

Micro-biocidal effects of fabrics and surgical gauze treated with essential oils of *Trachyspermum ammi* and *Cinnamomum tamala*

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ABSTRACT

Objective

The objective of the present study was to evaluate the antimicrobial effects of essential oils on fabrics and surgical gauze.

Materials and methods

The antimicrobial activity of essential oils of *Anethum graveolens*, *Cinnamomum tamala*, *Citrus bergamia*, *Pelargonium graveolans*, and *Trachyspermum ammi* was determined against Gram-positive & Gram-negative bacteria and a dermatophytic fungi. An antimicrobial evaluation was carried out by disc-diffusion, micro broth dilution, *in-vitro* dressing, and scanning electron micrographic methods. Percentage reduction of microorganisms and wash durability of essential oil treated fabric was also determined.

Results and discussion

The largest diameter of the inhibition zones was obtained against *Bacillus subtilis* by *Trachyspermum ammi* essential oil around the fabric disc. The value of MIC was, 0.031% v/v for *Bacillus subtilis* and both strains of *Staphylococcus aureus*, whereas MBC was determined to be 0.25% v/v and 0.5% v/v respectively. Sensory evaluation of the treated fabric and gauze revealed a gradual decline in odour intensity. The highest reduction in microbial counts has been observed in *Trachyspermum ammi* EO (0.125%) treated gauze disc both against *Bacillus subtilis* followed by *Trichophyton rubrum*.

Conclusion

High laundering durability, inhibition of microbial cells, and retention of EOs indicates the promising antimicrobial and aroma-therapeutic potential of *Trachyspermum ammi* and *Cinnamomum tamala* EOs in fabrics.

Keywords

antimicrobial; aroma-therapeutic; *Cinnamomum tamala* essential oil; *Trachyspermum ammi* essential oil; wash-durability

INTRODUCTION

Fabrics are the basic materials for clothing, these are either worn as garments or used for personal and housing items. Nowadays, the consumption of fabrics has been increased enormously because of huge demands of consumers, fashion allures and lack of durability.¹ In addition to the personal clothes, other clothing items such as bedding, bed covers, linens, tents, blinds, shower curtains, etc., gets contaminated as and when microorganisms enters in their pores; multiply by obtaining nutrients from the wearer's body

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and the fabric itself.² Moreover, in clinical and surgical settings, the wound care items such as bandages, wound dressings, absorbent items, and suturing supplies are frequently colonized by pathogenic microorganisms which leads to nosocomial infections.³⁻⁴ Antimicrobial finishings and coatings can be incorporated to the on fabrics so that the growth of microorganisms can be reduced, additionally solving the problems of bad odours and fabric deterioration.⁵ Although synthetic antimicrobial agents such as triclosan and Quaternary Ammonium Compounds (QACs) have been incorporated in fabrics; but are not eco-friendly as these cause skin irritation, are non-biodegradable.⁶

Former literature depicts the antimicrobial properties of natural products in multitude, which includes plant extracts⁷ and essential oils.⁸⁻⁹ Moreover, the natural anti-microbials such as plant extracts, plant pigments, and chitosan and their derivatives have also been explored for their micro-biocidal action in contaminated fabrics.^{5,10} Among the natural compounds, essential oils (EOs) are globally preferred for their aesthetic and aromatic properties.⁹ These odoriferous oils have also been previously reported to be inhibitory against bacterial, fungal, and viral pathogens^{8,11,12}, therefore can be incorporated in textile materials also.¹³ *Anethum graveolens* EO and its emulsion in water has been used in formulations for the treatment of gastrointestinal disorders, is an important ingredient of gripe water; also has been found inhibitory to gram-positive, gram-negative bacteria and fungi.¹⁴ *Cinnamomum tamala* (Bay Leaf), a culinary herb; its EO has shown antibacterial and antifungal properties.^{12,15} In addition to applications as natural preservatives, flavorings, and antioxidant, *Citrus bergamia* (Bergamot) peel EO also exhibits antibacterial and anti-fungal properties.¹⁶ *Pelargonium graveolans* (Geranium) EO shows remarkable antibacterial and anti-fungal properties, hence a component of food, cosmetic and pharmaceutical products.^{11, 17-18} Remarkable antimicrobial activity of *Trachyspermum ammi* (Ajwain) EO, justifies its application in food items and medicines.¹⁹ Considering the amalgam of antimicrobial and deodorizing properties; EOs of *Anethum graveolens* (Dill Seed), *Cinnamomum tamala* (Bay Leaf), *Salvia sclarea*, (Clary sage), *Pelargonium graveolans* (Geranium), and *Trachyspermum ammi* (Ajwain) are being assessed for their antimicrobial activities on surgical bandages and fabrics.

MATERIALS AND METHODS

Essential oils

Essential Oils (EOs) of *Anethum graveolens* (Dill Seed Oil), *Cinnamomum tamala* (Bay Leaf), *Citrus bergamia* (Bergamot), *Pelargonium graveolans* (Geranium oil) and *Trachyspermum ammi* (Ajwain oil). Essential oils were purchased from Sai Export India, India. Essential oils were stored in an amber-colored bottle and refrigerated at 4° C until use.

