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Wash-durability, surface, and antibacterial properties of wool fabric treated with nature-derived thymol and totarol under subcritical CO_2, aqueous, and ethanol media

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ABSTRACT

Traditional aqueous-based antibacterial treatments used for wool fabrics are not sustainable as they produce toxic effluent and therefore it is necessary to develop a zero-effluent treatment. In this work, wool fabrics were treated with two plant-derived non-toxic antibacterial agents, thymol and totarol, under subcritical carbon dioxide (subc-CO_2), and their antibacterial properties were measured. The wash-durability of the fabric treated with thymol under subc-CO_2 was compared with wool fabrics treated with thymol in aqueous and ethanol media. The change in the physicochemical properties of the wool fabric treated with thymol under subc-CO_2 at various concentrations was assessed by FTIR, contact angle measurement, and EDX spectroscopy. Thymol showed excellent antibacterial properties, but the wool fabric samples treated with totarol showed no antibacterial activity. The wool fabrics treated with thymol in the aqueous and ethanol media lost antibacterial activity after 5 times IWS 7A washes, but the fabric treated with thymol under subc-CO_2 showed excellent antibacterial activity even after 20 times IWS 7A washes (equivalent to 80 domestic washes) indicating its high durability to wash. The thymol treatment slightly improved the hydrophobicity of the treated fabric's surface as the contact angle at 240 s improved to 119° for the fabric treated with 5% thymol from 106° for the untreated control fabric.

1. Introduction

Textiles are known as the second skin as clothes are used to protect our bodies from the damaging effects of the surrounding environment. Our skin harbors verities of microbial communities and body sweat can transmit these microbes from human skin to clothes (Grice et al., 2009). As clothes are not sterile, they allow for harboring and growing bacteria on them (Callewaert et al., 2014). The types of pathogens and their counts on human skin can vary from one area of skin to others as human skin is heterogeneous (Byrd et al., 2018). It is assumed that natural fibers (especially protein fibers like wool fiber) are more easily affected by the microbiota than synthetic fibers due to the various nutrients present in natural fiber-made clothing and their ability to adsorb sweat components (Szostak-Kotowa, 2004). Wool fibers have exceptional characteristics, such as inherent warmth, wrinkle resistance, and fire retardancy (Hassan and Carr, 2019), and they are used for the manufacturing of next-to-skin apparel including undergarments. Untreated wool fibers show some level of bacterial resistance because of the presence of a polyethylene-like hydrophobic layer on their epicuticles (Caven et al., 2019), which does not allow the persistent deposition of bacteria on the fiber surface. However, wool keratin protein is a source of nitrogen for the growth of bacteria, and wool fibers have high moisture regain. Therefore, bacteria can grow on them compromising the health of wearers of wool garments and affecting the longevity and usability of wool fibers (Abu-Rous and Liftinger, 2019).

To hinder harboring bacteria on wool fabrics, they are frequently treated with several types of leaching and non-leaching antibacterial agents. Of the leaching type of antibacterial agents, quaternary ammonium compounds (Ran and Gao, 2011; Hassan and Sunderland, 2015), quaternized chitosan (Tian et al., 2015), metallic nanoparticles (Hassan, 2017; Hassan, 2019; Johnston and Nilsson, 2012), natural essential oils (Lee et al., 2009), and natural polyphenols and flavonoids from green tea (Tang et al., 2010), turmeric (Shahmoradi Ghaheh et al., 2014), polyphenols of feijoa skin and mango seed kernel (Hassan, 2021), and

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gallo-tannin of Chinese sumac gall (Hassan and Saifullah, 2021), have been studied to inhibit bacterial growth on wool textiles and also to protect human health. However, the treatment with many of these natural polyphenols may affect the inherent color of the treated fabrics. Quaternary ammonium compounds are quite popular in the textile industry as they are highly effective as an antibacterial agent and do not affect the color of the treated fabric (Hassan, 2014; Hassan, 2015). The antibacterial agents without fiber-reactive groups are difficult to bind to wool fiber and therefore they may leach to water during laundering causing a gradual loss of antibacterial activity. Many of the synthetic antibacterial agents used in the textile industry are quite toxic and produce toxic effluent. For example, quaternary ammonium compounds have an LC_{50} value of less than 1 mg/L (Di Nica et al., 2017; Lavorgna et al., 2016), which confirms their high toxicity. Published literature shows that quaternary ammonium compounds can cause reproductive and developmental problems in animals, and also can disrupt key cellular processes (Melin et al., 2014; Maher, 2008).

Of the non-leaching type antibacterial agents, fiber-reactive quaternary ammonium compounds and polymers are prominent (Hassan, 2014; Hassan, 2015), but they are relatively expensive. The nanoparticles of silver, gold, and copper are also found highly effective against various types of bacteria (Gonçalves et al., 2022; Hassan, 2019; Johnston and Nilsson, 2012), but their application also changes the color of the fabric. Moreover, there is a risk of the liberation of not only the metallic ions from nanoparticles but the whole nanoparticles may release from the treated fabrics during washing potentially contaminating watercourses (Mitrano et al., 2016; Lorenz et al., 2012). Wool fabric coated with nano TiO_2 and ZnO exhibits strong antibacterial and self-cleaning properties (Behzadnia et al., 2015; Montazer and Seifollahzadeh, 2011), but under visible light and solar irradiation, nano TiO_2 and ZnO can cause severe photocatalytic degradations of fibers with a considerable loss of fabric strength (Tang et al., 2021). Not only metallic nanoparticles, but most of these antibacterial agents also have some level of toxicity and can end up in the washing effluent. In this respect, natural antibacterial agents are preferable to synthetic antibacterial agents as they are generally recognized as safe. Natural mono and diterpene phenols, such as totarol and 2-isopropyl-5-methylphenol, are attractive alternatives to toxic synthetic antimicrobial agents as they are green and plant-derived.

