 90.00 ± 0.00 |
| Litsea | 52.80 ± 2.90 | 20.20 ± 0.40 | 47.20 ± 3.70 | 90.00 ± 0.00 | 90.00 ± 0.00 |
| Bitter Orange | 28.70 ± 1.63 | 0.00 ± 0.00 | 25.00 ± 2.00 | 46.19 ± 1.36 | 81.17 ± 10.01 |
| Sweet Orange | 0.00 ± 0.00 | 0.00 ± 0.00 | 22.20 ± 0.80 | 25.35 ± 1.92 | 46.50 ± 3.73 |
| Peppermint | 0.00 ± 0.00 | 20.40 ± 0.50 | 47.80 ± 1.80 | 44.33 ± 8.02 | 90.00 ± 0.00 |
| Rosemary | 90.00 ± 0.00 | 23.80 ± 1.90 | 50.50 ± 12.90 | 25.90 ± 2.70 | 90.00 ± 0.00 |
| Rosewood | 90.00 ± 0.00 | 0.00 ± 0.00 | 50.3 ± 10.90 | 58.00 ± 4.69 | 90.00 ± 0.00 |
| Wild Thyme | 56.50 ± 6.20 | 0.00 ± 0.00 | 50.5 ± 12.80 | 43.33 ± 2.58 | 90.00 ± 0.00 |
44.6% in 28 days and 50% in 42 days (Fig. 1a). However, microemulsions produced a CI over 60% within 3 cycles (15 min) of centrifugation (Fig. 1b), indicating that the long-term stability of the microemulsion is low. The percentage incorporation efficiency of the active compounds in the EOs is reasonably high (86.1 ± 3.44%) however large fluctuations in concentrations of citral present in the microemulsion were observed over 28 days storage at 15 or 40°C indicating inconsistent distribution of the active ingredients of the EOs within the chitosan-alginate assembly in the microemulsion (Fig. 1c). The concentration of citral present when stored at 15°C significantly decreased ($P \leq 0.05$) from 136.81 to 0.02 mmol l$^{-1}$, while when stored at 40°C there was an increase in citral concentration after 28 days from 114.33 to 254.23 mmol l$^{-1}$ (Fig. 1c).

### Treatment of polyester and cotton fabric with the Litsea-lemon EO microemulsion

The liquor pickup (LPU) of the microemulsion was 112.89 ± 1.59% for cotton and 97.45 ± 5.97% for polyester during the padding process. The amount of citral