Microorganisms

The following microbial cultures (i-vii) were procured from MTCC, Chandigarh (India):

- i. *Staphylococcus aureus* MTCC 3160
- ii. *Pseudomonas aeruginosa* MTCC 2453
- iii. *Klebsiella pneumoniae* MTCC 4030
- iv. *Escherichia coli* MTCC 614
- v. *Bacillus subtilis* MTCC 441
- vi. *Proteus vulgaris* MTCC 7299
- vii. *Trichophyton rubrum* MTCC 296
- viii. Methicillin-resistant *Staphylococcus aureus* (MRSA) was procured from the Department of Microbiology, Swami Rama Himalayan University, Dehradun, India.

Surgical gauze bandages and fabric- Surgical gauze bandages were purchased from a local chemist shop and the fabrics were obtained from AVConnect, Noida, Uttar Pradesh, India. The plain weaved fabrics were treated to remove impurities using the Soxhlet method (the solvent used was petrol ether followed by ethanol). The fabrics were then rinsed in three different water baths with distilled water, dried at ambient temperature and were cut as circular discs (diameter-10mm), and sterilized by autoclaving.²⁰ The fabric and gauze samples were soaked in different concentrations (0.25 % to 2.0%) of EOs for 18h at room temperature. The soaked samples were pressed in aseptic conditions and dried in the air to strain out the excessive oil.

Screening of antimicrobial sensitivity of essential oils

Disc diffusion method was followed for preliminary screening of antimicrobial activity; different concentrations of each essential oil (10µl) were checked against bacteria using Muller Hinton Agar (MHA, HiMedia, India) medium. Media was seeded with the bacterial culture (100µl) and then added

10 μ l of particular essential oil (2%) onto the sterile filter paper discs [6mm diameter, (HiMedia, India)], incubated at 37°C for 24h. For the screening of anti-fungal activity, Sabouraud's Dextrose Agar (SDA, HiMedia, India) medium was seeded with the fungal spore suspension, EO (10 μ l) was added, incubated at 25°C for 5-7 days. After the incubation, the diameter of the zone of inhibition (ZOI, in mm) was measured using an 'Antibiotic Zone Scale' (HiMedia) caliper; an average of three different readings was calculated. An inhibition zone of 14 mm or greater was considered as high antimicrobial activity, appropriate positive and negative controls were also included.^{8, 21} Further, EOs showing antibacterial activity were also assessed for antimicrobial activity on fabric and gauze discs.^{22, 23, 24}

According to the *in-vitro* Dressing Model, 10 μ l aliquot of EO (which showed the best antimicrobial activity by preliminary screening i.e. *T. ammi* (Ajwain) & *C. tamala* (Bay leaf) essential oils (100%) was applied as spots at four different areas on the sterile 10.2 x 10.2 cm, cotton gauze dressing pad. MHA plates were seeded with standardized inoculums and covered with a single layer of sterile bandage without touching the inoculated medium surface. The dressed petri plates were subsequently incubated at 37°C for 24 h and the visible zones of inhibition were noted.²⁵⁻²⁶

Statistical analysis was carried out to examine significant variations ($p < 0.05$) by applying one-

way ANOVA and subsequently following Tukey's Post hoc test (SPSS Ver. 16.0). For each

treatment, the analyses were done in triplicates. The result was shown as mean \pm standard

deviation and a set of critical difference (CD) values.

Determination of minimum inhibitory concentration & minimum bactericidal concentration of essential oils

As described previously, micro broth dilution method (in sterile microtitre plates), was performed to determine the minimum inhibitory concentration (MIC) of EO. Minimum bactericidal concentration (MBC) was determined by the subsequent sub-culturing from the wells of microtitre plate.²⁷

Sensorial evaluation

The gauze and fabric samples treated with essential oils were kept at room temperature and the fragrance was sensorially evaluated at a 5-day interval by 10 healthy

human subjects at the same time. The aroma intensity of the specimen was evaluated by rating on an ordinal scale (from 5 to 0); 5 representing a very strong aroma and zero representing a loss of scent.²⁸

Chromatic measurements

EO-treated fabric, gauze, and control samples were dyed with Sudan Red dye solution (2g/l of chloroform). After the particular period (24, 48, 72 & 96h), the fabric & surgical gauze were taken out and squeezed and the remnant solution was analyzed spectrophotometrically. The release of EO was measured as the difference between the absorbance of the treated and control samples.^{10, 29}

Wash Durability Testing

The essential oil-treated fabric samples were washed using a standard detergent (2% on weight of fabric) and sodium carbonate (1% on weight of fabric) at 60°C. The antimicrobial activity in the terms of percentage reduction of microbial growth was assessed after 5, 10, and 20 washes.^{22, 30}

Percentage Reduction Test

Fabric disc (diameter, 10mm) was soaked in EO solution (MIC/2) and kept overnight at 37°C. The treated gauze disc was added to the sterile Trypticase Soy Broth (HiMedia, India), and inoculated with the appropriate test microorganism, incubated at 37°C for 18h. The growth was monitored in the terms of absorbance and cfu/ml. The control experiment was performed with an untreated sterile fabric disc.

