Antimicrobial treatments with chitosan microencapsulated Angelica (*Angelica archangelica*) and Marsh Labrador Tea (*Rhododendron tomentosum*) supercritical CO_2 extracts in linen-cotton jacquard woven textiles

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Keywords

plant extracts, natural compounds, supercritical carbon dioxide extraction, GC-MS chromatography, antimicrobial activity, microencapsulation, textile functionalization

Abstract

In this study antimicrobial linen-cotton jacquard textiles were manufactured using green chemistry methods. The functionalization of the fabrics was executed by impregnating chitosan microencapsulated bio-based oils from Angelica (*Angelica archangelica* L.) and Marsh Labrador Tea (*Rhododendron tomentosum* Harmaja) obtained with pilot scale supercritical carbon dioxide extraction ($scCO_2$). The chemical compositions of the extracts (AA and MLT, respectively) were analyzed by a combination of gas chromatography and mass spectrometry (GC-MS). The antimicrobial activities of the extracts, AAC and MLTC microcapsules, and the microencapsulated textiles (AAC and MLTC textiles) were analyzed against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria, dimorphic yeast *Candida albicans* and filamentous mold *Aspergillus brasiliensis*. The AAC textile proved 40% inhibition against *S. aureus*, whereas the MLTC textile demonstrated 43.8% and 51.7% inhibition against both *S. aureus* and *E. coli*, respectively. Although the chitosan shell material itself indicated mild activity against both bacterial strains, the extracts increased the antibacterial activities in microencapsulated textiles. In addition, the antifungal impact of the MLTC textile was demonstrated against *A. brasiliensis*. According to the FTIR-ATR and FESEM analyses, covalent bonding between the microcapsules and textile fibers was

established with citric acid as a cross-linker. The antimicrobial activity was also shown to persist in the MLTC textiles after six domestic washing cycles.

Introduction

The textile industry is one of the major sources of global emissions, and most of the chemicals used in textile and clothing production are harmful or even dangerous to humans and nature.[1-3] Harmful synthetic chemicals are applied in textiles, especially during finishing procedures, e.g., to prevent mold and other microbial growth or insects in textiles during long transportation distances.[2-5] Due to the hydrophobic nature of synthetic fibers, they are more durable against the growth of microorganisms than natural fibers.[5] Climate neutrality goals and EU legislation[6-8], combined with the growing interest of consumers and competitiveness in the market, have led the textile industry to take steps towards environmentally friendly, safe, and sustainable materials and chemicals. Hence, in recent years, the study of the properties and applications of bio-based alternatives and green chemistry methods has grown significantly.[9-10]

The exploration of biomimicry or nature-inspired innovations is one approach to mimicking interesting properties from nature in textiles.[11] For example, plants defend themselves against insects and diseases by using their secondary metabolism products, such as essential oils and waxes.[12-14] These products are therefore commonly used in natural biocides, insect repellents, perfumes, cosmetics, and medicine.[15-16] Our interest lies in sustainable utilization of the protective properties of plants and substituting harmful chemicals, for instance, in textiles or aromatherapy textiles with natural compounds in a manner that aligns with their studied properties, defining their subsequent applications. Aromatherapy textile applications can encompass perfumed, antimicrobial, mosquito-repellent, and/or medical textiles.[17] However, it is crucial to note that bio-based chemical compounds and materials do not automatically guarantee sustainable production or non-toxicity. Nevertheless, if protective agents can be

safely, effectively, and profitably extracted from plants and used as textile coatings, many non-renewable and environmentally harmful protective agents would remain unused. The supercritical carbon dioxide extraction ($scCO_2$) method has many advantages in producing plant-based oils and waxes by using only non-toxic and recyclable solvents. The $scCO_2$ method enables the production of bioactive plant extracts with minimized degradation and without any co-solvent during a relatively short extraction time.[18-22] As an example of the attainable bioactivities, in a review by Da Silva et al.[23], it was concluded that natural compounds obtained by supercritical fluid extraction were mainly antioxidant (41%), antitumor (18%) and antibacterial (10%), while also antiviral, antimicrobial, anti-inflammatory, and anticholinesterase activities had been observed.

Antimicrobial textiles have two main functions. Firstly, they possess a hygienic finishing and protect the wearer against pathogens and odor-generating micro-organisms.[5] Secondly, they shield the textile from deterioration caused by mold and rot-producing microorganisms.[5] Coacervation microencapsulation methods are commonly employed to provide antimicrobial finishing on textiles, particularly in an oil-in-water and water-in-oil medium.[5,24-28] The polymeric shell material, such as chitosan or gelatin, safeguards the sensitive core material from heat, pH changes, evaporation, and moisture. Additionally, microencapsulation enables controlled and sustained release of the active agents through the shell.[5, 28-29] The microcapsules are typically attached to the textile fibers using binders like synthetic resins, latexes, rubbers, and silicones. However, more recently, biodegradable citric acid and 1,2,3,4-butanetetracarboxylic acid have become common cross-linking agents between the chitosan shell and cellulose fiber.[5, 30] Due to the volatile nature and poor lasting effect of essential oils, they have not been in common use as antimicrobial agents in textiles, but the microencapsulation of essential oils could enhance the durability.[17, 28] The textiles with antimicrobial properties of encapsulated essentials oils have previously been prepared mainly

from bulk oils, such as vanillin, limonene, eucalyptus and sandal wood oils[24, 26, 28, 31] , but studies have also covered the process from extractions to textile finishings [32-34].

In earlier studies, Angelica (*Angelica archangelica* L.) and Marsh Labrador Tea (*Rhododendron tomentosum* Harmaja) proved to be highly applicable plant materials for laboratory scale $scCO_2$ extractions. According to the results, the extracts exhibited a broad spectrum of antimicrobial activities against selected microbes.[35] In this study, our objective was to manufacture linen-cotton jacquard textiles with antimicrobial activity by incorporating these two plant extracts into the textiles using green chemistry methods with non-toxic and recyclable chemicals. To the best of our knowledge, no larger scale supercritical fluid extractions had been previously conducted on these two selected plant materials, and no previous studies utilizing these plant extracts in textile applications exist to date. Consequently, we scaled up the Angelica and Marsh Labrador Tea $scCO_2$ extraction methods to the pilot scale. Recognizing that compositions and antimicrobial activities may vary with a change in scale, we characterized the Angelica and Marsh Labrador Tea extracts (AA and MLT, respectively) through a combination of gas chromatography and mass spectrometry (GC-MS). Additionally, we analyzed their antimicrobial properties using bacterial biosensor imaging methodology and a modified agar well diffusion method. Moreover, the AA and MLT extracts were microencapsulated with chitosan using the coacervation method. The resulting microcapsules (AAC and MLTC microcapsules, respectively) were impregnated onto the linen- and cotton-based jacquard fabrics using citric acid as a cross-linker to establish covalent bonding between the chitosan microcapsule shell and cellulose textile material.

The primary objective of this study was to demonstrate the retention of antimicrobial activity in the textiles during the microencapsulation treatment. Furthermore, we present field emission scanning electron microscopy (FESEM) images of the produced textiles, and Fourier-transform

infrared spectroscopy with attenuated total reflection (FTIR-ATR) technique was also employed for the microcapsules and textiles to indicate the successful impregnation. We also examined the antimicrobial properties of the microencapsulated fabrics (AAC textile and MLTC textile) and executed a domestic washing durability testing for the MLTC textile. The six domestic washing cycles were followed by FESEM analysis, antibacterial assays, and solid phase microextraction analysis with GC-MS (SPME-GC-MS) to establish the washing fastness of the microcapsules and indicate retention of antimicrobial activity.

Materials and methods

Chemicals

Unless otherwise stated, the chemicals were purchased from VWR International. For the preparation of microcapsules, high molecular weight chitosan (Sigma-Aldrich) was used as a shell material, and decyl β-D-glucopyranoside (>98.0%, Lutensol30 (TCI)), was used as a non-ionic surfactant. Ledol, an antifungal agent (>95.0% (Abcam)), was used as the standard and reference material in antimicrobial and SPME-GC-MS analyses. In addition, deionized water was used to prepare the microcapsules and impregnate them onto the fabrics.

Plant materials

The roots of Angelica were cultivated and collected by Arctic Warriors Oy (Arctic Warriors Oy, Narkaus, Lapland, Finland) in Northern Finland, specifically in Narkaus village in Rovaniemi (66.36263, 26.3551), in September 2021. The roots were thoroughly washed with water intended for industrial food processing, chopped into smaller pieces, and then freshly frozen at -20 °C. Subsequently, the frozen plant material underwent drying using a condensing circulating warm air dryer (VegeDryer 100, Siikaisten Ykkösmyymälät Oy) at 37.5 °C for four days (Arctic Warriors Oy, Rovaniemi, Finland).

The stems and leaves of Marsh Labrador Tea were collected from wild populations in Finland, specifically in Northern Ostrobothnia Ylikiiminki, from the Rekikylä (65.04779, 25.95942) and Vähävuotto (64.92893, 26.52503) areas. The sample collection took place in July 2021 after the blossoming had ended. The Marsh Labrador Tea plant material was freshly frozen to -20 °C and then air-dried at 25 °C for 18 days using a Heraeus UT 5100E D-6450 Hanau, Binder Gmbh dryer in Ruukki, Finland.

For the $scCO_2$ extractions, the particle size of both the aforesaid plant materials was reduced using a pulverisette cutting mill (Type 15.903, Fritsch GmbH) with a 2 mm sieve cassette.

Textile materials

The textile material was a jacquard woven fabric with a thin natural white mercerized 100% cotton yarn as the warp yarn (59×2 tex, Toika Oy) and an unbleached Esito 100% linen yarn (half wet spun, Nel 6, tex 280, Lankava Oy) as the weft yarn. The fabric was woven using a digital weaving TC1 loom (Thread Controller 1., Digital Weaving Norway, Tronrud Engineering Moss) at the University of Lapland, Faculty of Art and Design. The woven fabric was designed using the Arahne Textile design software (Arahne CAD/CAM for weaving: ArahWeave Personal Edition 9.5i*, ArahPaint, ArahDrape).