| Organism                      | EO      | MIC alone ($\mu$ l ml$^{-1}$) | MIC in combination ($\mu$ l ml$^{-1}$) | FICI  | Interaction |
|-------------------------------|---------|-------------------------------|---------------------------------------|-------|-------------|
| *Staphylococcus aureus*       | Litsea  | 1.25                          | 0.60                                  | 0.50  | Synergistic |
|                               | Rosemary| 5                             | 0.11                                  |       |             |
|                               | Litsea  | 1.25                          | 0.10                                  | 0.09  | Synergistic |
|                               | Lemon   | 20                            | 0.10                                  |       |             |
|                               | Rosemary| 5                             | 0.10                                  | 0.50  | Synergistic |
|                               | Lemon   | 20                            | 1.25                                  | 0.66  | Indifferent |
|                               | Litsea  | 1.25                          | 0.6                                  |       |             |
|                               | Rosemary| 5                             | 0.6                                  |       |             |
| *Pseudomonas aeruginosa*      | Litsea  | 10                            | 40.0                                  | 4.06  | Antagonistic |
|                               | Rosemary| 20                            | 1.25                                  |       |             |
|                               | Litsea  | 10                            | 2.5                                   | 0.31  | Synergistic |
|                               | Lemon   | >40                           | 2.5                                   |       |             |
|                               | Rosemary| 20                            | 10.0                                  | 0.75  | Indifferent |
|                               | Lemon   | >40                           | 10.0                                  |       |             |
|                               | Litsea  | 10                            | 10.0                                  |       |             |
|                               | Rosemary| 20                            | 20.0                                  |       |             |
| *Escherichia coli*            | Litsea  | 2.5                           | 0.10                                  | 0.28  | Synergistic |
|                               | Rosemary| 2.5                           | 0.60                                  |       |             |
|                               | Litsea  | 2.5                           | 0.10                                  | 0.17  | Synergistic |
|                               | Lemon   | 40                            | 5.0                                   |       |             |
|                               | Rosemary| 2.5                           | 0.10                                  | 0.17  | Synergistic |
|                               | Lemon   | 40                            | 5.0                                   |       |             |
|                               | Lemon   | 40                            | 2.50                                  | 0.50  | Synergistic |
|                               | Litsea  | 2.5                           | 0.60                                  |       |             |
|                               | Rosemary| 2.5                           | 0.60                                  |       |             |
| *Staphylococcus epidermidis*  | Litsea  | 0.6                           | 0.60                                  | 1.25  | Indifferent |
|                               | Rosemary| >40                           | 10                                    |       |             |
|                               | Litsea  | 0.6                           | 1.25                                  | 2.15  | Indifferent |
|                               | Lemon   | 20                            | 1.25                                  |       |             |
|                               | Rosemary| >40                           | 1.25                                  | 0.75  | Indifferent |
|                               | Lemon   | 20                            | 1.25                                  |       |             |
|                               | Lemon   | 20                            | 5.0                                   | 0.79  | Indifferent |
|                               | Litsea  | 0.6                           | 0.10                                  |       |             |
|                               | Rosemary| >40                           | 10.0                                  |       |             |
| *Trichophyton rubrum*         | Litsea  | 2.5                           | 0.60                                  | 1.24  | Indifferent |
|                               | Rosemary| 10                            | 10.0                                  |       |             |
|                               | Litsea  | 2.5                           | 0.60                                  | 0.49  | Synergistic |
|                               | Lemon   | 10                            | 2.50                                  |       |             |
|                               | Rosemary| 10                            | 0.60                                  | 0.56  | Indifferent |
|                               | Lemon   | 10                            | 0.5                                   |       |             |
found in the EO microemulsion-coated polyester and cotton decreased significantly \((P \leq 0.05)\) over time, for example from 21.03 to 16.03 mmol l\(^{-1}\) on 16 cm\(^2\) polyester (Fig. 2a) and 18.41 to 12.03 mmol l\(^{-1}\) on 16 cm\(^2\) cotton (Fig. 2b). The concentration of limonene was significantly lower than citral, however there was also a significant \((P \leq 0.05)\) reduction in citral within the fabrics over 7 days storage (Fig. 2a,b).

Antimicrobial activity of the litsea-lemon EO microemulsion

**Screening for antimicrobial activity**

The disc diffusion method indicated that the microemulsion inhibited E. coli, S. aureus and S. epidermidis, with ZoIs of 22.73–38.00 mm (Fig. 3a). All individual components of the microemulsion possessed antimicrobial activity except 1% w/v sodium alginate (Fig. 3a). The EO blend alone produced similar ZoIs to the microemulsion, ranging 24.04–34.94 mm (Fig. 3a).

**Time-kill assay**

The microemulsion (1.0% v/v in water) showed rapid antimicrobial activity against S. epidermidis, S. aureus and E. coli, with a complete reduction of all bacterial test species \((7.31–7.54 \log_{10} \text{CFU per ml})\) within 5 min (Fig. 3b). A complete reduction \((7.13 \log_{10} \text{CFU per ml})\) of T. rubrum was achieved within 2 h (Fig. 3b).

**Qualitative antimicrobial efficacy test of Litsea-lemon EO microemulsion treated textile**

The microemulsion-treated cotton and polyester inhibited the growth of E. coli, S. aureus, S. epidermidis, and T. rubrum according to the qualitative antimicrobial textile efficacy test (Fig. 4a). Cotton and polyester (24 h post-treatment) produced ZoIs against S. epidermidis clinical isolate \((13.29–15.09 \text{ mm})\) and MRSA \((7.65–17.64 \text{ mm})\), and although ZoIs were not observed for E. coli and S. aureus, growth of microorganisms in direct contact with the textile was inhibited. S. epidermidis type strain was not inhibited by polyester 24 h post-treatment. Polyester and cotton \((7 \text{ days post-treatment})\) inhibited all test species, with ZoIs ranging from 0.53 mm against E. coli clinical isolate to 65.74 mm against T. rubrum (Fig. 4a). Microemulsion-treated cotton and polyester samples did not retain their antimicrobial activity following washing at 40°C against all test species \((\text{ZoIs} = 0.00 \pm 0.00 \text{ mm})\) except for cotton against T. rubrum \((\text{ZoI} = 0.52 \pm 0.24 \text{ mm})\).