The percentage reduction was calculated as below:

$$\text{Percentage reduction} = \frac{C-T}{C} \times 100$$

Where; C = control, T= test³¹

Scanning Electron Micrographic Analysis

Fabric disc, previously treated with *T. ammi* EO, inoculated with *P. aeruginosa*, kept at 37°C for 18h. The surface morphological study of untreated and treated fabric samples was carried out at different magnifications by scanning electron microscopy SEM 450 electron microscope at an accelerating voltage of 20kV, Resolution: 30nm at different magnifications³⁰, at Wadia Institute of Himalayan Geology (WIHG), Dehradun, Uttarakhand, India.

Ethical clearance: Not applicable

RESULTS

Table 1. Antimicrobial activity of essential oil treated paper, fabric and surgical gauze disc shown as zone of inhibition (in mm, ± SD)

Microorganism	Essential oil														
	Anethum graveolens			Citrus bergamia			Cinnamomum tamala			Pelargonium graveolens			Trachyspermum ammi		
	Paper disc	Fabric disc	Surgical gauze	Paper disc	Fabric disc	Surgical gauze	Paper disc	Fabric disc	Surgical gauze	Paper disc	Fabric disc	Surgical gauze	Paper disc	Fabric disc	Surgical gauze
<i>Bacillus subtilis</i> MTCC 441	14.1 ^a ± 0.9	16.4 ^b ± 0.9	15.8 ^b ± 0.8	15.7 ^b ± 1.2	15.9 ^b ± 0.5	14.8 ^b ± 0.8	22.8 ^a ± 1.3	23.7 ^b ± 1.1	21.8 ^a ± 0.9	19.9 ^a ± 0.5	20.9 ^a ± 0.9	19.8 ^a ± 1.2	24.1 ^b ± 1.6	26.2 ^a ± 0.6	22.8 ^a ± 1.3
<i>Escherichia coli</i> MTCC 614	13.2 ^a ± 0.7	14.4 ^a ± 0.7	12.2 ^a ± 0.2	14.8 ^a ± 0.9	13.9 ^a ± 0.8	16.1 ^b ± 0.4	21.0 ^a ± 0.9	19.8 ^a ± 1.9	20.8 ^a ± 1.5	16.1 ^b ± 1.2	15.9 ^b ± 0.8	15.2 ^a ± 0.4	17.4 ^b ± 0.9	17.2 ^b ± 0.9	16.8 ^b ± 1.0
<i>Klebsiella pneumoniae</i> MTCC 4030	12.8 ^a ± 1.1	13.9 ^a ± 0.8	11.2 ^a ± 0.9	14.7 ^a ± 1.1	13.9 ^a ± 0.4	13.6 ^a ± 0.7	13.9 ^a ± 1.1	13.8 ^a ± 0.9	12.8 ^a ± 1.4	15.9 ^b ± 0.8	14.9 ^a ± 0.6	14.2 ^a ± 0.7	16.2 ^b ± 0.7	15.8 ^a ± 0.7	15.3 ^a ± 0.9
<i>Proteus vulgaris</i> MTCC 7299	13.3 ^a ± 0.8	13.4 ^a ± 0.6	16.2 ^b ± 0.5	15.9 ^b ± 0.9	16.9 ^b ± 0.7	16.6 ^b ± 0.8	17.0 ^b ± 1.0	17.6 ^b ± 1.3	18.8 ^b ± 1.7	16.8 ^b ± 0.9	17.0 ^b ± 0.8	16.2 ^b ± 0.4	16.8 ^b ± 1.1	17.1 ^b ± 0.9	16.9 ^b ± 1.0
<i>Pseudomonas aeruginosa</i> MTCC 2453	13.9 ^a ± 0.9	14.4 ^a ± 0.8	12.2 ^a ± 0.9	15.4 ^a ± 0.9	15.1 ^a ± 0.4	11.8 ^a ± 0.7	13.9 ^a ± 0.6	14.3 ^a ± 0.9	12.8 ^a ± 1.3	15.9 ^b ± 0.9	15.0 ^b ± 0.6	14.7 ^b ± 0.7	16.1 ^b ± 0.9	15.6 ^b ± 0.7	15.1 ^a ± 0.9
<i>Staphylococcus aureus</i> MTCC 3160	15.9 ^b ± 1.1	15.7 ^a ± 0.9	14.5 ^a ± 0.8	14.7 ^a ± 0.7	16.5 ^b ± 0.7	15.8 ^b ± 0.9	20.8 ^a ± 0.7	21.7 ^b ± 1.0	21.6 ^a ± 0.7	20.8 ^a ± 0.8	21.9 ^a ± 0.6	19.4 ^a ± 1.1	21.2 ^b ± 0.9	23.2 ^a ± 0.8	22.6 ^a ± 1.4
<i>Staphylococcus aureus</i> (Methicillin resistant, MRSA)	16.0 ^b ± 0.8	15.2 ^b ± 0.9	15.3 ^b ± 0.7	15.2 ^b ± 0.7	15.6 ^b ± 0.8	15.4 ^b ± 0.9	19.4 ^a ± 1.1	20.4 ^b ± 1.0	19.6 ^a ± 0.9	21.9 ^a ± 1.0	22.9 ^a ± 1.2	20.4 ^a ± 1.2	20.9 ^a ± 1.2	22.9 ^a ± 0.9	22.8 ^a ± 1.1
<i>Trichophyton rubrum</i> MTCC 296	19.8 ^a ± 0.7	20.4 ^a ± 0.7	19.2 ^a ± 0.2	15.7 ^a ± 1.2	16.6 ^b ± 0.9	16.9 ^b ± 0.7	18.6 ^b ± 1.2	20.8 ^a ± 1.3	20.5 ^a ± 1.2	16.2 ^b ± 0.9	16.9 ^b ± 0.7	16.7 ^b ± 1.4	21.6 ^a ± 1.3	23.6 ^a ± 1.1	23.1 ^a ± 0.9