Totarol, a golden yellow-colored naturally produced diterpene, is known to have good antibacterial activity (Shi et al., 2018), but has never been studied for the antibacterial treatment of textiles. It is usually extracted from the heartwood of *Podocarpus totara*, a coniferous tree found in New Zealand but also can be extracted from other plants (e.g., *Podocarpus affinis*, *Podocarpus rumphii*, *Biota orienetalis*, etc.). On the other hand, 2-isopropyl-5-methylphenol, a natural monoterpene phenol can be extracted from *Thymus vulgaris* (thyme), ajwain, and various other plants, and its antibacterial activity is already known (Wang et al., 2022; Marchese et al., 2016). The aqueous antibacterial treatment of textiles with thymol produced textiles with poor durability of the treatment to washing, but also produces effluent containing thymol. To enhance the wash durability, thymol was encapsulated with β-cyclodextrin and bonded to cotton fibers by an acrylic binder, which improved the durability only to 10 washes (Türkoğlu et al., 2022). The polymeric grafting with β-cyclodextrin and plasma treatment of cotton fabrics further enhanced the durability to washing of the thymol-treated fabrics (Shahidi et al., 2014; Rukmani and Sundrarajan, 2012). However, such kind treatments are costly, difficult for industrial applications, and also produce effluent containing the applied antibacterial agents. The release of antibacterial agents into the environment may negatively affect the population of aquatic microorganisms and fishes and may play a role in the evolution of antibacterial resistance (Larson and Flach, 2022; Kümmerer, 2003). Moreover, all of these antibacterial agents have some level of toxicity and their aqueous treatment produces effluent containing antibacterial agents.

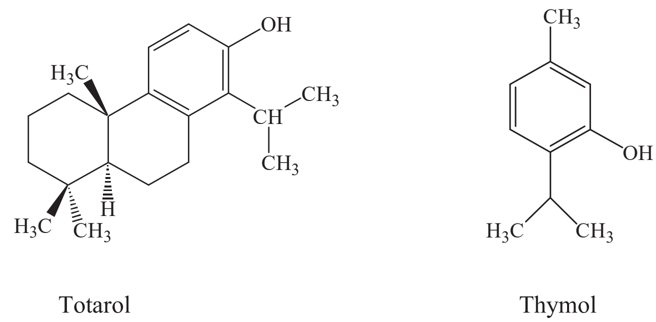


Fig. 1. Chemical structure of totarol and thymol.

Supercritical carbon dioxide (sc-CO_2)-based zero-effluent treatments are considered sustainable and eco-friendly as CO_2 is a non-toxic and non-flammable gas. The sc-CO_2 is extensively studied as a medium for the dyeing of textiles with disperse and reactive classes of dyes (Penthala et al., 2022; Gong et al., 2021). The production of an antibacterial cotton gauge, corona-treated polypropylene non-woven fabric, and cellulose acetate film with thymol in the supercritical CO_2 (sc-CO_2) at 20–35 MPa are reported (Milovanovic et al., 2016; Milovanovic et al., 2013), but the durability of the treatment to washing was not assessed as the treated materials are for single use. Although the supercritical point of CO_2 is 8 MPa pressure and 40 °C, sc-CO_2 treatments are mostly carried out at 20–30 MPa pressure making the treatment machinery unsafe and highly costly to manufacture. If the treatment is carried out under sub-critical conditions at 5–6 MPa, the treatment machinery becomes less risky and the manufacturing cost of such kind of machinery becomes low (Laboureur et al., 2015). A recent published article reported a sub-critical CO_2 (subc-CO_2)-based insect-resist treatment of wool fabric with permethrin that considerably improved the durability of the treatment to washing compared to the aqueous emulsion-based treatment (Hassan and Brorens, 2023). In this work, for the first time, we are reporting the development of antibacterial wool fabric by treating it with thymol under subcritical CO_2 (subc-CO_2). The wash-durability of the antibacterial finish of the wool fabric treated with thymol under subc-CO_2 was compared to the wool fabrics treated with thymol and totarol in aqueous and ethanol media. In subc-CO_2-based treatment, considerably lower pressure is used compared to the sc-CO_2 medium, which improves the safety of the process.

2. Experimental methods

2.1. Materials

A plain-woven wool fabric of 136 g/m^2 (29 ends/cm and 25 picks/cm) made from untreated merino wool fibers (not shrink-resist treated) with an average diameter of 19 μm was used throughout this work. The wool fabric was treated with 2 g/L Sandoclean PC (a detergent) and 0.2 g/L Sandozin MRN (a wetting agent) at 60 °C for 20 min to remove any dirt and spinning oil present in the fabric before conducting the treatment with thymol and totarol in subc-CO_2. Of the antibacterial agents studied in this work, thymol was purchased from Sigma-Aldrich Limited (USA) and Totarol, extracted from the *Podocarpus totara* plant was supplied by Essentially New Zealand Limited (Auckland, New Zealand). Their chemical structure is shown in Fig. 1. Arkema Chemicals (Switzerland) supplied the Sandozin MRN and Sandoclean PC. The surfactant, Termul 5370 (HLB = 14.5) was supplied by Huntsman Chemicals (USA).

2.2. Treatment of wool fabric with thymol and totarol in subc-CO_2

A 50 ml pressure vessel (Polaron Critical Point Dryer, Quorum Technologies Limited, UK) made of brass and chrome with a glass window was used to treat the wool fabric with thymol and totarol. The

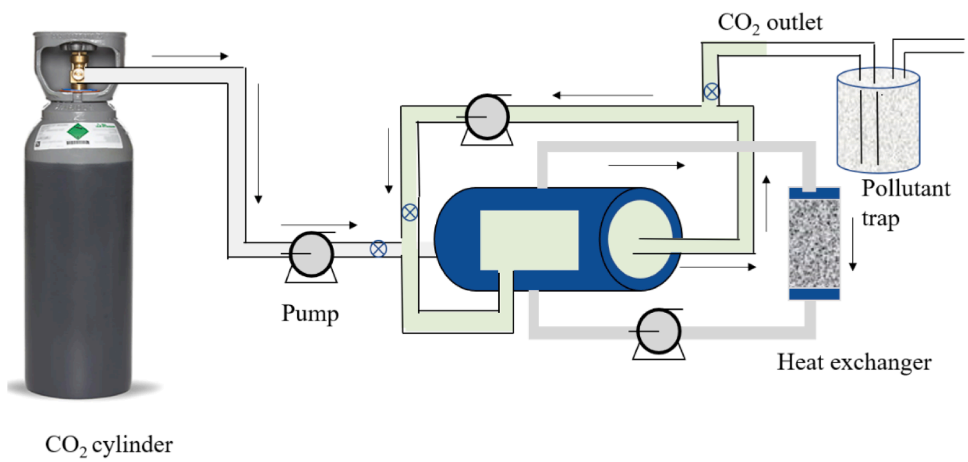


Fig. 2. The set-up used for the subc-CO_2 treatment of wool fabric with totarol and thymol.