The jacquard fabric, with a weight per unit area of 650 g/m^2 (chain width 70 cm, chain and weft density 12 yarns/cm, satin weave), was cut into approximately 3 cm × 3 cm (3.2 ± 0.3 g) pieces. These test fabric pieces were washed for 30 minutes in a deionized water/acetone solution (10:90, v/v) and dried overnight at 20 °C before impregnating the microcapsules onto the fabrics. The moisture content of the fabric after drying was 4.8%.

Supercritical carbon dioxide extraction

Plant materials with reduced particle size were extracted using a supercritical fluid pilot plant (Chematur Ecoplanning). Air-dried plant material was loaded into a vessel and placed within a closed reactor. The approximate weights of plant material were 2.5 kg for oven-dried Angelica root and 1.4 kg for air-dried Marsh Labrador Tea stems and leaves. The pressure in the reactor was gradually increased to 350 bar, and the temperature of the reactor was adjusted to 70 °C, with a supercritical CO_2 flow rate of 0.45 L/min. The separation chamber temperature for collecting Angelica root oil extract (AA) was set to 40 °C. For the Marsh Labrador Tea wax extract (MLT), separation occurred in two stages, the first at 55 °C and the second at 40 °C, using two separation chambers in series. The extracts were collected in a bottle and weighed at 15-minute intervals to determine the extraction endpoint. Because it is known that the changes in the solvation properties of supercritical carbon dioxide depend on temperature and pressure, the relatively high extraction pressure and temperature were chosen to achieve the maximum extraction intensity.[36]

GC-MS analyses of the extracts

Extractives / Silylated samples

A precisely weighed sample of 30 mg was dissolved in methyl tert-butyl ether (MTBE). Cholesterol, dissolved in MTBE at approximately 5% (w/v), was added as standard. An aliquot corresponding to 1.0 mg was pipetted into a test tube, and the solvent was evaporated using a stream of nitrogen gas. The samples were silylated with a 160 µL solution containing 120 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, Sigma-Aldrich), 20 µL of trimethylsilyl chloride (TMCS, Sigma-Aldrich), and 20 µL of pyridine (Sigma-Aldrich). The mixture was heated for 50 min at 70 °C. After derivatization, samples were allowed to cool to room temperature and then transferred to a GC vial for analysis by GC-FID and GC-MS.

The samples were parallelly analyzed using a GC-FID system for quantification and a GC-MS system for identification under nearly identical conditions. The GC analysis employed a Shimadzu GC-2010Plus gas chromatograph with an FID detector and a Shimadzu GCMS-QP2010Plus mass selective detector. Both systems utilized a Zebron ZB-1HT column (Phenomenex; 20 m × 0.18 mm × film thickness 0.18 µm). For the GC-FID, the oven temperature program was as follows: the initial temperature was either 35 °C for highly volatiles or 80 °C, followed by an increase of 8 °C/min to 360 °C, with a 20-minute hold time. The injector temperature was maintained at 250 °C. Hydrogen served as the carrier gas, with a 40 cm/s linear velocity.

GC-MS identification involved comparing spectra and retention indices in our in-house database, as well as standard commercial databases (Wiley/NIST/FFNSC/SHIM2205).

Hydrolysis of the samples for GC analysis

An aliquot of the sample, along with the added standard corresponding to 1.0 mg, was pipetted into a test tube. The solvent was then evaporated using a stream of nitrogen gas. Subsequently, 2 mL 2 M KOH in EtOH:water (90%, v/v) was added, and the sample was shaken. Following this, the sample was heated in an oven at 70 °C for three hours, with intermittent shaking. The hydrolysis process was halted by introducing 3 mL of water and neutralizing the solution with a dropwise addition of 9% (v/v) HCl (approximately 1 mL) until reaching a pH 3, as determined using bromocresol green as an indicator. The resulting solutions were extracted with 3 × 3 mL portions of n-hexane. The combined hexane solution was then evaporated and silylated as described above.

Volatiles

The most volatile components, primarily monoterpenes and sesquiterpenes, were determined from an MTBE solution without derivatization and quantified against 5% dodecane as an internal standard. All GC chromatographic parameters remained the same as those for the extractives analysis, with the exception that the temperature program was initiated at 35 °C.

Production of microcapsules

The preparation of chitosan microcapsules with AA and MLT extracts was modified from previous studies, which utilized commercial bulk oils as a core material.[24, 28] Briefly, a 0.5 % (w/w) solution of chitosan and surfactant was prepared by dissolving chitosan (0.2 g) in a 1% (v/v) acetic acid solution (39,6 g) under magnetic stirring at 1,500 rpm for two hours. Thereafter, the surfactant, Lutensol30 (0.2 g) was added to the solution, and the stirring speed was increased to 11,000 rpm using an Ultra turrax (IKA®-WERKE, GMBH & CO.). Consequently, the concentration of chitosan in the formed solution was 5 g/L. Next, 4 mL of AA or 4 mL of MLT, mixed with paraffin oil (0.75 g/mL respectively) to reduce the viscosity of MLT extract, was added dropwise into the solution, and the mixture was stirred at 13,000 rpm for 10 min. The resulting emulsion was carefully poured into 200 mL of 1% (w/v) NaOH, and the solution was left for slow agitation. After standing for 30 min, a small sample of the obtained microcapsule mixture was removed from each batch and taken for optical microscopy.

As the AAC microcapsules tended to settle at the bottom of the formed microcapsule-NaOH mixture, the mixture was centrifuged (2,000 rpm, 5 min) prior to filtration. Both the AAC and MLTC mixtures were filtered through a monofilament nylon filter (NMO-50-P03S-E-50S, Eaton) using a Büchner funnel and vacuum suction. Finally, both AAC and MLTC microcapsules were washed three times with 30 mL of deionized water.

Optical microscopy analysis of the microcapsules

The particle sizes of the AAC and MLTC microcapsules were examined using an Olympus DSX1000 digital microscope and DSX software. Approximately 70 microcapsules were imaged, and the average particle diameter calculated.

Impregnation of the Microcapsules onto Fabrics

The AAC and MLTC microcapsules were attached to the linen-cotton jacquard textile using citric acid as a cross-linker to ensure the formation of covalent bonding. Each test fabric was immersed in a 10 mL of the finishing liquid bath (pH 2.37 at 25.2 °C) containing 8% (w/v) of microcapsules, 3% (w/v) citric acid, and 1.5% (w/v) NaH_2PO_4 for a few minutes. The microcapsules were gently pressed onto the linen side of the textile material. In addition, microcapsules were attached to the textile without citric acid and NaH_2PO_4 to be used as reference samples (AAC and MLTC textile water). The procedure was the same as above, but the 10 mL of finishing liquid bath contained deionized water with 8% (w/v) of microcapsules. After impregnating microcapsules onto fabrics, the treated textile pieces were dried overnight at room temperature, washed twice in 300 mL of deionized water for 5 min, and dried again overnight at room temperature. The mass difference between the washed and dried microencapsulated textiles and the textiles before impregnation was 0.32 ± 0.03 g, indicating a microcapsule uptake of approximately 10% (w/w). The formulated AAC and MLTC textiles were analyzed using FESEM, FTIR-ATR, and antimicrobial methods. In addition, reference linen-cotton jacquard textiles without microcapsules were prepared for antimicrobial tests by immersing them in a 10 mL of water bath (REF textile water) or a 10 mL liquid bath containing 3% (w/v) citric acid and 1.5% (w/v) NaH_2PO_4 (REF textile citric acid) for a few minutes, followed by overnight drying at room temperature.

FESEM analysis

The surfaces of the textile samples, with and without AAC and MLTC microencapsulation treatments, were examined using a field emission scanning electron microscope (FEI Quanta FEG450, FEI Company) equipped with xT Microscope Control software (version 4.1.6.2080, FEI Company). For FESEM imaging, a subsample was prepared by cutting an approximately 10 × 10 mm piece of the fabric and attaching it, linen side up, to a sample stub with two-sided conductive carbon tape. No carbon coating was applied to the samples to avoid errors in the potential subsequent analyses. To ensure sufficient resolution and magnification without accumulating electric charge in the sample, a low acceleration voltage of 1.25 kV with a spot size of 5.5–6.5 was used. Multiple images at various magnifications were captured from each sample.

FTIR-ATR spectroscopy

To examine the surfaces of the textiles and the impregnation of AAC and MLTC microcapsules onto linen-cotton jacquard textiles, FTIR analysis with a single reflection ATR technique (attenuated total reflectance, ZnSe crystal plate) was employed. The studied samples included AAC and MLTC microcapsules, AAC and MLTC textiles microencapsulated with and without citric acid, untreated jacquard fabric (a control sample), and the source chemicals of the AAC and MLTC microcapsule finishing liquid bath, such as citric acid, chitosan, AA, and MLT. FTIR-ATR analyses were carried out using a Shimadzu IR Prestige-21 spectrometer (OrdiorBerner Oy, Helsinki) equipped with a DLATGS detector and Shimadzu IRsolution 1.40 2007 software. Spectra were acquired in absorbance mode with 60 scans at a resolution of 4 cm^{-1} in the range of 4,000–400 cm^{-1}. For repeatability and consistent analysis, all samples were randomly selected and analyzed with three parallel measurements.

Antibacterial assays

The plant extracts AA, MLT, and MLT in paraffin oil (0.75 g/mL, used in the preparation of microcapsules, see section 2.6.), AAC and MLTC microcapsules, and linen-cotton jacquard textiles with and without the AAC and MLTC treatments in citric acid and water bath were investigated for their antibacterial activities using bacterial biosensor imaging methodology first published by Jyske et al. [37]. In addition, chitosan and citric acid, both used in the microencapsulation process, were tested as references. For MLT antibacterial analyses, the ledol standard was used also as a reference sample.