*Note- The superscript 'a' denoted CD values <0.25, 'b' denotes CD values which lies between 0.25 and 0.5, 'c' denotes the values > 0.5; Different superscript on the values in the same row denotes difference.

Table 2- Sensorial evaluation for odour intensity of essential oil treated fabrics and surgical gauze

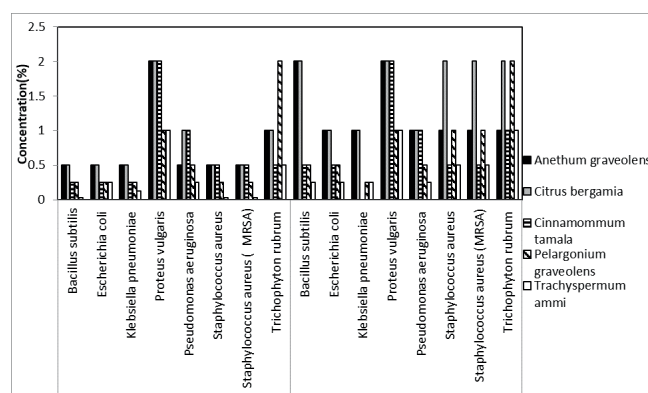
Essential Oil	1 Day		5 Days		10 Days		15 Days	
	Fabric disc	Surgical gauze	Fabric disc	Surgical gauze	Fabric disc	Surgical gauze	Fabric disc	Surgical gauze
<i>Anethum graveolens</i>	+++++	+++++	++++	++++	+++	+++	++	++
<i>Citrus bergamia</i>	+++++	+++++	++++	++++	+++	+++	++	++
<i>Cinnamomum tamala</i>	+++++	+++++	++++	++++	+++	+++	+++	+++
<i>Pelargonium graveolens</i>	+++++	+++++	++++	++++	+++	+++	+++	+++
<i>Trachyspermum ammi</i>	+++++	+++++	+++++	+++++	++++	++++	+++	+++

The antimicrobial activity of essential oils (EOs) namely, *Anethum graveolens* (Dill Seed Oil), *Citrus bergamia* (Bergamot), *Cinnamomum tamala* (Bay Leaf), *Pelargonium graveolans* (Geranium oil), and *Trachyspermum ammi* (Ajwain oil) on paper, fabric and surgical gauze discs are represented in terms of the diameter of zone of inhibition. All the essential oils under study displayed activity; around the fabric disc, the diameter of ZOI ranged from 13.3mm (*Anethum graveolens* EO against *Proteus vulgaris*) to 26.2mm (*Trachyspermum ammi* EO against *Bacillus subtilis*) Whereas around the surgical gauze disc the lowest value 11.2mm (*Anethum graveolens* EO against *Proteus vulgaris*) and highest 23.7mm (*Cinnamomum tamala* EO against *Bacillus subtilis*) (Table 1).

Trachyspermum ammi EO exhibited the greatest antimicrobial activity against all test species, the value of MIC was, 0.031% v/v for *Bacillus subtilis* and *Staphylococcus aureus*. The values of MIC & MBC of essential oils of *Cinnamomum tamala* and *Pelargonium graveolens* against the microorganisms were also remarkably lower. *Proteus vulgaris* was the least susceptible among all the tested microorganisms, as determined by higher values of MICs and MBCs (Fig. 1).

Dressing model study showed the complete inhibition of *S. aureus*, *Proteus* and *E. coli* (Fig. 2). The results of *in-vitro* dressing model showed the complete absence of growth of all the test microorganisms by all the essential oils except *K. pneumoniae*.

The sensorial evaluation depicted a very strong odour rating (+++++) on the first day following the application of aromatic oils on the fabric and surgical gauze discs. Further, the sensory evaluation showed a general trend of reduction in the odour intensity for all the essential oils. No significant difference in the ratings was judged

**Fig 1.** Minimum Inhibitory Concentration (%v/v) and Minimum Bactericidal Concentration (%v/v) of essential oils against microorganisms

for the various types of EOs. However, a comparatively lesser reduction in the odour was determined for the *Trachyspermum ammi*-treated fabric and surgical gauze (Table 2).