schematic diagram of the subc-CO_2 treatment system used for the wool fabric is shown in Fig. 2. The wool fabric was wrapped on a perforated steel cylinder, and it was placed inside the vessel. The required quantity of thymol/totarol was taken in a small stainless-steel boat, and it was placed inside the perforated steel cylinder inside the pressure vessel. The vessel was filled with liquid CO_2 at the desired pressure and the desired temperature (°C) was maintained by circulating warm water through the outer shell of the vessel as shown in Fig. 2. The liquid CO_2 was recirculated through the vessel by a high-pressure pump to have some level of liquor movement through the wool fabric to achieve uniform treatment. The concentration of thymol and the vessel pressure and temperature were varied to acheive the optimum thymol absorption by wool fabric. The pressure and temperature were adjusted to 6 MPa and 50 °C respectively, and impregnation was continued for up to 3 h, which we found the optimum conditions for the treatment of wool fabric with thymol and totarol under subc-CO_2. After the completion of treatment, the pressure of the vessel was released, the treated fabric was recovered, and the vessel was cleaned for the next treatment.

2.3. Treatment of wool fabric with thymol in aqueous and ethanol media

The durability of the wool fabric treated with thymol under subc-CO_2 to washing was compared with wool fabrics treated with thymol in aqueous and ethanol media. For this purpose, a nano-emulsion of thymol was prepared according to published literature, but the thymol was initially dissolved in ethanol (Li et al., 2017). In brief, 5 g thymol was dissolved in 10 ml ethanol, 1 g of Termul 5370 was added to it, and then slowly they were mixed with stirring. Then 90 ml water was added to it under high-speed stirring that produced a milky thymol nano-emulsion. The treatment of wool fabric with this thymol emulsion was carried out in an Ahiba laboratory dyeing machine (Model: Turbomat 1000, Datacolor International, Switzerland). The bath was prepared with water and the required amount of thymol emulsion and was run for 60 min at 30 °C. After completion of the treatment, the liquor was drained, the fabric was squeezed to remove extra liquor, and dried at room temperature. Wool fabric was also treated with thymol (5% owf) in ethanol in a 3-neck beaker fitted with a condenser at the top at 60 °C for 120 min with magnetic stirring. The sample was then removed and dried under a fume hood.

2.4. Assessment of antibacterial activity

The assessment of antibacterial activity of the thymol-treated wool fabric against Gram-positive *Staphylococcus aureus* and Gram-negative *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* bacteria was carried according to the *AATCC Test Method 147–1998: Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method*. 1 ml of 24 h broth culture of bacteria was added to 9 ml of sterile distilled water and one loopful of that inoculum was transferred to the surface of the sterile agar plate and 5 streaks of 60 mm long were made approximately covering the central area of a standard Petri dish. Each streak was 10 mm apart from the other streaks. A wool fabric sample of 25 mm × 50 mm dimension was placed transversely across the inoculum streaks on each agar plate so that the samples are in contact with the inoculum streaks. They were then placed inside an incubator at 37 ± 2 °C for 24 h. After which, the agar plates were removed from the incubator and the zone of inhibition beyond its edge and the interruption of bacterial growth along the streaks of inoculum under the specimens were examined. No growth of bacteria under the test sample indicates antibacterial activity and the zone of inhibition indicates the leaching of the antibacterial agent from the test samples to the inoculum.

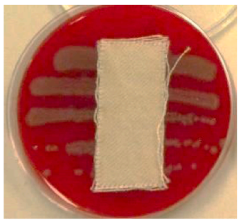
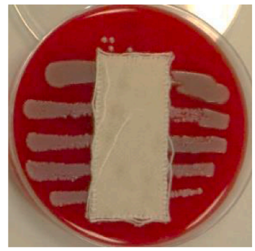
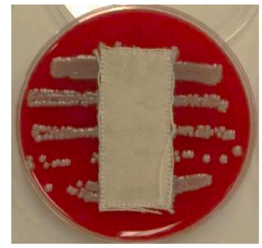
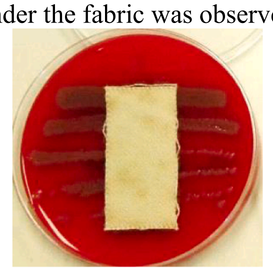
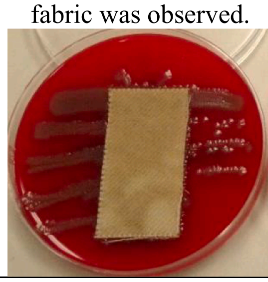
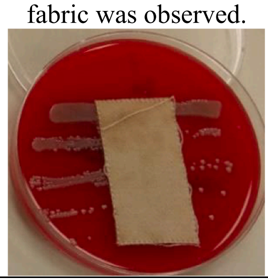
2.5. Surface characterizations

The surface of the thymol-treated fabric was characterized by Fourier transform infrared spectroscopy, contact angle measurement, energy-dispersive X-ray, and scanning electron microscopy. The presence of thymol was identified by the Fourier transform infrared (FTIR) spectroscopy by recording IR spectra on a Nicolet Summit Pro FTIR spectrometer (Thermo Fisher Scientific, USA) equipped with an attenuated total reflectance (ATR) attachment at a resolution of 4 cm^{-1} in the range from 450 to 4000 cm^{-1} by using a diamond crystal and 128 scans were signal-averaged. The contact angle of untreated and thymol-treated wool fabrics was measured by a KSV Contact Angle Measurement Apparatus (Model: CAM 100, KSV Instruments, Finland). At least 10 measurements were taken on the face and back of each treated fabric and the average has been reported. The surface of thymol-treated fabrics was examined by a scanning electron microscope (Model: TM 3030 Plus, Hitachi Corporation, Tokyo, Japan). The elemental analysis of C, N, O, and S was carried out by an energy dispersive X-ray (EDX) using the same Hitachi SEM equipped with Quantax75 energy dispersive X-ray attachment.