The bacterial strains *Escherichia coli* K12+pcGLS11 and *Staphylococcus aureus* RN4220+pAT19 have been genetically manipulated to produce a constant luminescent light signal as part of their normal metabolism.[38] They indicate the presence of antibacterial substances with a dose-responsive reduction of the luminescent light signal, caused by disturbed metabolism and cell death, which can be monitored via luminescent readers or imaging devices. The bacterial inoculation is reported in detail by Tienaho et al.[39] In brief, the bacterial strains were stored at -80 °C in glycerol and cultivated for approximately 16 h at 30 °C (*E. coli*) and 37 °C (*S. aureus*) on lysogeny agar plates (LA) (tryptone 10 g/L; yeast extract 5 g/L; NaCl 10 g/L; and agar 15 g/L). The *E. coli* plates were supplemented with 10% (v/v) sterile filtered phosphate buffer (PB) (1 M, pH 7.0) and 100 µg/mL of ampicillin and *S. aureus* plates with 5 µg/mL erythromycin. Bacterial stock solutions were prepared by inoculating a single colony in lysogeny broth with similar supplementations to the LA plates. Stocks were again cultivated for approximately 16 h at 300 rpm shaking at 30 °C (*E. coli*) and 37 °C (*S. aureus*).

In the imaging methodology, an aliquot of 7 mL of bacterial inoculum grown overnight was added for every 100 mL of soft agar (LA but with 7.5 g/L (50%) agar content) supplemented with a 10% (v/v) sterile filtered phosphate buffer (PB) (1 M, pH 7.0) and 100 µg/mL of

ampicillin (*E. coli*) and with 5 µg/mL erythromycin (*S. aureus*) at approximately 50 °C. The soft agar containing bacteria were mixed gently and rapidly poured into 6-well plate triplicates containing approximately 1 × 1 cm pieces of the test Jacquard textiles or plant extracts and other samples on a thin layer of LA before the soft agar started to solidify. The plates were then inoculated at 30 °C (*E. coli*) or 37 °C (*S. aureus*) overnight (approximately 16 h) and finally scanned using the SPECTRAL Lago X *in vivo* imaging system (Spectral Instruments Imaging, AZ, United States) with luminescence and an image overlay mode. The exposure time was set to one second, and small to medium binning (2×–4×) was used, with field of view (FOV) 25 × 25 cm (for 3–4 6-well plates at once) and an object height of 1.5 cm. The data were then handled using Aura Spectral Instruments Imaging Software version 3.2. Circular regions of interest (ROIs) were chosen for the whole 6-well plate well and the results were obtained in photons/s/cm^2/sr (sr=steradian, unit of solid angle). To change the results into more comparable units between bacterial inoculations, the sample result averages from the three plate replicates were normalized by dividing them with the control plate ones (containing only bacterial inoculation soft agar and no sample) and finally expressed in inhibition percentages (inhibition-%).[40] The antibacterial inhibition can, therefore, vary from 100%, where no luminescence light is detected in the well, indicating complete loss of metabolism and cell death, to 0%, where the bacteria is growing and emitting luminescence as well as the undisturbed control. Everything above 0% indicates antibacterial effects. Because whole-well ROIs are used, even 20% decrease indicates significant antibacterial effects. Error bars represent the coefficient of variation between the samples on three 6-well plate replicates.

Antifungal assays

Fungal test organisms consisted of *Aspergillus brasiliensis* (ATCC® 16404™, Microbiologics, Inc.) and *Candida albicans* (ATCC® 10231™, Microbiologics, Inc.) strains, pre- and subcultured on Malt Extract Agar (MEA) (malt extract 30 g/L; and agar 15 g/L; pH 5.6) plates.

Assessment of plant extracts

The modified agar well diffusion method[41-42] was utilized to determine the antifungal capacity of AA and MLT extract in paraffin oil (0.75 g/mL, used in the preparation of microcapsules, see section 2.6.) and MLT in 99.5% EtOH (1 g/mL). In preliminary testing, EtOH was confirmed not to disturb the growth of the *A. brasiliensis* and *C. albicans*, and EtOH was therefore excluded from further assays. Amphotericin B (PanReac AppliChem) was utilized as a positive control for both fungal species (10 µg/mL in DMSO). The culturing of test organisms, as well as the production of the test suspensions, was conducted following the EN 1650 standard. The test plates were prepared by spreading 0.5 mL of *A. brasiliensis* and *C. albicans* test solutions (1.8−3.6 × 10^6 CFU/mL and 1−3 × 10^5 CFU/mL, respectively) onto MEA plates. The plates were subsequently punched with the head of a glass Pasteur pipette to create 5 mm diameter holes, which were filled with approximately 100 µL of the tested solutions. The plates were first kept in a refrigerator overnight and thereafter incubated for two days at 25 °C (*C. albicans*) or 28 °C (*A. brasiliensis*). The area of inhibition zones was determined with a planimeter (PLANIX 10S "Marble") from three replicates of the plant extracts. The testing was conducted in triplicate, and the mean and standard deviations were calculated.

Examination of textiles

Solely MLTC textiles were chosen for the antifungal activity testing, as AA did not inhibit the growth of the test organisms based on the results generated in the current study. The protocol

for antifungal activity was modified from the transfer method of the plate count methodology (ISO 13629-2). The *A. brasiliensis* sub-culturing and spore suspension preparation were conducted according to ISO 13629-2. One mL of the spore suspension (2.28–2.62 × 10^5 CFU/mL) was spread onto a Sabouraud dextrose agar (SDA) plate (pepsic meat peptone 10 g/L; dextrose 40 g/L; agar 15 g/L; pH 5.6) and kept on the desk of the flow hood for 5 min. The 1 × 1 cm pieces of microcapsule-treated and reference samples without the microcapsule treatment were weighed down onto a Petri dish with a decanter (filled to 200 g) for 1 min. Three jacquard textile samples (time point 0 h, before incubation) were each transferred to a Falcon tube and vortexed for 5 × 1 min in 2 mL of neutralizer solution (polysorbate 80 30 g/L; lecithin 3 g/L; pH 7.2). Subsequently, this solution (10^0) and 10^{-1} dilution prepared with 0.05% polysorbate 80 solution were spread onto the SDA plates supplemented with chloramphenicol (25 µg/mL) and incubated at 30 °C for 48 h. Another three jacquard textile pieces of each specimen (time point 48 h) were transferred to an empty Petri dish and incubated in a growing chamber at 30 °C with a relative humidity of 90%. After 48 h, the jacquard textile pieces were neutralized similarly (to the time point 0 h textiles), but aliquots of 10^0 to 10^{-4} dilutions were plated on SDA chloramphenicol plates and kept for 48 h at 30 °C. The number of colonies was recorded from each plate.

SPME-GC-MS analyses of the textiles

The main volatile components of the MLTC textile, palustrol, and ledol, were analyzed using SPME-GC-MS technique. Before analyses, glass bottles and septa were cleaned overnight in an oven at 200 and 105 °C, respectively, and verified to be free of contaminants. Three replicate MLTC textile samples were placed inside the glass bottles overnight to stabilize the system. The SPME fiber, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm Stableflex, SUPELCO), was inserted through the septum into the glass chamber, kept for

1 h, and thereafter manually injected to GC-MS system (Agilent 7980B GC with 5997A MS-detector) containing a GC-column (Agilent HP-5 ms ultra-inert 30 m × 250 μm × 0.25 μm) for 60 s. The temperature of the GC run started from 30 °C and changed 10 °C/min to 230 °C. Thereafter, there was a five-min hold, and then heating (40 °C/min) to 300 °C for two min. After detection, SPME fibers were cleaned inside another GC injector at 260 °C in nitrogen flow for 30 min.

The results were calculated against a calibration curve of ledol and according to its mass spectra. Ledol was dissolved in 5 μL of hexane and injected inside a calibration vessel, shaken thoroughly, and stabilized there for 1 h. The SPME fiber was held inside the glass bottle with the ledol calibration vessel for 1 h and analyzed with the GC-MS.

Domestic washing and drying durability test

To study the washing durability of the microencapsulated textile samples, six subsequent domestic washing and drying cycles were performed on the MLTC textiles with cotton ballast (type I, 100% cotton) according to the standard SFS-EN ISO 6330-2021. The washing procedure, using ECE Electrolux Wascator FOM 71CLS, included gentle washing program (3M) at 30 °C with 500 rpm and 50 mL of standard detergent (ECE), followed by flat drying of the textiles in room temperature according to the standard. The drying time between each cycle was at least 24 hours. After six washing and drying cycles, FESEM imaging analysis, antibacterial analyses against *S. aureus* and *E. coli*, and SPME-GC-MS analysis were performed for the textile samples.

Results and discussion

Supercritical carbon dioxide extraction

The Angelica root yielded significantly less extract than the Marsh Labrador Tea (Table 1). Similar observations have been previously reported. [35] The differences in standard deviations of the yield are most likely attributed to variations in material homogeneity and composition. Angelica roots, with moisture content 5.3% (w/w), were milled to fine particle size and mixed before extraction from a single batch. The Marsh Labrador Tea was derived from two batches with different moisture contents of 9.2% (w/w) and 10.8% (w/w). A higher extract yield was obtained from the batch with a greater moisture content.

Table 1. Air-dried (a.d.) and oven-dried (o.d.) supercritical carbon dioxide extraction yields of Angelica roots and Marsh Labrador Tea.

Sample	Repetitions	A.d. extraction yield (%)	O.d. extraction yield (%)[1]
Angelica roots	3	4.0 ± 0.1	4.3 ± 0.1
Marsh Labrador Tea	3	9.3 ± 2.4	10.3 ± 2.8

[1] The values were calculated from the air-dried extraction yield by taking the moisture content into account.