**Quantitative antimicrobial efficacy test of litsea-lemon EO microemulsion treated textile**

The microemulsion-treated polyester reduced S. epidermidis by 6.69 \(\log_{10}\) CFU per ml immediately after inoculation (Fig. 4b); similarly, S. aureus and S. epidermidis were reduced by 6.56–6.96 \(\log_{10}\) CFU per ml immediately upon contact with the microemulsion-treated cotton (Fig. 4b). Microemulsion-treated polyester and cotton were strongly antimicrobial against all test species after 24–48 h, with reductions ranging 6.84–8.91 \(\log_{10}\) CFU per ml \((8 \log_{10} \text{CFU per ml inoculum})\) against E. coli, S. aureus and S. epidermidis and 6.76–6.77 \(7 \log_{10} \text{CFU per ml inoculum})\) against T. rubrum (Fig. 4b,c).
Repellency of Litsea-lemon EO blend and microemulsion-treated cotton against *A. aegypti* mosquitoes

The mean repellency of cotton samples treated with the microemulsion (24 h post-treatment) against *A. aegypti* mosquitoes was $72.9 \pm 13.9\%$ (mean, $n = 3 \pm$ standard deviation [SD]) compared to $52.3 \pm 4.2$ (mean, $n = 3 \pm$ SD) repellency for cotton samples impregnated with the litsea-lemon EO blend (1 : 2 ratio).

**Figure 2** Mean concentration (mmol l$^{-1}$) of limonene and citral extracted from of litsea-lemon microemulsion-treated polyester (a) and cotton (b) samples 24 h and 7 days post-treatment ($n = 3 \pm$ SD). (■) limonene (24 h post-treatment), (△) limonene (7 days post-treatment) (□) citral (24 h post-treatment) (○) citral (7 days post-treatment).

**Figure 3** Antimicrobial activity of the litsea-lemon EO microemulsion. (a) ZoIs (mm) of microemulsion components against *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis* according to the disc diffusion method ($n = 3 \pm$ SD). (b) log$_{10}$ CFU per ml reductions of *E. coli*, *S. aureus*, *S. epidermidis* and *Trichophyton rubrum* treated with the microemulsion in the time-kill assay ($n = 4 \pm$ SD). (■) *E. coli*, (□) *S. aureus*, (○) *S. epidermidis*, (△) *T. rubrum*.

Repellency of Litsea-lemon EO blend and microemulsion-treated cotton against *A. aegypti* mosquitoes

The mean repellency of cotton samples treated with the microemulsion (24 h post-treatment) against *A. aegypti* mosquitoes was $72.9 \pm 13.9\%$ (mean, $n = 3 \pm$ standard deviation [SD]) compared to $52.3 \pm 4.2$ (mean, $n = 3 \pm$ SD) repellency for cotton samples impregnated with the litsea-lemon EO blend (1 : 2 ratio).
Antimicrobial textile finishings have a potential application for wound dressings or sportswear to control bacterial and fungal contamination. Natural products could offer an environmentally friendly and biodegradable alternative to currently employed biocides for antimicrobial textile finishes. The aim of this study was to develop an antimicrobial textile coating loaded with EOs using a novel green polyelectrolyte micro-assembly process.

Here, 10 EOs were screened for antimicrobial activity against two Gram-positive bacteria (S. aureus and S. epidermidis), two Gram-negative bacteria (E. coli and P. aeruginosa) and a dermatophyte (T. rubrum) associated with skin conditions. All EOs tested demonstrated antimicrobial activity against S. aureus, S. epidermidis and T. rubrum while bergamot, lemon, litsea and rosemary EOs inhibited all the tested species (Table 1). The broad-spectrum antimicrobial activity of the EOs in this study are consistent with previous research, as outlined by a literature by Orchard and Van Vuuren (2017); it was reported that bergamot, lemon, litsea and rosemary EOs possess antimicrobial activity against S. aureus, while rosemary EO also inhibited E. coli and T. rubrum, lemon EO inhibited E. coli and P. aeruginosa and bergamot

![Diagram](image_url)

**Figure 4** Antimicrobial activity of litsea-lemon EO microemulsion-treated cotton or polyester fabrics. (a) ZoIs (mm) of microbial growth on the treated fabrics, 24 h or 7 days post-treatment or after washing at 40°C with domestic detergent. [Escherichia coli], [E. coli clinical isolate], [Staphylococcus aureus] MRSA, [Staphylococcus epidermidis], [S. epidermidis clinical isolate]; Trichophyton rubrum spores. (b) log$_{10}$ CFU per ml reduction of bacteria by the treated fabrics. [E. coli], [S. aureus], [S. epidermidis]. (c) log$_{10}$ CFU per ml reduction of T. rubrum by the treated fabrics.