2.6. Assessment of durability of the antibacterial treatment to washing

The durability of the antibacterial activity of the wool fabric treated with thymol was measured according to the International Wool Secretariate (IWS) developed 7A washing protocol by washing in a wascator at 40 °C using a commercial wool washing detergent (Softly®, marketed by Pental Products Limited, Australia). Each cycle of such washing is equivalent to four domestic washes. The fabric samples were washed for 5, 10, 15, and 20 cycles wet-on-wet basis without in-between drying.

Table 1
Antibacterial performance of wool fabric treated with various dosages of totarol against three types of Gram-positive and Gram-negative bacteria.

Concentration of thymol (% owf)	Antibacterial performance against *Escherichia coli*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
0	No zone of inhibition. An extraordinarily strong growth of bacteria under the fabric was observed. 	No zone of inhibition. Robust growth of bacteria under the fabric was observed. 	No zone of inhibition. Robust growth of bacteria under the fabric was observed. 
1	No zone of inhibition. Robust growth of bacteria under the fabric was observed. 	No zone of inhibition. Robust growth of bacteria under the fabric. 	No zone of inhibition and robust growth of bacteria under the fabric. 
3	No zone of inhibition and weak growth of bacteria under the fabric was observed. 	No zone of inhibition. Robust growth of bacteria under the fabric was observed. 	No zone of inhibition. Robust growth of bacteria under the fabric was observed. 

3. Results and discussion

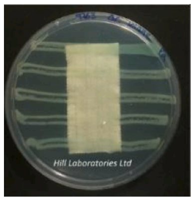
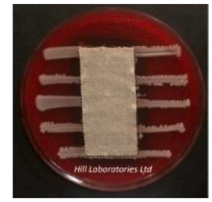
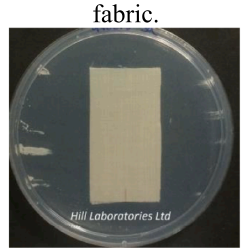
3.1. Solubility of the antibacterial agents in sub-CO_2

Totarol is a yellowish-brown colored compound, and several companies in New Zealand commercially extract totarol using sc-CO_2. The glass window on one side of the high-pressure vessel allowed us to clearly observe the solubility of the active compounds in subc-CO_2. The solubility of totarol is already reported in a previously published work (Hassan and Brorens, 2023). The treated fabric produced a uniformly golden color shade. In the case of thymol, a clear water-like solution was produced, and no thymol particles were visible suggesting its good solubility in subc-CO_2. The wool fabric treated with thymol retained its natural color as thymol is colorless and the treated fabric had a thyme-like smell. Published articles show that diterpenes extracted from various sources, such as ground coffee beans, are soluble in sc-CO_2 (Zhang et al., 2022; Barbosa et al., 2014). Therefore, it can be anticipated that totarol is soluble in the subc-CO_2 medium, although the pressure applied in subc-CO_2 is considerably lower compared to the sc-CO_2 system. On the other hand, the wool fabric treated with thymol retained its natural color as thymol is colorless and the treated fabric had a thyme-like smell. The antibacterial activity of the fabrics achieved with totarol, and thymol treatment was assessed by the standard method.

3.2. Antibacterial performance

Table 1 shows the antibacterial performance of the untreated wool fabric as well as wool fabric treated with 1% and 3% on the weight of wool fiber (owf) totarol in subc-CO_2 for 3 h at 6 MPa and 40 °C against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* bacteria. As expected, the untreated fabric showed no antibacterial performance against the studied bacteria, and high growth of bacteria was observed around the edge of samples as well as underneath the fabric. Although the antibacterial activity of totarol is previously reported (Shi et al., 2018), the totarol-treated fabric in this work did not show any antibacterial activity. In other studies, totarol powders were dispersed in the medium so that the small particles of totarol could come in contact with bacteria. The reason for the no antibacterial activity observed by the totarol-treated fabric in this work could be due to the total absorption of totarol by the fabric and also due to no leaching of totarol from the treated fabric during the antibacterial test because of its no solubility in water. Therefore, no totarol particles were released to come in contact to interact and kill the bacteria. The other reason could

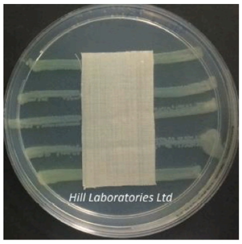
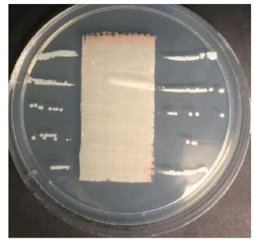
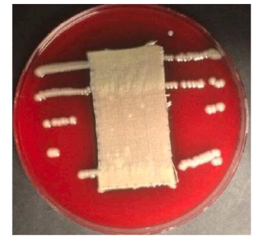
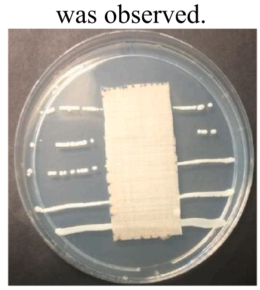
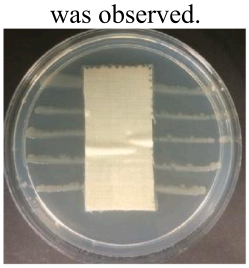
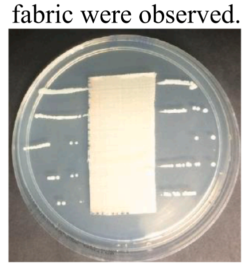
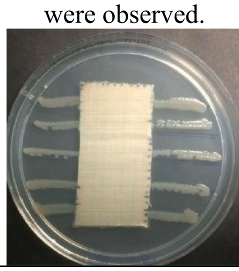
Table 2
Antibacterial performance of wool fabric treated with various dosages of thymol against various bacteria.