GC-MS analyses of the extracts

The results of the combined GC analysis are presented in Tables 2 and 3. The primary compound group of the Angelica extract (AA) consisted of coumarins, comprising over 40% of the extract. The most prominent identified coumarins were oxypeucedanin (4.37% of AA extract), imperatorin (2.41%) and osthole (2.07%).[43-44] In addition to the identified coumarins, AA contained several coumarin compounds whose exact structure could not be determined (over 30%). The free fatty acid content in the AA was low (1.02%). Hydrolysis of the extract revealed a high content of esterified fatty acids (15.58%). The major esterified fatty acids were 18:2 and 16:0 fatty acids (11.26 and 2.20%, respectively). Furthermore, dozens of compounds belonging to mono- and triterpenes/terpenoids, cyclic lactones, fatty alcohols, and sterols were

identified, but all these individual compounds were present in less than 1% in the AA extract. The presence of these components has been reported in earlier studies.[45-48]

The main compound groups in the Marsh Labrador Tea extract (MLT) were sesquiterpenes/terpenoids (18.10%) and sterols (16.19% in free form). The sesquiterpenoids palustrol (7.21%) and ledol (5.18%) were the major components of the MLT extract, and their presence has been reported in earlier studies.[49-50] The main identified sterols were sitosterol and lupeol (both 1.49% as free form and <1% as bound). Lupeol has also been previously identified.[51] Several sterols were left unidentified, comprising a total of 10.14% of the MLT extract. Long-chain alkanes comprised 9.27% of the extract, with C31 and C29 being the main alkanes (4.81 and 2.81%, respectively). Fatty acids, hydroxy fatty acids, and fatty alcohols were identified, but only the content of free 24:0 OH fatty alcohol exceeded 1% (1.83% in free form and 2.09% in total after hydrolysis). In addition, mono-, di-, and triterpenes/terpenoids were identified, but their combined content was low (altogether 3.38%).

Table 2. Composition of air-dried Angelica root $scCO_2$ extract (AA) in percentages.

Monoterpenenes monoterpenoids	**Underivatized**
α-Thujene	trace
α-Pinene	0.05
Camphene	trace
Sabinene	0.03
β-Pinene	0.01
Myrcene	0.03
α-Phellandrene	0.05
3-Carene	0.05
p-Cymene	0.05
β-Phellandrene	0.13
Limonene (syn. Dipentene)	0.04
β-Ocimene (*cis*)	trace
β-Ocimene (*trans*)	0.01
γ-Terpinene	trace
Sabinene hydrate	trace
Terpinolene	trace
Verbenol	0.03

Borneol	0.01
Cryptone	0.03
p-Cymen-8-ol	0.01
Bornyl acetate	0.08
Verbenyl acetate	0.09
Piperitol acetate (*cis*)	0.08
Unknown monoterpenoids	0.56
	1.35

Sesquiterpenes and sesquiterpenoids	**Underivatized**
α-Copaene	0.15
β-Elemene	0.01
β-Cedrene	0.05
β-Caryophyllene	0.02
β-Copaene	0.03
β-Barbatene	0.07
α-Muurolene	0.08
β-Bisabolene	0.10
β-Sesquiphellandrene	0.03
α-Copaen-11-ol	0.03
Elemol	0.03
Germacrene B	0.03
Unknown sesquiterpenoids	0.35
	0.98

Cyclic lactones	**Underivatized**
Tridecanolide	0.07
12-methyl-tridecanolide	0.02
Pentadecanolide	0.20
Heptadecanolide	0.08
	0.37

Fatty acids	**Silylated**	**Hydr. + Sil.**
14:0 fatty acid		0.07
15:0 fatty acid	0.02	0.22
15:1 fatty acid		0.07
16:0 fatty acid	0.27	2.47
17:0 fatty acid		0.10
18:2 fatty acid	0.48	11.74
18:1 fatty acid	0.04	0.39
18:1 (11) fatty acid		0.14
18:0 fatty acid	0.09	0.22
20:0 fatty acid	0.04	0.17
21:0 fatty acid		0.03
22:1 fatty acid (Erucic acid)		0.10
22:0 fatty acid		0.33

23:0 fatty acid		0.10
24:0 fatty acid		0.24
25:0 fatty acid		0.04
C16:0-mono glyceride	0.08	0.13
C18:0-mono glyceride		0.05
	1.02	**16.60**

Fatty alcohols	**Silylated**	**Hydr. + Sil.**
C15:0 OH	0.02	0.06
C16:0 OH	0.02	0.05
C17:0 OH	0.11	0.07
C18:0 OH	0.02	0.03
	0.18	**0.21**

Sterols	**Silylated**	**Hydr. + Sil.**
Campesterol		0.05
Stigmasterol	0.19	0.26
Sitosterol	0.51	1.16
Cycloartenol		0.15
Methylene cycloartanol		0.07
Citrostadienol		0.03
	0.70	**1.72**

Coumarins	**Silylated + underivatized***
Isosoralen (syn. Angelicin)	0.50
Psoralen (syn. Ficusin)	0.07
Isobergapten	0.09
Bergapten	0.21
Ostohole	2.07
Imperatorin	2.41
Isopimpinellin	0.05
Oxypeucedanin	4.37
Unidentified underivatized coumarins	11.08
Unidentified silylated coumarins	20.14
	40.98

Sum	**45.58**	**62.21**

trace: composition less than 0.01%, *combined data from silylated and underivatized samples

Table 3. Composition of air-dried Marsh Labrador Tea $scCO_2$ extract (MLT) in percentages.

Monoterpenenes monoterpenoids	**Underivatized**
Camphene	0.01

β-Pinene	trace
Myrcene	0.01
3-Carene	trace
p-Cymene	0.01
α-Phellandrene	trace
β-Phellandrene	trace
Limonene (syn. Dipentene)	trace
Ipsidienol	0.01
Borneol	0.01
Citronellol	0.02
Geraniol	0.02
Citronellyl acetate	0.01
Geranyl acetate	0.04
Unidentified monoterpenoids	2.98
	3.13

Sesquiterpenes and sesquiterpenoids	**Underivatized**
Aromadendrene	0.42
Ledol	5.18
Viridiflorol	0.15
Palustrol	7.21
Cyclocolorenone	1.27
Unidentified sesquiterpenes	3.88
	18.10

Other volatiles	**Underivatized**
Phytol (diterpene)	0.23
Squalene (triterpenoid)	0.02
	0.25

Fatty acids	**Silylated**	**Hydr. + Sil.**
14:0 fatty acid	0.07	0.14
15:0 fatty acid	0.01	0.03
16:0 fatty acid	0.26	2.24
17:0 fatty acid	trace	0.03
18:2 fatty acid	0.08	0.53
18:1 fatty acid	0.06	0.32
18:0 fatty acid	0.04	0.28
19:0 fatty acid	0.01	0.02
20:1 fatty acid	0.01	0.06
20:0 fatty acid	0.06	0.68
22:0 fatty acid	0.03	0.07
23:0 fatty acid	0.01	0.06
24:0 fatty acid	0.13	0.41
26:0 fatty acid	0.05	0.12
28:0 fatty acid	0.14	0.26

34:0 fatty acid	0.43	0.70
36:0 fatty acid	0.27	0.47
	1.64	**6.41**

Hydroxy fatty acids	**Silylated**	**Hydr. + Sil.**
16-OH-16:0 fatty acid	0.02	0.13

Fatty alcohols	**Silylated**	**Hydr. + Sil.**
16:0 OH	0.02	trace
18:0 OH	trace	0.03
20:0 OH	0.01	0.04
22:0 OH	0.14	0.52
23:0 OH	0.06	0.04
24:0 OH	1.83	3.92
25:0 OH	0.09	0.10
26:0 OH	0.66	1.01
27:0-OH	0.03	0.04
29:0-OH	0.13	0.10
34:0-OH	0.08	0.08
10-nonacosanol (C29:10-OH)	0.05	0.04
10-hentriacontanol (C31:10-OH)	0.93	0.93
	4.05	**6.84**

Alkanes	**Silylated**	**Hydr. + Sil.**
C23 alkane	0.02	0.02
C25 alkane	0.02	0.02
C27 alkane	0.10	0.10
C28 alkane	0.04	0.04
C29 alkane	2.81	2.81
C30 alkane	0.15	0.15
C31 alkane	4.81	4.81
C32 alkane	0.07	0.07
C33 alkane	1.25	1.25
	9.27	**9.27**

Sterols	**Silylated**	**Hydr. + Sil.**
Sitosterol	1.49	1.93
β -Amyrin	1.06	1.56
Lupeol	1.49	2.35
Olean-12-ene-3,28-diol	1.08	1.27
Urs-12-ene-3,28-diol	0.93	0.96
Other unidentified sterols	10.14	6.21
	16.19	**14.28**

Other unidentified	**Silylated**	**Hydr. + Sil.**
	5.98	3.82

Sum	**58.63**	**62.24**

trace: composition less than 0.01%.

Optical microscopy analysis of the microcapsules

Based on optical microscopy studies, the AAC microcapsule size range was 1.1–30 μm in diameter, while the MLTC microcapsule size range was 1.0–7.7 μm in diameter. The AAC microcapsules exhibited a larger size range than MLTC microcapsules, as visualized in Figure 1. This is likely one reason why AAC microcapsules settled at the bottom of the formed mixture during the production process, while MLTC microcapsules formed on the surface of the liquid solution. The commonly reported encapsulation efficacy (EE%) is often determined using GC-FID for single pure substances such as vanillin or limonene, as outlined by Sharkawy et al.[31] However, due to the diverse array of compounds present in the $scCO_2$ extracts, seen in Tables 2-3, the GC-FID method was not applicable in this study. It is highly probable that the ratio between the concentrations of the different compounds would not remain constant during the microencapsulation process.