Fabric and gauze samples with Sudan red for different time intervals showed the analysis patterns which differ due to the type of EO. The controlled release of EO oil at the concentrations ranging from 0.25% to 2.0% is shown in (Fig 3).

Significant percentage reduction by sub-minimal concentrations of all EOs under study has been observed. The highest reduction in CFU has been observed in *Trachyspermum ammi* EO (0.125%) treated gauze disc both against *Bacillus subtilis* and *Trichophyton rubrum*. At 0.125%, *Cinnamomum tamala* EO showed maximum percentage reduction for both *Staphylococcus aureus* and MRSA (Fig 4).

Wash-durability assessment of EO treated fabric discs was performed to check the effect of repeated laundry cycles on the reduction of microorganisms by sub-

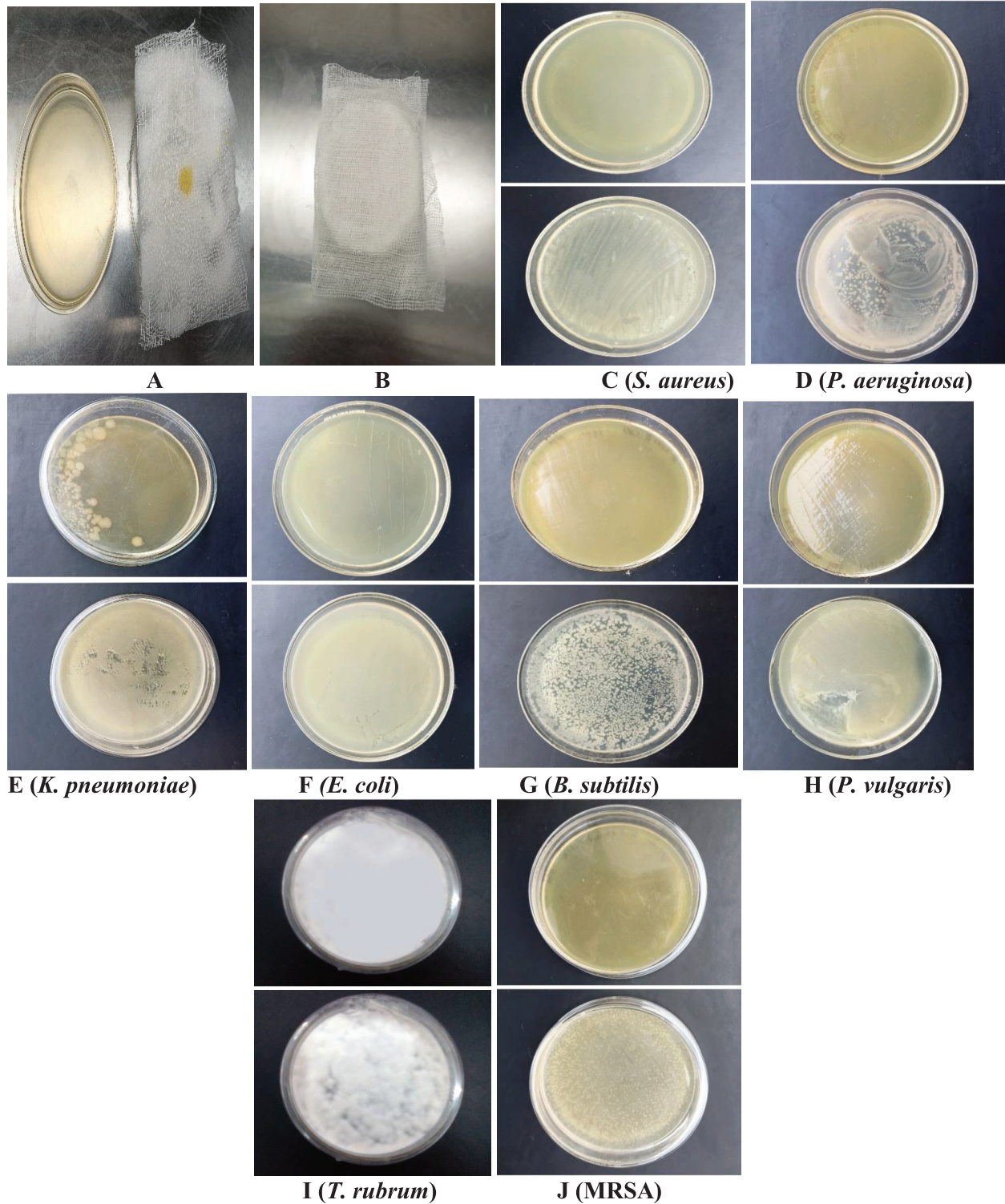


Fig 2. Dressing model experiment; (A) *C. tamala* essential oil spot on sterile gauze, (B) Sterile bandage dressing on petriplate inoculated with microorganism, (C) –(J) upper petri-plate medium showing absence/lesser growth after incubated with essential oil spotted dressing, lower petri-plate medium with complete growth (control)