Dosage of thymol (% owf)	Antibacterial performance against		
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Klebsiella pneumoniae*
0	No zone of inhibition. Robust growth of bacteria directly under the fabric. 	No zone of inhibition. Robust growth of bacteria directly under the fabric. 	No zone of inhibition. Robust growth of bacteria directly under the fabric. 
2	No zone of inhibition. Weak growth of bacteria under the fabric was observed. 	0.1 cm zone of inhibition. No growth of bacteria under the fabric. 	0.1 cm zone of inhibition. No growth of bacteria under the fabric. 
3	No zone of inhibition. Weak growth of bacteria under the fabric was observed. 	0.3 cm zone of inhibition. No growth of bacteria under the fabric was observed. 	0.5 to 1.5 cm zone of inhibition. No growth of bacteria under the fabric. 
4	No zone of inhibition but no growth of bacteria under the fabric was observed. 	0.3-1.0 cm zone of inhibition. Strong growth of bacteria under the fabric was observed. 	0.1-1.0 cm zone of inhibition. No growth of bacteria under the fabric was observed. 
5	No zone of inhibition. Weak growth of bacteria under the fabric was observed. 	2.0 cm zone of inhibition. No growth of bacteria under the fabric. 	0.6 to 1.0 cm zone of inhibition. No growth of bacteria under the fabric. 

be that totarol might be effective only against a certain type of bacteria or may not have high antibacterial potency to kill the bacteria. As totarol did not exhibit any antibacterial activity, it was excluded from further works.

Table 2 shows the antibacterial performance of thymol-treated wool fabric against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* bacteria. The control untreated fabric failed to hinder the growth of bacteria underneath the test sample or around the sample

Table 3
Antibacterial performance of wool fabric treated with 5% owf thymol in subc-CO_2 after 5, 10, 15, and 20 times 7A washing.

No. of washing	Antibacterial performance against *Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
5	No zone of inhibition but no growth of bacteria under the fabric was observed. 	0.4 cm zone of inhibition. No growth of bacteria under the fabric was observed. 	0.1-0.4 cm zone of inhibition but no growth of bacteria under the fabric was observed. 
10	No zone of inhibition but no growth of bacteria under the fabric was observed. 	0.4 cm zone of inhibition. No growth of bacteria under the fabric was observed. 	No zone of inhibition but no growth of bacteria under the fabric was observed. 
15	No zone of inhibition and weak growth of bacteria under the fabric was observed. 	0.5 cm zone of inhibition and weak growth of bacteria under the fabric were observed. 	No zone of inhibition and weak growth of bacteria under the fabric was observed. 
20	No zone of inhibition and no growth of bacteria under the fabric were observed. 	0.1 cm zone of inhibition and weak growth of bacteria under the fabric were observed. 	No zone of inhibition and weak growth of bacteria under the fabric was observed. 

and the bacterial growth was quite spontaneous for all bacterial types. The control fabric did not show any antibacterial activity and no zone of inhibition was also observed. The wool fabric treated with thymol at 2% owf did not show any zone of inhibition against *Pseudomonas aeruginosa,* but the growth of bacteria underneath the fabric was weak. However, in the case of *Staphylococcus aureus* and *Klebsiella pneumoniae*, a very small (0.1 cm) zone of inhibition and no growth of bacteria under the test specimens were observed suggesting good antibacterial activity. The increase in the dosage of thymol to 3% owf also did not provide any zone of inhibition against *Pseudomonas aeruginosa* but the growth of bacteria directly under the test specimen was stopped and the size of zone inhibition was increased in the case of the other two types of bacteria. Further increase in the dosage of thymol further increased the size of zone inhibition but in the case of *Pseudomonas aeruginosa*, still, no zone of inhibition was observed but also did not show any growth of bacteria under the specimens. Overall, the thymol-treated fabrics showed excellent antibacterial activity against all three types of bacteria and the dosage of 3% owf was enough to achieve adequate antibacterial activity against all three types of bacteria.

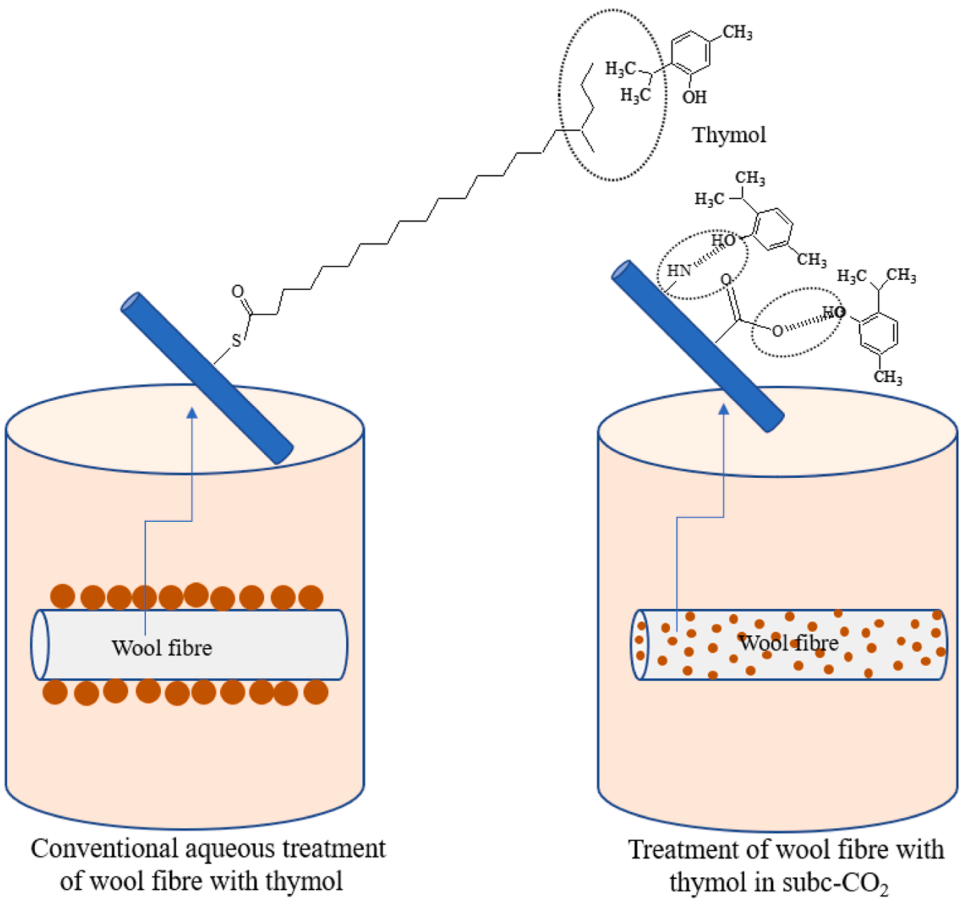


Fig. 3. Mechanism of binding of thymol onto wool in aqueous treatment and into wool fiber in sub-CO_2.