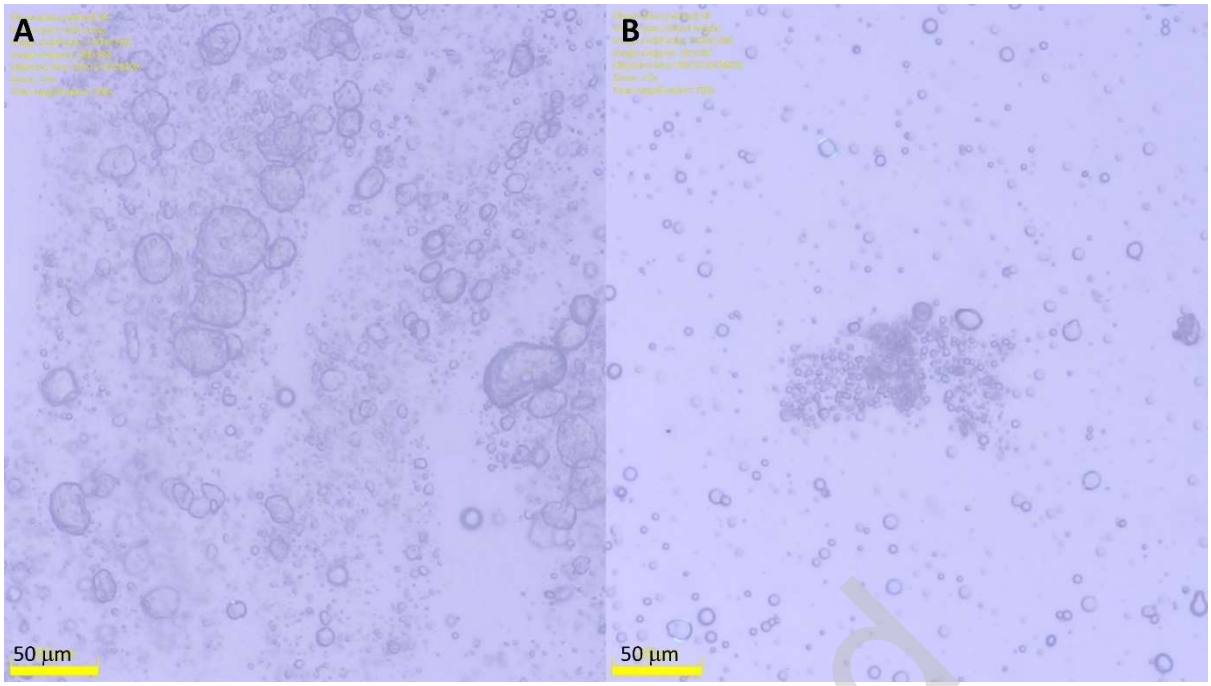

Figure 1. Optical microscopy images of the A) AAC and B) MLTC microcapsules.

FESEM analyses of the microencapsulated textiles

The FESEM images of the untreated linen-cotton jacquard textile material, as well as AAC and MLTC textiles produced in citric acid and in water solution, illustrate the external morphology of the microcapsules and their attachment to textile fibers (Figure 2). The AAC and MLTC textiles, prepared in a water bath without citric acid and subsequently washed multiple times with water, displayed identical FESEM images to those where the microcapsules were attached to the textile fibers with citric acid. Consequently, these images do not allow for an estimation of the interactions between the microcapsules and the fibers.

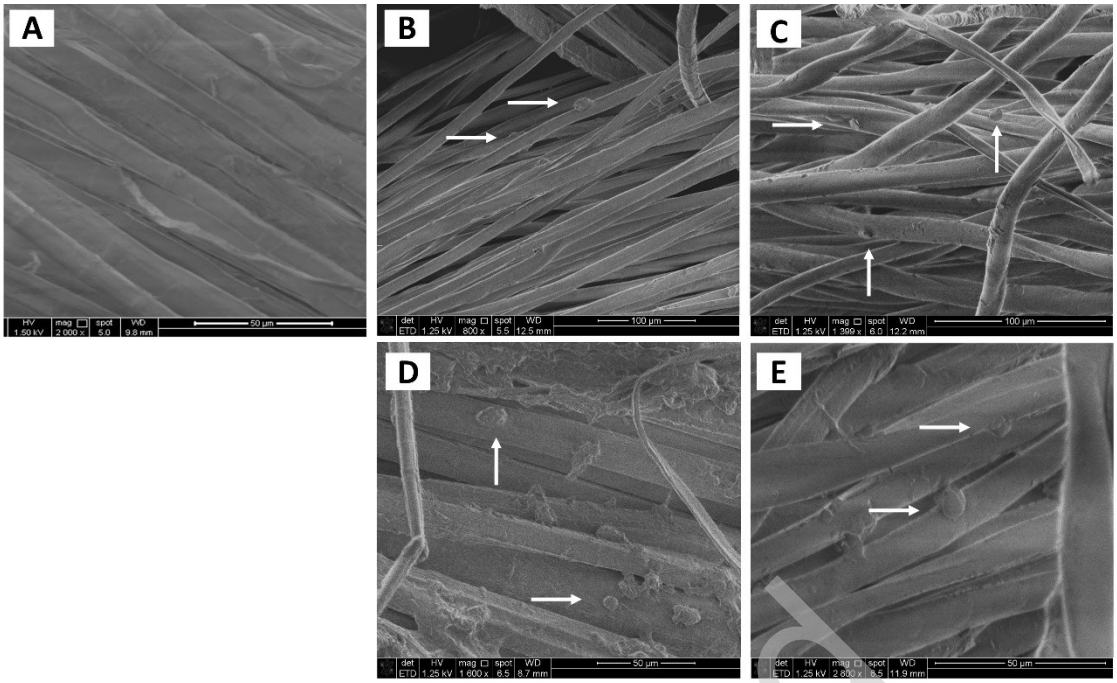

Figure 2. FESEM images of the jacquard linen-cotton textile A) without the microencapsulation treatment, B) with AAC, and C) with MLTC impregnation treatments in citric acid, D) with AAC, and E) with MLTC impregnation treatments in water.

FTIR-ATR spectroscopy

The bonding of the microcapsules and the jacquard textile material, with and without a citric acid linker, was studied using FTIR-ATR. As seen in Figures 3 and 4, all samples, including AAC and MLTC microcapsules, untreated textiles, and AAC and MLTC textiles microencapsulated in a citric acid solution and in water (without citric acid), exhibited a broad band at approximately 3,300 cm^{-1}. This band was attributed to O-H stretching vibrations for chitosan and cellulose, overlapping with the N-H stretching vibration of chitosan.[24,31] In addition, the cellulose and chitosan hydrocarbon stretching vibrations $\nu_{as}(CH_3)$, $\nu_{as}(CH_2)$, and $\nu_s(CH_3)$ were observed at approximately 2,918 cm^{-1}, 2,895 cm^{-1}, and 2,851 cm^{-1}, respectively, in all spectra. A weak C=O stretching vibration signal, likely indicating the presence of trace oil extract on the surface of both AAC and MLTC microcapsules, was identified in the region

of aliphatic carboxylic acids at 1,734 cm^{-1}.[52] This peak was also present in the spectra of the original source extracts, AA and MLT. Characteristic peaks of chitosan were identified in the spectra of microcapsules and microencapsulated textiles at 1,624 cm^{-1} (C=O stretching, Amide I), 1,560 cm^{-1} (N-H bending, Amide II), and 1,458 cm^{-1} (C-N-H bending, Amide III).[53] Several characteristic weak peaks of cellulose in untreated and microencapsulated textile materials were found in the region of 1,680 – 900 cm^{-1}, such as at 1,420, 1,364, 1,339, 1,024, and 893 cm^{-1} attributed to stretching and bending vibrations of the CH_3 and CH_2, and C-O bonds.[54] Notably, new peaks in the spectra of both AAC and MLTC textiles produced in a citric acid were observed at 1,719 cm^{-1} and at 1,618 cm^{-1}. The presence of the former peak and its intensity indicated covalent bonding, specifically esterification of the carboxylic group of citric acid and the OH group of cellulose.[31] This peak was not found in the spectrum of the untreated control fabric nor the citric acid itself. However, very weak absorptions at 1,719 cm^{-1} were identified in the spectra of AAC and MLTC textiles produced in a water bath without citric acid. As similar absorptions with very weak intensity were also found in the spectra of AAC and MLTC microcapsules, they may be attributed to traces of esterified acetic acid from the microcapsule production process. The peak at 1,618 cm^{-1} was attributed to the reaction between the NH_2 group of the chitosan microcapsule shell and the citric acid COOH group.[31] These findings in AAC and MLTC textiles suggest covalent bonding between the microcapsules and the textile surface via citric acid.

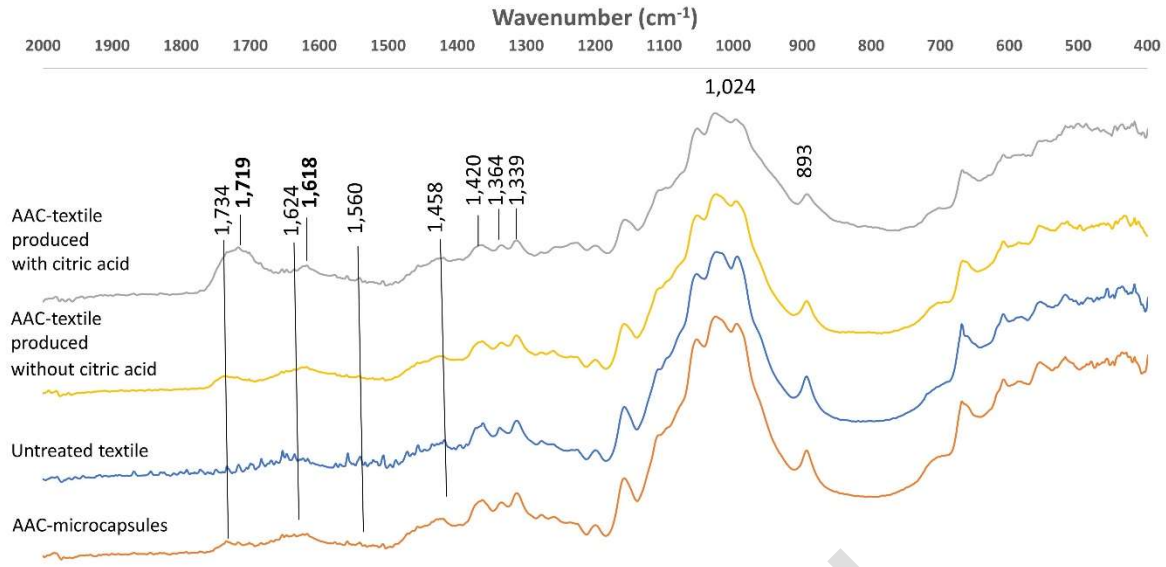

Figure 3. The FTIR-ATR spectra of untreated linen-cotton textile, AAC microcapsules, and AAC textiles produced with and without citric acid.