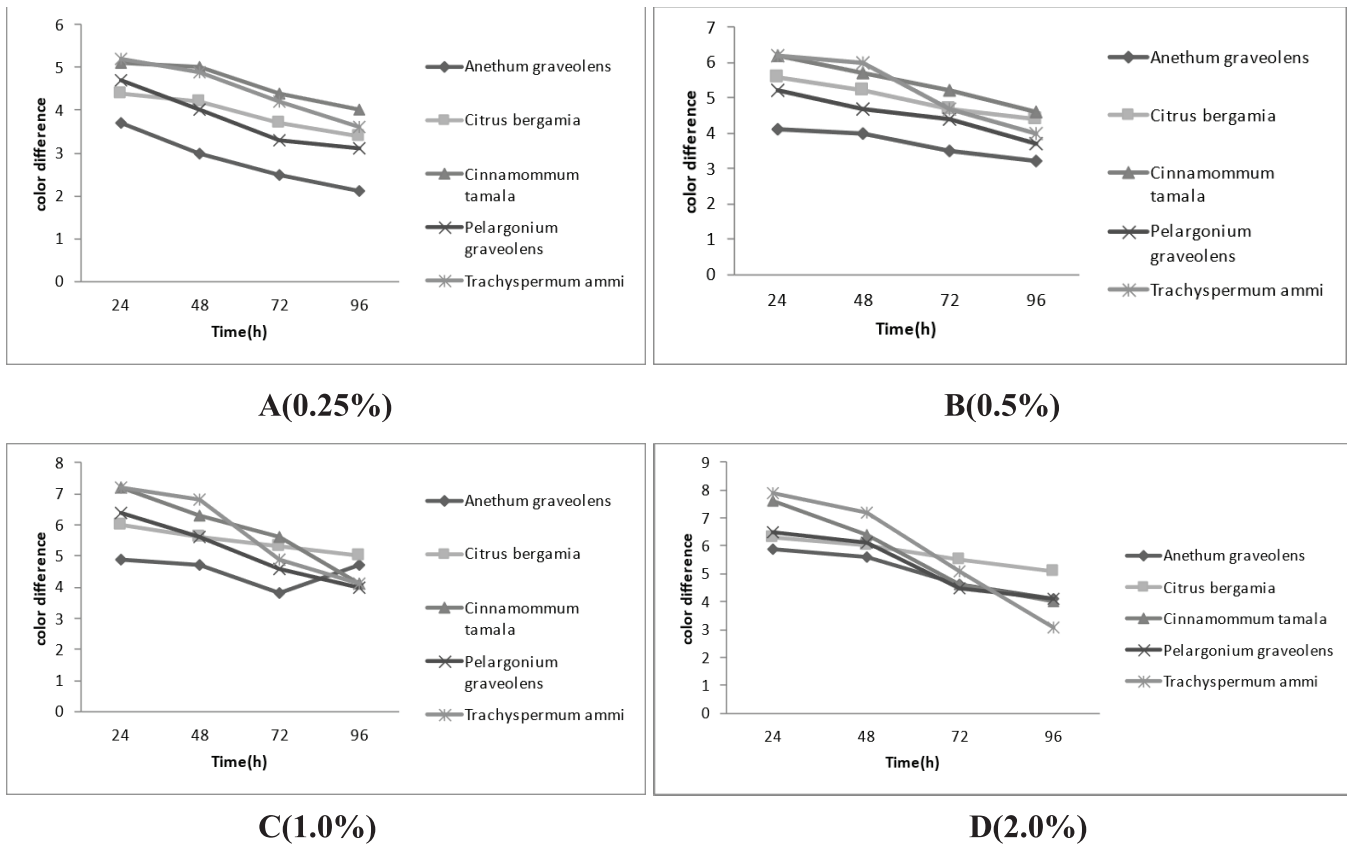


Fig 3- Colour difference for fabrics treated with essential oils for different time periods