3.3. Durability of the antibacterial treatment to washing

Table S1 (Supplementary Materials) shows the durability of the antibacterial activity of the wool fabrics treated with 5% owf thymol aqueous emulsion to 5 washes. The aqueous-treated wool fabric before washing showed excellent antibacterial activity against *Staphylococcus aureus*, and *Pseudomonas aeruginosa* bacteria as no growth of bacteria was observed under the test specimens or around the fabric samples. In the case of *Staphylococcus aureus,* a 0.1–1.0 cm sized zone of inhibition was observed but no zone of inhibition was observed against *Pseudomonas aeruginosa*. However, after 5 times IWS 7A washing, the aqueous-treated wool fabric sample totally lost its antibacterial activity. Strong growth of bacteria was observed directly underneath the thymol-treated test specimens and obviously also did not show any zone of inhibition against any type of bacteria. The loss of antibacterial activity suggests little absorption of thymol into wool fiber as almost all of the thymol deposited on the fiber surface was washed off during washing resulting in a loss of antibacterial activity. The wool fabric treated with thymol in ethanol also exhibited similar antibacterial activity before and after washing like the aqueous-treated wool fabric.

Table 3 shows the antibacterial activity of wool fabrics treated with 5% owf thymol in sub-CO_2 after washing for various washing cycles. After 5 washing, the treated fabric barely lost any antibacterial activity as the washed fabric showed 0.4 and 0.1–0.4 cm zone of inhibition against *Staphylococcus aureus*, *Klebsiella pneumoniae* respectively, and no growth of bacteria was observed under the test specimens for all three types of bacteria. The antibacterial activity only marginally decreased with an increase in the number of washes. Even after 20 cycles of washing (equivalent to 80 times domestic washing) the washed fabric still showed excellent antibacterial activity against all three types of bacteria. The size of the zone of inhibition was still 0.1–0.2 cm in the case of *Staphylococcus aureus*, and *Klebsiella pneumoniae* and no growth of bacteria was observed directly under the sample even after 20 washes, which is enough to provide a lifetime guarantee of antibacterial activity as wool garments are less frequently washed compared to the garments made with cellulosic, regenerated cellulosic, and synthetic fibers.

3.4. Mechanism of enhanced wash-durability and antibacterial activity

The wool fabric treated with thymol in subc-CO_2 showed considerably higher durability of antibacterial activity to washing than the fabric treated in the aqueous medium. The thymol nano-emulsion has a larger particle size compared to the thymol dissolved in subc-CO_2, and therefore in the case of the aqueous treatment instead of absorption, thymol deposited onto the fiber surface as illustrated in Fig. 3. The cuticles of untreated wool fiber surface are coated with a polyethylene-like 18-methyleicosanoic acid layer and hydrophobic thymol is mostly deposited onto the wool fiber surface and bonded by weak hydrophobic-hydrophobic interaction. Therefore, after 5 washes, the aqueous-treated wool fabric lost almost all of the applied thymol and did not exhibit any antibacterial activity. On the other hand, in the case of wool fabric treated with thymol in subc-CO_2, under high pressure, thymol was absorbed into wool fiber and bonded to amino and carboxyl groups of wool keratin protein by hydrogen bonding inside wool fibers. Therefore, the fabric treated in subc-CO_2 exhibited high durability of antibacterial activity to washing even after 20 times 7A washes. Only a small quantity of applied thymol was lost during washing that hardly impaired the antibactrial activity of the treated fabrics.

The antibacterial agent, thymol, has a phenolic hydroxyl group on the phenolic ring that allows their very low level of solubility in water