**Discussion**

Antimicrobial textile finishings have a potential application for wound dressings or sportswear to control bacterial and fungal contamination. Natural products could offer an environmentally friendly and biodegradable alternative to currently employed biocides for antimicrobial textile finishes. The aim of this study was to develop an antimicrobial textile coating loaded with EOs using a novel green polyelectrolyte micro-assembly process.

Here, 10 EOs were screened for antimicrobial activity against two Gram-positive bacteria (S. aureus and S. epidermidis), two Gram-negative bacteria (E. coli and P. aeruginosa) and a dermatophyte (T. rubrum) associated with skin conditions. All EOs tested demonstrated antimicrobial activity against S. aureus, S. epidermidis and T. rubrum while bergamot, lemon, litsea and rosemary EOs inhibited all the tested species (Table 1). The broad-spectrum antimicrobial activity of the EOs in this study are consistent with previous research, as outlined by a literature by Orchard and Van Vuuren (2017); it was reported that bergamot, lemon, litsea and rosemary EOs possess antimicrobial activity against S. aureus, while rosemary EO also inhibited E. coli and T. rubrum, lemon EO inhibited E. coli and P. aeruginosa and bergamot.
inhibited *E. coli* and *T. rubrum* (Orchard and Van Vuuren, 2017).

Previous studies have reported synergistic interactions between EOs, resulting in reduced effective doses (Lee *et al.* 2020) which in turn may decrease the risk of toxicity of EOs towards the user due to potential concentration-dependent skin irritation of EOs (Lee *et al.* 2013). In this study, the majority of combinations were indifferent (Table 2), however litsea and lemon EOs were synergistic against *S. aureus*, *E. coli* and *T. rubrum* (FICIs = 0.09–0.49), reducing the MICs of the individual EOs as indices of enhanced antimicrobial potency. Triple combinations of the EOs in this study only showed synergism against *E. coli* but did not show any significant effect on the MIC values compared to the double combinations against *S. aureus*, *S. epidermidis* and *P. aeruginosa* (Table 2). In contrast, a triple combination of litsea, clove and rosewood EOs was more potent than double combinations in terms of MIC against the acnec vulgaris associated pathogen *Cutibacterium acnes* (Owen *et al.* 2017). Overall, the MICs of litsea and lemon EOs in a combination that inhibited all test species was 2.5 and 5.0 μl ml⁻¹ respectively, representing a litsea : lemon ratio of 1 : 2, which was carried forward for further investigations.

EOs have limited applications due to their volatility (Volić *et al.* 2020). Microencapsulation has previously been used to improve the durability of natural products on fabrics (Juliahea *et al.* 2021). The litsea and lemon EO blend (1 : 2 ratio) identified in this study was micro-emulsified and stabilized with a chitosan-sodium alginate polyelectrolyte assembly within the microemulsion, showing moderate stability over 14 days and a CI of around 10% (Fig. 1a). Though flocculation/creaming of the microemulsion was observed within 24 h, this is reversible by shaking, allowing for treatment of fabrics despite creaming. The chemical stability of the microemulsions was monitored via the major components of litsea and lemon EOs, citral and limonene. The citral concentration within microemulsions varied after 28 days, with a significant increase observed at 40°C but a near complete decrease at 15°C (Fig. 1c). The initial high EO concentration at 15°C followed by a decrease is ascribed to a burst release or loss of the loosely bonded EOs at the surface of the chitosan-alginate self-assembly whereas higher temperatures (40°C) will breakdown the chitosan-alginate self-assembly leading to the release of higher concentrations of the EOs from the inner core of the self-assembly. It is evident that the release of the EO incorporated into the chitosan-alginate microemulsion can be controlled. Citral is reportedly prone to autooxidation (Bailly 2020), and storage conditions such as temperature, humidity and light can affect the concentration of EO compounds (Soltanbeigi 2020), whereas as shown in this study degradation of citral at 40°C was not evident. In order to prevent any signs of physical or chemical instability of the EOs, only fresh microemulsions were used during the treatment and analysis of cotton and polyester fabrics; indeed, a significant difference (*P* ≤ 0.05) in limonene and citral concentrations were noted on the treated cotton at 24 h post-treatment compared to 1-week post-treatment (Fig. 2).