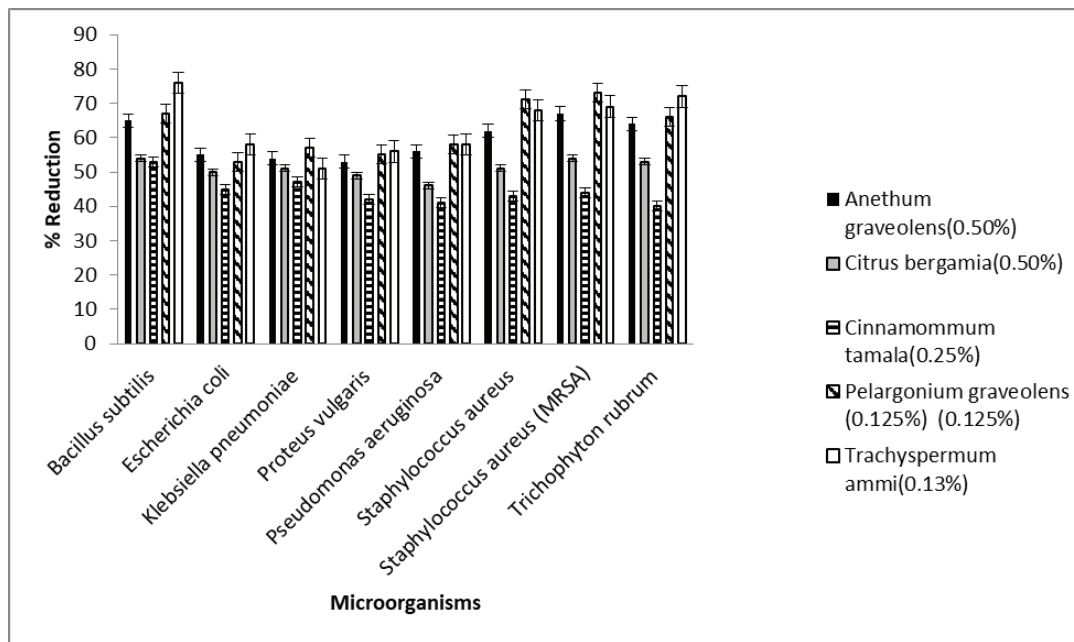
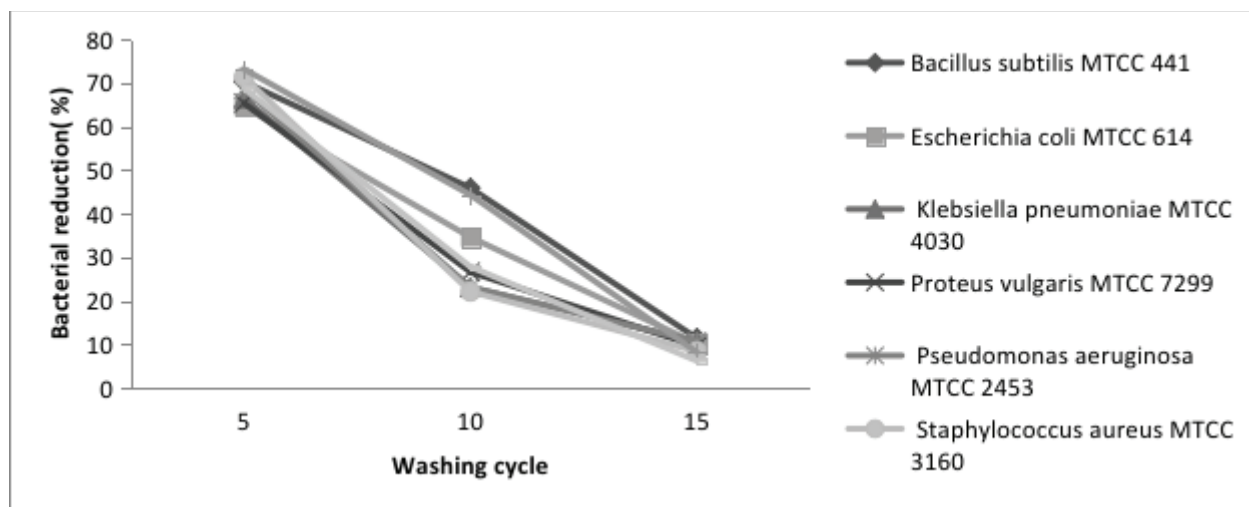
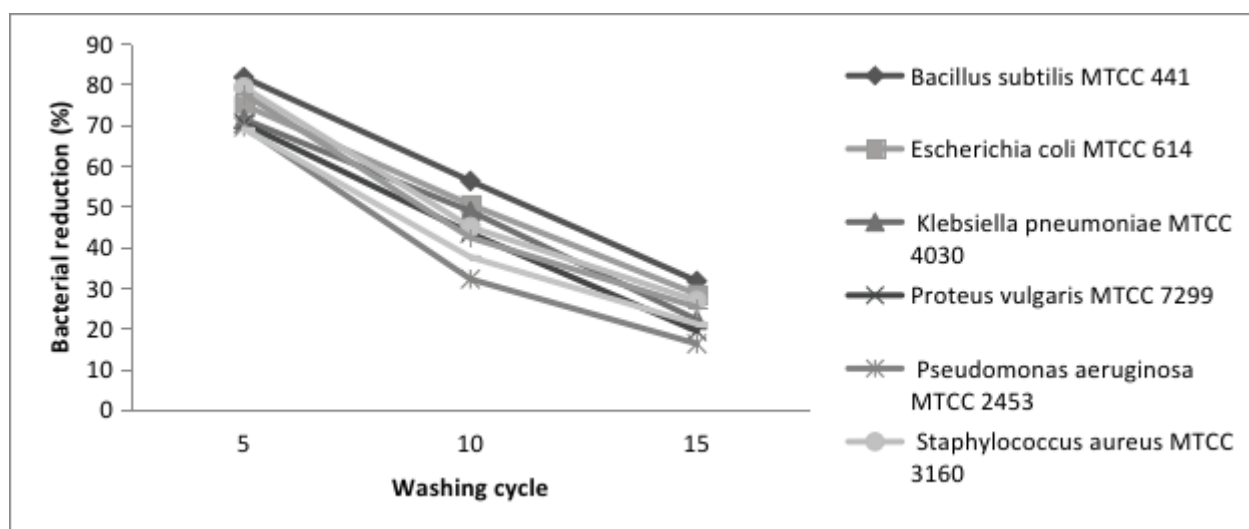