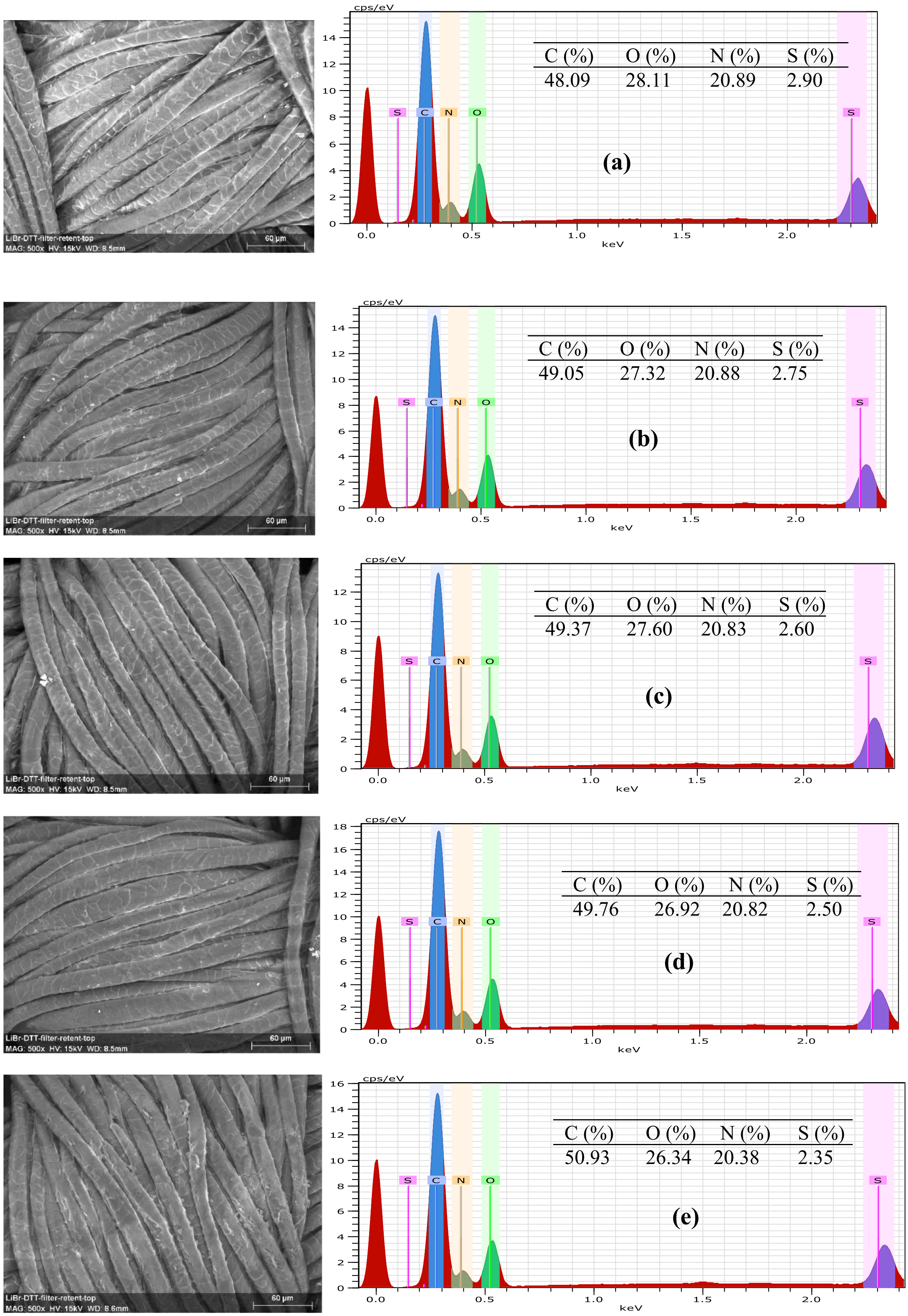


Fig. 4. EDX spectra of 0 (a) and 2 (b), 3 (c), 4 (d), and 5% owf thymol-treated wool fabrics.

Table 4
Dynamic contact angle of the surface of wool fabrics treated with various concentrations of thymol under subc-CO_2 treatment.

Thymol concentration (% owf)	Average contact angle (°) at				
	0 s	60 s	120 s	180 s	240 s
0	120.0	114.7	110.6	106.9	105.8
2	120.4	116.9	112.6	108.1	108.3
3	120.8	119.1	118.1	117.4	113.8
4	121.5	118.6	116.9	116.0	116.4
5	122.9	121.7	121.2	118.4	118.7

and helps thymol to enter the membrane of bacteria and kill them (Lambert et al., 2001). The wool fabric treated with higher concentrations of thymol showed a zone of inhibition suggesting that thymol leached from the treated samples to the bacterial medium and suppressed their growth.

3.5. Distribution of C, N, O, and S elements on the wool fabric surface

Fig. 4 shows the elemental analysis of C, O, N, and S on the surface of wool fabrics with various concentrations of thymol. The elemental compositions of wool fabrics treated with various concentrations of

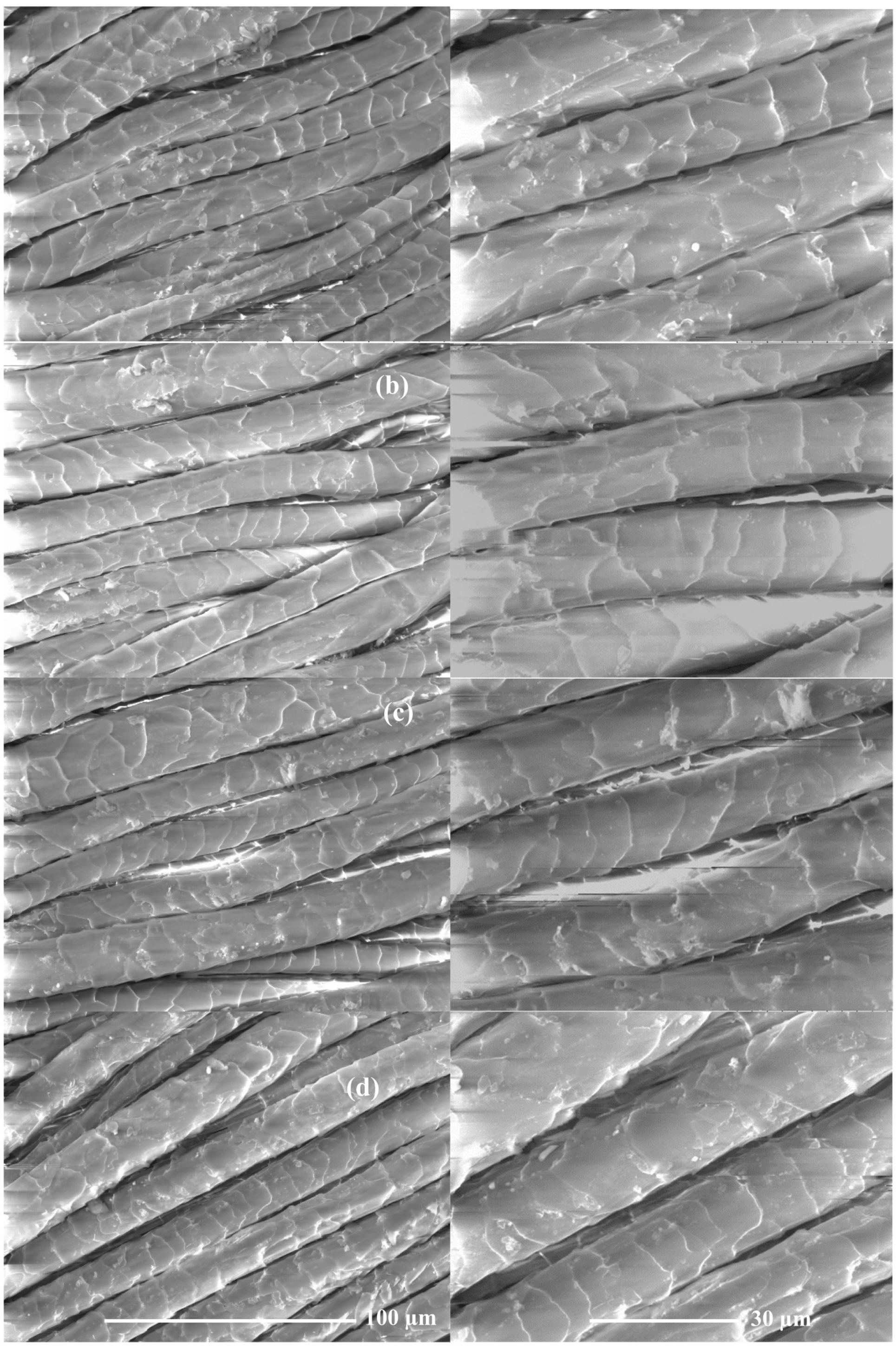


Fig. 5. SEM micrographs of wool fabric treated with 2%, 3%, 4%, and 5% owf thymol by the subc-CO_2 method.