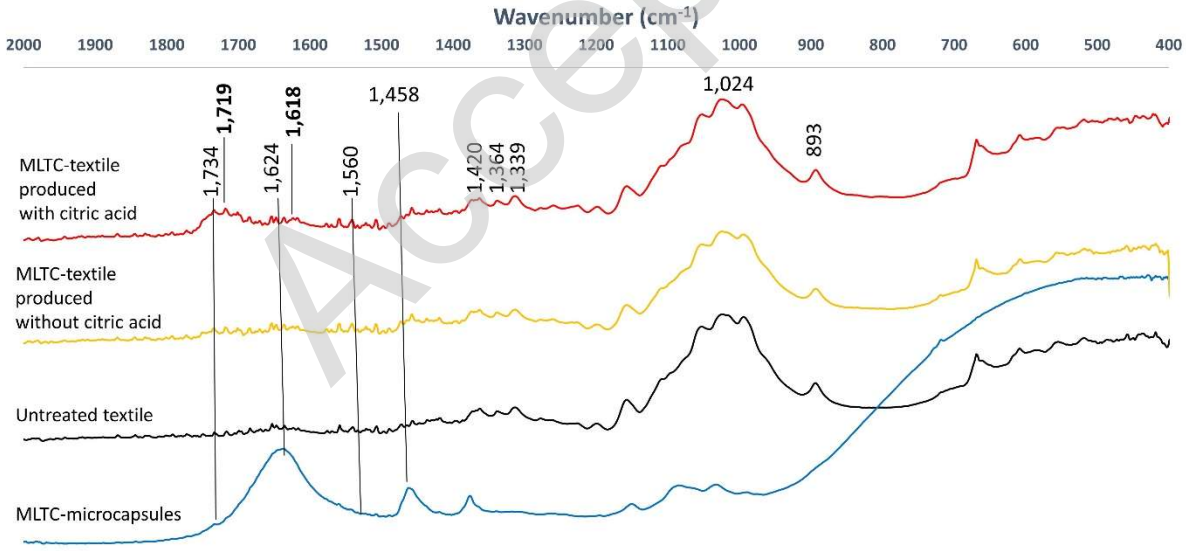

Figure 4. The FTIR-ATR spectra of untreated linen-cotton textile, MLTC microcapsules, and MLTC textiles produced with and without citric acid.

Antibacterial assays

The antibacterial activities of *A. archangelica* plant extract (AA), microcapsulated plant extract (AAC microcapsules) and treated linen-cotton jacquard textiles (AAC textiles) are illustrated in Figure 5. The extract exhibited no antibacterial activity against the *E. coli* strain but displayed high activity (82% inhibition) against the *S. aureus* strain. Chitosan, utilized in the microencapsulation process, demonstrated moderate antibacterial activity against the *E. coli* strain used, and this activity likely contributed to the observed effects in AAC (Figure 5A). The relatively low activity witnessed in the linen-cotton jacquard textile without microcapsules (REF textile citric acid) is likely attributed to the pH change induced by citric acid treatment. While some antibacterial activity against *S. aureus* is lost in the microencapsulation process (AA 82%; AAC 41%), considering that chitosan itself exhibits 21% inhibition against the strain, AAC textiles demonstrated antibacterial behavior with both water attachment (63%) and citric acid attachment (40%) (Figure 5B). Notably, jacquard textiles with the microcapsules attached in a water bath exhibited higher antibacterial activity, possibly because the microcapsules were more reactive or readily available, as they likely did not attach to the textile surface via covalent bonding.

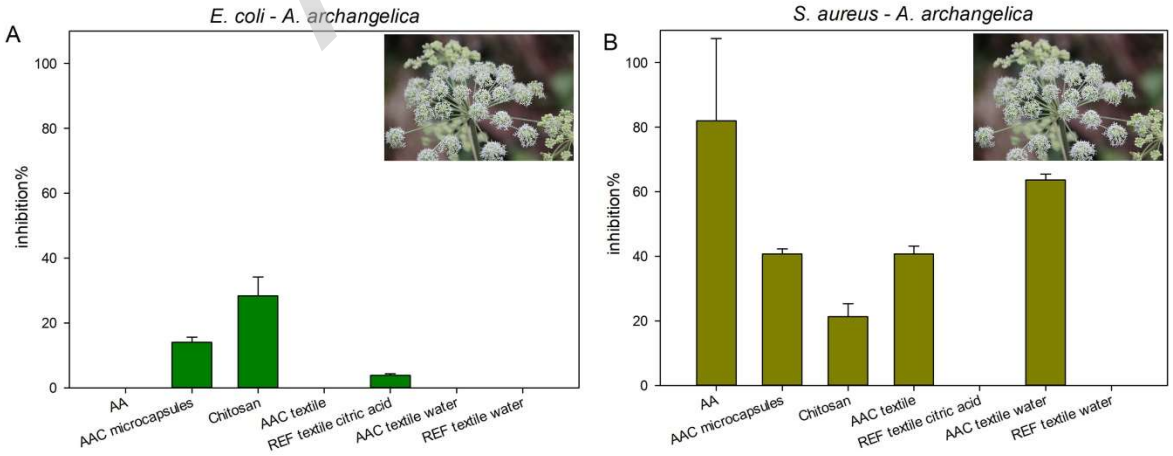

Figure 5. Antibacterial activity in inhibition-% of AA, AAC microcapsules, AAC textiles produced in citric acid and in water, and reference samples (chitosan, REF textile citric acid, REF textile water) against *E. coli* (A) and *S. aureus* (B) biosensors. Error bars represent the coefficient of variation between sample triplicates. Plant pictures: Pixabay.

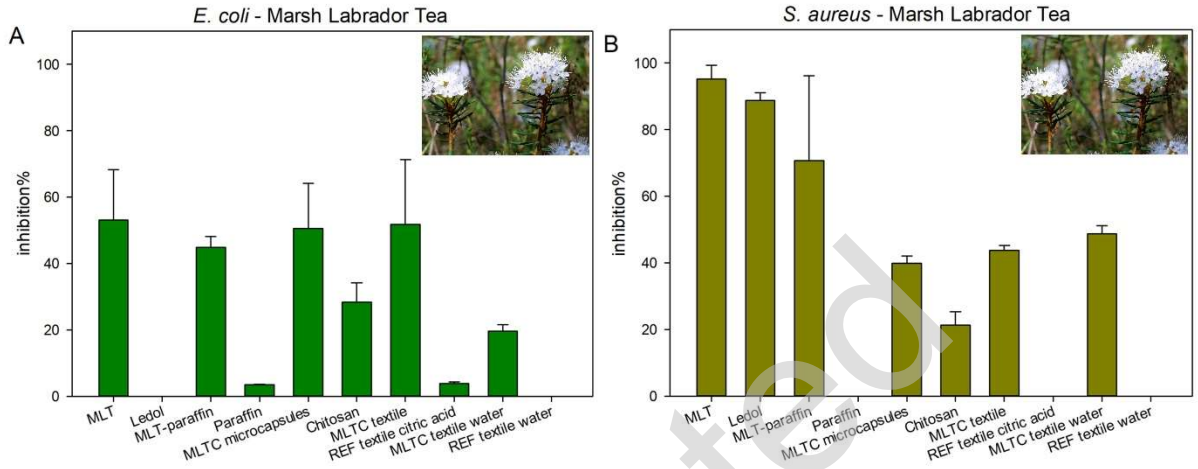

Figure 6. Antibacterial activity in inhibition-% of MLT, MLTC microcapsules, MLTC textiles produced in citric acid and in water, and reference samples (ledol, MLT-paraffin, paraffin, chitosan, REF textile citric acid, REF textile water) against *E. coli* (A) and *S. aureus* (B) biosensors. Error bars represent the coefficient of variation between sample triplicates. Plant pictures: Pixabay.

The antibacterial activities of MLT, MLTC microcapsules, and MLTC treated jacquard textiles are depicted in Figure 6. The extract demonstrated significant antibacterial efficacy against both strains, exhibiting 53% inhibition against *E. coli* and 95% against *S. aureus*. Ledol, a sesquiterpene present in the *R. tomentosum* essential oil and scCO$_2$ extract,[35] displayed notable activity against gram-positive *S. aureus* (89%), while no activity was detected against the gram-negative *E. coli* strain. MLT in paraffin exhibited nearly comparable activity to the extract, whereas paraffin itself exhibited minimal to no activity (3.8% inhibition against *E. coli* and 0% *S. aureus*). MLTC displayed mediocre activity (50.6% inhibition against *E. coli* and 39.9%

against *S. aureus*). Textiles with MLTC attached in citric acid also exhibited 3.8% inhibition without extract and 51.7% inhibition with extract against the *E. coli* strain, but showed no inhibition without extract and 43.8% inhibition with extract against *S. aureus*. Similar to the Angelica extract, textiles with microcapsules attached in a water bath demonstrated higher antibacterial activity against *S. aureus* (48.8% inhibition). However, for *E. coli*, those attached in citric acid were more active (19.7% inhibition with water), presumably due to the strain's pH sensitivity, as observed in the *E. coli* strain used, making it more responsive to decreases in pH levels. Because luminescence light production of the entire well is measured, even a 20% decrease is considered significant, indicating noteworthy antibacterial effects.

Some variation was observed between the linen and cotton fabric sides of the textiles, and the textile pieces tended to curl up at the corners when bacterial media were poured on them. The differences in bacterial activities on the two sides of the jacquard textile may be attributed to variation in the adherence of the microcapsules. Although errors appeared to be reasonably low, some variation in both Figures 5 and 6 is likely resulted from the challenging handling of the viscous substances. Achieving a constant amount of the sample in each 6-well plate well proved troublesome. Textile samples were weighed before placement on the plates, ranging from 60 mg to 118 mg in weight, while maintaining the size at approximately 1 cm × 1 cm. However, minor differences in size seemed to have little impact in results, with more significant variation stemming from factors such as the behavior of the textile sample in the plate (curling up) and the amount of the poured bacterial cultivation agar needed to cover the sample adequately.