Quantification of citral and limonene within the treated fabric demonstrated that there is a direct relationship between the entrapment of the EOs (within the microemulsion) on the fabric and the fabric area (Fig. 2), suggesting that the EOs were evenly distributed across the treated textile. This in turn indicates that the treatment of the fabric will also be potentially successful at both a small and larger scale, making the method scalable.

The microemulsion showed significant antimicrobial activity; *E. coli*, *S. aureus* and *S. epidermidis* were reduced by 7 log₉ CFU per ml within 5 min by the microemulsion (Fig. 3b). *T. rubrum* was less susceptible to the microemulsion, with a complete inactivation (7 log₁₀ CFU per ml) after 120 min (Fig. 3b). Previous research has demonstrated the antimicrobial activity of EO emulsions, for example bergamot EO nanoemulsions reduced *E. coli* by up to 4.5 log₁₀ CFU per ml after 5 h (Marchese *et al.* 2020). Textile challenge tests show that microemulsion-treated polyester and cotton also possessed good antimicrobial activity, with a complete reduction achieved by both treated fabrics after 24 h for all test bacteria (Fig. 4b) and 48 h for *T. rubrum* (Fig. 4c). In a similar study, Khodary *et al.* (2017) reported that a cotton wound dressing treated with microencapsulated geranium extract completely reduced *E. coli* and *S. aureus* after 4 h. No antimicrobial activity was retained for microemulsion treated textiles after washing at 40°C with a domestic detergent (Fig. 4a), this can be attributed to the lack of cross-linking chemicals or fixation agents used in the application of the microemulsion to textiles in this study. Further research into the use of environmentally friendly fixation techniques to improve the durability of the finish are therefore warranted, which would improve the cost and sustainability of the treatment. The current treatment could nonetheless be suitable for single use applications such as wound care, however further studies on toxicity would need to be carried out to determine the human sensitivity to the micro-emulsified EO blend when used topically. In addition, there can be batch-to-batch variation in the chemical composition of EOs, and standardization of EO composition is required for successful application of novel EO based formulations (Baptista-Silva *et al.* 2020).

In common with many natural volatile oils this blend did demonstrate some repellent properties against...
mosquitoes, with the microemulsion indicating superiority to the EO alone. However, the longevity and usefulness of this property needs to be further demonstrated with laboratory and field work utilizing human volunteers.

In conclusion, natural products could be a potential source of environmentally friendly and effective antimicrobial fabric finishes. Micro-emulsified litsea and lemon EOs stabilized in a chitosan-sodium alginate polyelectrolyte assembly in this study showed significant antimicrobial activity against the skin associated microorganisms E. coli, S. aureus, S. epidermidis and T. rubrum, indicating that EOs are potentially useful as finishing agents for the creation of environmentally friendly functional antimicrobial textiles. Further research is required to improve the stability of micro-emulsified EOs and investigate their attachment on the textile fibers to enable their use in wider applications beyond single-use treatments.

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Conflict of Interest

The authors declare no conflict of interest.

Author contributions

Microbiology—Anita Soroh: Conceptualization, Investigation, Formal analysis and Writing—original draft. Lucy Owen: Formal analysis, Writing—original draft and Writing—review and editing. Katie Laird: Conceptualization, Formal analysis, Writing—original draft and Writing—review and editing: Chemistry—Amos Abiye: Conceptualization, Formal analysis and Writing—review and editing. Omar Qutachi: Conceptualization, Formal analysis, Writing—review & editing. Jinit Masania: Investigation, Formal analysis and Writing—review & editing. Textile Technology—Jinsong Shen: Conceptualization, Formal analysis and Writing—review and editing. Entomology—Noor Rahim: Investigation, Formal analysis and Writing—original draft. Larry Goodyer: Conceptualization, Formal analysis and Writing – review and editing.

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