thymol changed with an increase in thymol dosage compared with the untreated control wool fabric. Control wool fabric has C, O, N, and S elements but thymol mainly has C and H elements, which is consistent with previously published results (Hassan and Leighs, 2017; Hassan and McLaughlin, 2013). Therefore, by the adsorption of thymol, the C content of the fabric should increase, and the S content of the fabric should decrease with an increase in the thymol concentration. From Fig. 2, it is evident that the C content of the fabric increased, and the S content decreased with an increase in the dosage of thymol suggesting an increase in thymol adsorption by the treated fabric with an increase in its applied dosage. The C and S contents of control wool fabric were 48.09% and 2.9% respectively but for the wool fabrics treated with 4% and 5% owf thymol, the C content increased to 49.76% and 50.93% respectively but the S content decreased from 2.9% for the control to 2.50% and 2.35% respectively. The change in the C and S contents of the fabric indicates the presence of thymol in the treated fabrics.

Fig. S1 (Supplementary Materials) shows the distribution of C, O, N, and S elements on the surface of wool fabric treated with various concentrations of thymol. Various elements are uniformly distributed on the surface of wool fabrics treated with various concentrations of thymol. The uniform distribution of all elements suggests uniform treatment of the fabric with thymol, which is important to achieve a uniform antibacterial activity in the treated fabric. The consistency of the antibacterial activity of samples cut from various treated fabrics shown in antibacterial tests also suggests uniform treatment of the fabric samples by the subc-CO_2 method.

3.6. Dynamic contact angle

The dynamic contact angle of untreated and thymol-treated wool fabrics from 0 s (i.e., just after placing the water droplet on the sample surface) to 240 s is presented in Table 4. The optical images of the water droplet placed on various treated fabrics at 0–240 s are presented in Fig. S2 (Supplementary Materials). The contact angle at 0 s was almost similar for the untreated and the fabrics samples treated with 1–5% owf thymol, but the contact angle at 4 min after placing the droplet quite increased with an increase in the thymol dosage. The contact angle at 0 s for the untreated fabric was 120.2° which is consistent with our previously published results (Hassan and Leighs, 2017). However, the contact angle of the surface of wool fabric treated with 5% owf thymol, the contact angle increased to 122.9°. The contact angle at 240 s for the untreated control fabric was 105.2°, but for the fabric treated with thymol, the contact angle at 240 s increased with an increase in the thymol concentration. The contact angle at 240 s for the fabric treated with 5% thymol increased to 118.7°, suggesting a slight increase in the hydrophobicity of the wool fabric with an increased thymol dosage.

3.7. Surface morphologies

SEM images of the surface of unwashed wool fabrics treated with various thymol dosages in subc-CO_2 are presented in Fig. 5. All the treated fabrics show similar surface morphologies, i.e., they exhibit the typical scaly structure of wool fibers as the fabric used in this work was made from non-shrink-resist-treated wool fibers. The surface of thymol-treated fabric shows the presence of debris of wool scales that were formed because of abrasion during their processing, but no other surface deposition is visible indicating good adsorption of thymol into wool fibers was achieved and also confirms the good solubility of thymol in the subc-CO_2 medium. If thymol was not soluble in subc-CO_2, it could be adsorbed onto the surface of wool fibers and visible on the fiber surface. Therefore, the fabric treated with thymol in a subc-CO_2 medium exhibited high durability to washing compared to the fabric treated with thymol in an aqueous medium in the case of treatment under subc-CO_2, the applied thymol was absorbed by the wool fiber and reached the interior of the fiber. Therefore, during washing, they did not come out of the fiber compared to the thymol mainly adsorbed on the fiber surface when the treatment was carried out in the aqueous medium and therefore it was easily washed out during the washing test. The marginal improvement in hydrophobicity was provided by the thymol molecules adsorbed that were near the surface of the fiber.

3.8. ATR-FTIR

The change in surface functionality of wool fabric treated with various concentrations of thymol was determined by FTIR and the spectra are shown in Fig. S3 (Supplementary Materials). The ATR-FTIR spectrum of wool fabric shows common IR bands associated with amide groups of wool fabric, such as IR bands at 1235, 1450, and 1650 cm^{-1} due to amide (III), amide (II) and amide (I) of wool keratin respectively. The IR bands at 2866 and 2925 cm^{-1} are associated with the symmetric and asymmetric $–CH_2$ groups and the broad IR band at 3260 cm^{-1} is related to hydroxyl groups of wool keratin protein (Hassan and McLaughlin, 2013). In this case, wool fabric treated with thymol shows the same IR band as thymol has only hydroxyl functional groups like wool keratin, but the intensity of the amide (I), amide (II), and hydroxyl bands increased and moved towards lower wavenumber compared to the untreated wool because of the interaction of hydroxyl groups of thymol with amino and carboxyl groups of wool keratin proteins through the formation of hydrogen bonding.

4. Conclusions

This work demonstrated that the durability of the thymol treatment can be considerably improved by carrying out the treatment in the subc-CO_2 medium. The ethanol and aqueous-based treatments showed very poor durability to washing and the treated fabrics completely lost antibacterial activity after 5 washing but the subc-CO_2 treatment considerably enhanced the durability of the treatment to washing. The fabric treated with totarol did not show any antibacterial activity against the three types of bacteria studied in this work, but the thymol treatment provided excellent antibacterial activities against all three studied bacteria. The thymol treatment in the subc-CO_2 medium had a marginal effect on the hydrophobicity and surface morphologies of wool fabric as the hydrophobicity of wool fabric only marginally improved by the treatment, even at 5% thymol dosage. The developed zero-effluent treatment using a green antibacterial agent can be used in the textile industry to make durable antibacterial fabrics for various applications including hospital gowns and other medical textiles.

Declaration of Generative AI and AI-assisted technologies in the writing process

The author declares that no AI and AI-assisted technologies have been used in the writing process.

Declaration of Competing Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psep.2023.06.075.

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