The obtained antibacterial results of the extracts align with previous findings. In our prior article[35], we demonstrated that essential oils and laboratory scale supercritical carbon dioxide extracts from both *A. archangelica* and *R. tomentosum* were highly active against gram-positive *S. aureus*, while only *R. tomentosum* extract was active against the gram-negative

Pseudomonas aeruginosa strain. A similar observation was made in the essential oil of *A. archangelica* roots by Fraternale et al.[55], where growth inhibition was observed for gram-positive bacteria but not for gram-negative ones. This effect is likely explained by the lipopolysaccharide layer of gram-negative species' cell outer layer providing greater protection against antibacterial effects. The peptidoglycan outer layer of the gram-positive bacterial species' cells can absorb the antibacterial compounds more easily. We also demonstrate a clear difference between reference textiles (without extracts) and textiles with microcapsules. Therefore, it appears that the antibacterial features of the microencapsulated sample extracts are retained in the linen-cotton jacquard textiles.

Antifungal assays

The antifungal activity of AA and MLT was assessed using an agar well diffusion method[41-42], where the impact of the samples on the growth of test organisms was determined by measuring inhibition zones around a well containing the tested extract. According to the results (Table 4) obtained in three separate assays, MLT showed some influence on the growth of both *A. brasiliensis* and *C. albicans*, while a similar effect was not detected for AA. In the case of *C. albicans*, which was more susceptible to the amphotericin B antibiotic, the inhibition of AA dissolved in EtOH or paraffin was 63% and 24% of the inhibition recorded for the antibiotic, respectively. For *A. brasiliensis*, MLT affected the spore germination and fungal growth, as evidenced by a more than twofold increase in the inhibition area for both MLT-EtOH and paraffin solutions compared to amphotericin B. Consequently, only MLTC textiles were selected for antifungal activity testing, involving inoculating textiles with an *A. brasiliensis* spore suspension and subsequently inspecting the colony number of *A. brasiliensis* on agar plates. MLTC textiles attached in water exhibited comparable *A. brasiliensis* growth, albeit with considerable variation between technical replicates (Table 5). In the case of MLTC textile

crosslinked with citric acid, the colony numbers were lower than in the reference textile. It can be ruled out that this effect was caused by differences in the textile structure (resulting in variance in the spore count on the specimen's surface) or an ineffective neutralization solution, as no difference was observed between textiles in the colony numbers immediately after inoculation (time point 0 h; Table 5). According to our preliminary testing (data not shown), linen-cotton textiles with a lower amount of MLTC microcapsules did not exhibit antifungal activity. However, it remains uncertain whether the antifungal effect is attributed to the interaction of chitosan, citric acid, and MLT, as the antifungal activity of chitosan and citric acid without MLT was not determined.

Table 4. The effect of Angelica (AA) and Marsh Labrador Tea (MLT) extracts on the growth of *Aspergillus brasiliensis* (ATCC® 16404™) and *Candida albicans* (ATCC® 10231™).

	C. albicans		*A. brasiliensis*	
	Inhibition area (mean ± SD)[1]	Inhibition-% (mean ± SD)[2]	Inhibition area (mean ± SD)[1]	Inhibition-% (mean ± SD)[2]
Amphotericin B[3]	1.5 ± 0.7		0.8 ± 0.7	
Paraffin[4]	0 ± 0	0 ± 0	0 ± 0	0 ± 0
AA	0 ± 0	0 ± 0	0 ± 0	0 ± 0
MLT	1.0 ± 0.5	63.0 ± 13.5	2.6 ± 1.9	300.5 ± 58.6[5]
MLT-paraffin	0.4 ± 0.2	23.9 ± 9.3	3.4 ± 2.3	339.9 ± 199.9[5]

[1] The area (cm^2) of the inhibition was determined with PLANIX 10S (mean and standard deviation)
[2] The inhibition percentage compared to amphotericin antibiotic
[3] 100 µl of antibiotic amphotericin (10 µg/ml) was used as a positive control
[4] The testing was repeated twice
[5] One testing of the inhibition percentage calculation was omitted, as amphotericin did not inhibit the growth of *A. brasiliensis*

Table 5. The effect of MLTC textiles produced in water and in citric acid on the colony number of *Aspergillus brasiliensis* (ATCC® 16404™).

	Time point 0 h[1]				Incubation 48 h[2]			
	Replicate				Replicate			
Textile[3]	1	2	3	Mean ± SD[4]	1	2	3	Mean ± SD[4]
REF textile water	23	27	78	43 ± 31	171	115	91	126 ± 41
REF textile citric acid	42	116	41	66 ± 43	165	150[5]	66	127 ± 53
MLTC textile water	54	37	53	48 ± 10	69	15	77	54 ± 34
MLTC textile	124	51	34	70 ± 48	15	0	1	5 ± 8

[1] *A. brasiliensis* colony number on three replicate plates after spores were subjected to the textile for a minute, neutralized, and the solution was diluted to 10^{-1} prior to plating
[2] *A. brasiliensis* colony number on three replicate plates after spores were subjected to the textile for a minute, the textile was incubated for 48 h at 30 °C, neutralized, and the solution was diluted to 10^{-4} prior to plating
[3] The textile specimens were ~1 cm × 1 cm in size

[4] The average and standard deviation (SD) of the *A. brasiliensis* colony numbers
[5] The colony number was beyond 150, but the exact number could not be determined as the uneven distribution of colonies on the plate

Previously, both Angelica and Marsh Labrador Tea essential oils and laboratory scale supercritical carbon dioxide extracts have been found to restrict the growth of yeast as well as filamentous fungi.[35,55] In the present study, no effect was found on the AA against the studied test organisms. This is in accordance with recent paper,[35] in which the essential oils of Angelica exhibited antifungal activity, whereas supercritical carbon dioxide extracts did not. On the contrary, the supercritical carbon dioxide extracts of MLT had increased antifungal activity against *C. albicans* (ATCC® 10231™), *Aspergillus niger*, *Cladosporium cladosporioides*, and *Penicillium venetum* compared to essential oil preparation.[35] Likewise, in the present study, MLT extract in paraffin was found to restrict the growth of the filamentous mold *A. brasiliensis* and to some extent the growth of the yeast *C. albicans*. We therefore proceeded to examine the antifungal efficiency of textiles microencapsulated with MLTC using *A. brasiliensis*. To our knowledge, there are no previous studies with MLTC coated textiles. Our initial results indicate that MLTC textile crosslinked with citric acid has the potential to prevent the spore germination and growth of *A. brasiliensis*, but further studies are needed to resolve the longevity of antifungal properties.

Antimicrobial mechanism

Plant secondary metabolites exhibit diverse mechanisms of antimicrobial action, encompassing the disruption of membrane function and structure, interference with DNA/RNA synthesis and function, modulation of intermediary metabolism, induction of cytoplasmic coagulation, and interruption of quorum sensing.[56] Drawing a correlation between mechanisms of resistance to antifungals and antibacterials, commonalities arise, such as the modification of enzymes targeted by antimicrobials and the involvement of membrane pumps in drug extrusion,

characteristics observed in both eukaryotic and prokaryotic cells.[57] Typically, the antimicrobial activity of plant extracts cannot be attributed to a single compound or a set of compounds, as numerous studies suggest that the synergistic interactions within the extract are likely responsible for the observed effects.[56]

The efficacy and mode of action of various plant metabolites are contingent upon factors such as the characteristics of target cells (bacterial/fungal, gram-positive/gram-negative) and the specific environmental conditions in which the antimicrobial activity is manifested. [56] Essential oils, for instance, exhibit heightened activity against gram-positive bacteria due to the presence of a peptidoglycan layer outside their outer membrane, while gram-negative bacteria possessing an outer membrane composed of a double layer of phospholipids connected to the inner membrane via lipopolysaccharides seems to offer enhanced protection.[58]

While the mechanisms of action for many compound groups remain incompletely understood, certain speculations have been made. Terpenes, for instance, are postulated to disrupt membranes due to their lipophilic nature.[59] On the other hand, polyphenols, according to various studies, bind to bacterial enzymes through hydrogen bonds, inducing modifications in cell membrane permeability and cell wall integrity.[60] Plant-derived compounds, including terpenes and polyphenols, are thought in general to act through diverse mechanisms, such as efflux pump inhibition, bacterial membrane destruction, biofilm formation inhibition, and interference with DNA and protein synthesis.[60]

Durability analyses of the MLTC textile after domestic washing and drying

FESEM & FTIR-ATR spectroscopy

The FESEM image of MLTC textile after six washing and drying cycles is presented in Figure 7. The FESEM image shows that the microcapsules are still attached to the textile fibers and

their spherical shape is the same as seen in Figure 2. In addition, the characteristic FTIR-ATR peaks of MLTC textile before and after 6 washing cycles at 1,719 cm^{-1} and at 1,618 cm^{-1} are shown in Figure 8. These results confirm the indication about the covalent bonding between the chitosan shell of microcapsules and the cellulose of textile fibers via citric acid and the microcapsules remain attached to the fabric after six washing cycles.

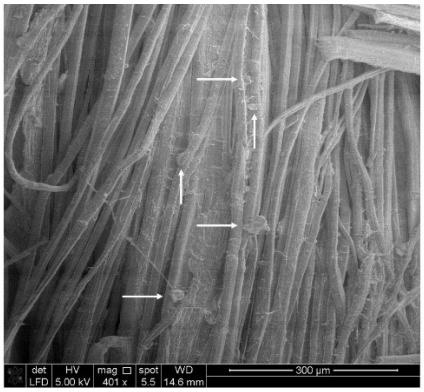

Figure 7. FESEM image of MLTC textile with microcapsules after six domestic washing and drying cycles.

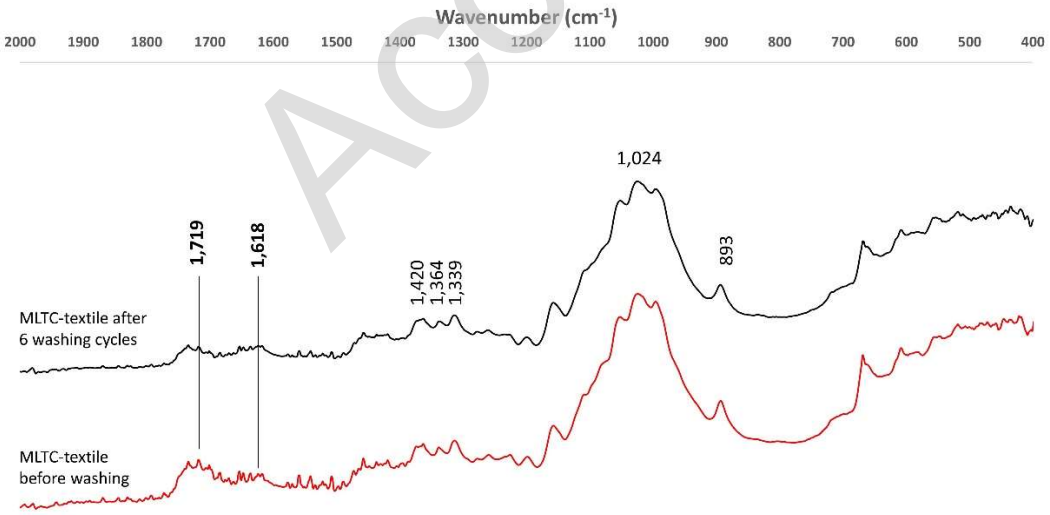

Figure 8. The FTIR-ATR spectra of MLTC textile before and after 6 washing cycles.

SPME-GC-MS analysis

The concentrations of major volatile compounds of Mars Labrador tea extract, palustrol and ledol (Table 3), in the MLTC textile samples were profiled using the SPME-GC-MS technique before and after six domestic washing cycles. The results are presented in Table 6.

Table 6. The concentrations of palustrol and ledol before and after six domestic washing cycles.

Sample	Palustrol (μg/L)	Ledol (μg/L)
MLTC textile before washing	35.6 ± 10.1 (100%)	22.7 ± 9.9 (100%)
MLTC textile after 6 washing cycles	0.127 ± 0.005 (0.4%)	0.060 ± 0.005 (0.3%)

The results show the decrease of the evaporation of palustrol and ledol from the microcapsules attached to the textile fiber surface after the domestic washing treatments. However, the other components shown in Table 3, were not studied.

Antibacterial assays

To establish that the functionalization of the MLTC textile can retain after washing, the antibacterial activities were analyzed before and after six subsequent domestic washing and drying cycles (Figure 9).

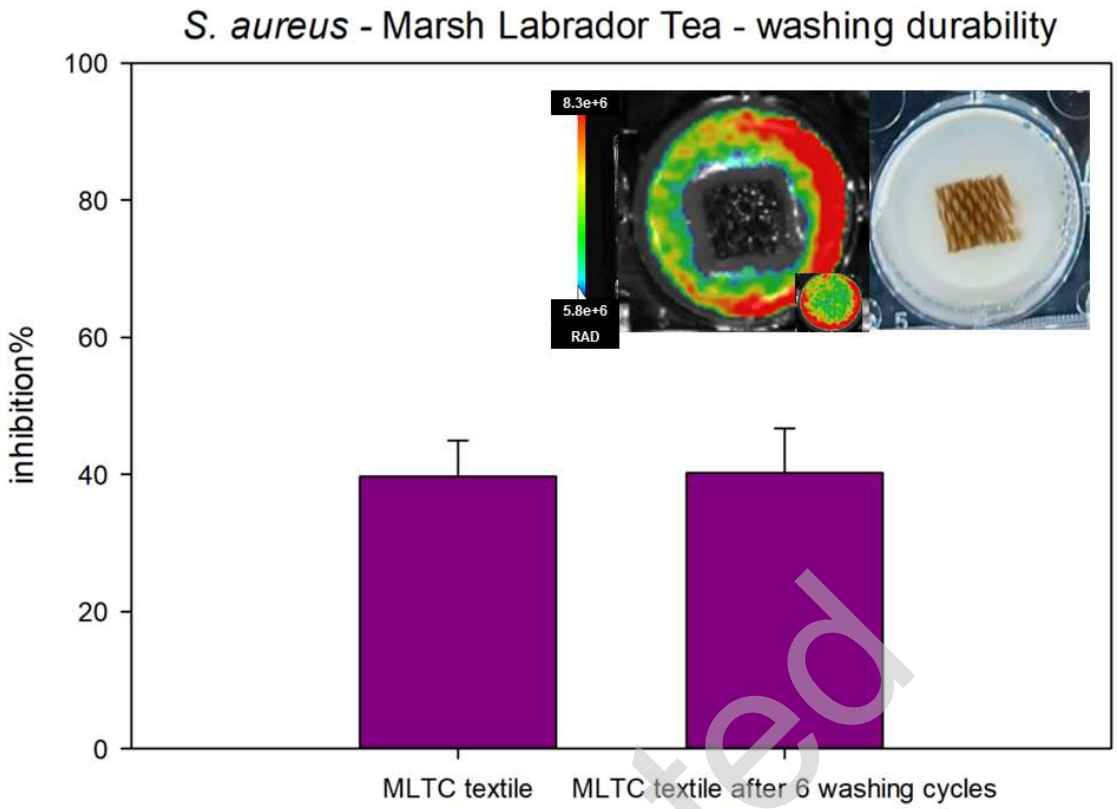

Figure 9. Antibacterial activity in inhibition-% of MLTC textiles against *S. aureus* biosensor before and after 6 domestic washing cycles. Error bars represent the coefficient of variation between sample triplicates. Pictures present the MLTC textile after 6 washing cycles luminescence (left, smaller picture in the low right shows the undisturbed bacterial control) and the normal photograph of the same textile (right). Clear luminescence light signal decrease round the sample is detected indicating antibacterial effects.

As seen in Figure 9 it is evident that the MLTC textile maintained its antimicrobial activity against the gram-positive *S. aureus* strain without significant differences between the textile before and after wash cycles. Similar test against *E. coli*, however, showed that one wash decreased the activity to half and after 6 washes no activity was no longer detected (data not shown). In this study, we showed that ledol was not active against *E. coli*, while having high activity (90%) against *S. aureus* (Figure 6) while GC-FID/MS analysis of the extracts (Table

3) indicated the presence of multiple other mono- and sesquiterpenoid structures. For example, purified palustrol fraction from Red Sea soft coral has been found to possess activity against multiple bacterial strains, including *S. aureus* and *E. coli*.[61] Guimarães et al. (2019)[62] tested various terpene and terpenoid structures from essential oils against bacteria and found that oxygenated terpene structures were especially showing activities against gram-negative bacteria. One could thus speculate that washing cycles reduced the amount of non-capsulated compounds from the textile surfaces or compounds released from broken microcapsules. This hypothesis is also supported by Table 6, which shows the reduction of volatile compounds, palustrol and ledol, after washing. However, the chitosan itself does not explain the retained activity shown in Figure 9 indicating that the intact microcapsules (Figure 7) must also contain other antibacterial compounds than palustrol and ledol found in the extract.

Conclusions

The eco-friendly production process of antimicrobial linen-cotton jacquard textiles included the 1) weaving of the textile material, 2) collection, pretreatments and pilot-scale $scCO_2$ extractions of the plant materials, Angelica and Marsh Labrador Tea, 3) the microencapsulation of the oil extract AA and wax extract MLT with chitosan by the coacervation method, and 4) the impregnation of the AAC and MLTC microcapsules onto the textile fibers via covalent bonding, with citric acid as a linker. The pilot-scale $scCO_2$ extraction method developed in this study was established as an effective green separation method, for fractionating bioactive oil and wax components from Angelica and Marsh Labrador Tea plant materials with desirable yields. The AA indicated high activity (82% inhibition) only against the *S. aureus* strain, whereas the MLT showed antibacterial activity against *E. coli* (53%) and *S. aureus* (95%) and suppressed the growth of both *A. brasiliensis* and *C. albicans*. The microencapsulation of the extracts and their attachment to the textile fibers were studied with FESEM images and FTIR-

ATR, which indicated the formation of covalent bonding between the chitosan shell of the microcapsules, citric acid, and cellulose of the AAC and MLTC textile materials. The AAC textiles proved antibacterial activity of 40% against *S. aureus*, and the MLTC textiles proved antibacterial activity of 43.8% against *S. aureus* and 51.7% against the *E. coli* strains. While chitosan itself indicated mild activity against both bacterial strains (28.3% inhibition against *E. coli* and 21.3% inhibition against *S. aureus*), it is evident that AA and MLT increased the antibacterial activities in the textiles in all cases except AAC textile against the gram-negative *E. coli* strain, in which no inhibition was detected. In addition, the results indicate that MLTC textile restricted the growth of *A. brasiliensis*.

The formation of covalent bonds between the microcapsules and the textile fibers via citric acid was confirmed by performing six domestic washing and drying cycles for MLTC textile. The FESEM images showed the attachment of the microcapsules on textile fibers and the antibacterial activity against the *S. aureus* retained after washing procedures.

In future studies, we will focus in more detail on the properties of the microencapsulated natural fiber textile materials for example, the stability of the antibacterial and antifungal activities, the volatile compounds evaporating through the chitosan shell of the microcapsules, and weather resistance of the textiles. Moreover, the industrial scale-up, business and utilization potentials, and the environmental impacts of the natural plant bio-oils will be studied.

Overall, we have successfully demonstrated the laboratory-scale production and antimicrobial effectivity of cotton-linen jacquard woven textile materials using plant-based $scCO_2$ extracts, which were microencapsulated with chitosan.

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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