Fig 4-Relative inhibition of micro-organisms by sub-minimal concentrations of essential oils



(A)



(B)

Fig 5- Wash durability of fabric treated with essential oils for microorganisms (A) *C. tamala*, (B) *T. ammi*

minimal concentrations of *Cinnamomum tamala* (0.25%) and *Trachyspermum ammi* EO (0.125%) EO. Laundering durability was found to be the best for *Cinnamomum tamala* (0.25%) and *Trachyspermum ammi* EO (0.125%) coated fabric since the significant reduction in microbial counts was determined even after 15 cycles of repeated washing (Fig 5). SEM results showed the confluent growth of *P. aeruginosa* cells on untreated fabric samples, while the fabric treated with 0.5% v/v of *T. ammi* EO only had a few bacterial cells (Fig 6).

DISCUSSION

Keeping in view the futuristic application of essential oils in textile and surgical care items, the antimicrobial potential of selected EOs has been assessed. The preliminary screening of EOs evaluated by disc (paper, fabric, and gauze) diffusion method indicated the highest inhibition by *Trachyspermum ammi* and *Cinnamomum tamala* EOs both against *Bacillus subtilis*. High antimicrobial activity by *Trachyspermum ammi* EO is in accordance with the previous studies.³²⁻³³ *T. ammi* EO (2µl) showed the diameter of the inhibitory

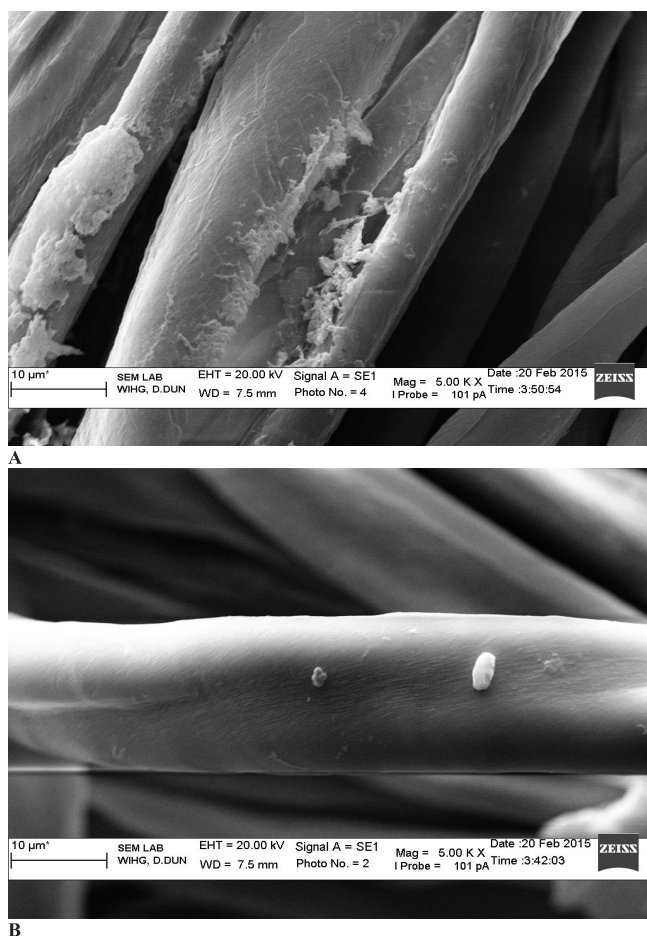


Figure 6: Scanning Electron Microscopy (SEM) images (5,000X) of (A) Fabric seeded with *Pseudomonas aeruginosa* after treatment with *Cinnamomum tamala* essential oil, (B) Fabric seeded with *Pseudomonas aeruginosa* (control), magnification- 5000X

zone against different bacteria ranging from 17 to 20 mm.³⁴ Further, the micro-broth dilution studies have also depicted inhibition of the tested microorganisms; which corroborates with the previous report³⁵; according to which MIC of *T. ammi* EO was found to be 500 µg ml⁻¹ against *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus*. In another previous study, the values of MIC for methicillin-sensitive and resistant *S. aureus* were 250 µg/ml and 500 µg/ml respectively by different chemotypes of ajowain (*T. ammi*) EO.¹⁹ The lower values of MICs, confirms a strong inhibition of microbial cells by *T. ammi* EO.

Analogously, the antimicrobial effectiveness of *Cinnamomum tamala* EO can be corroborated with investigations of previous researchers.^{15,36} 1000 ppm

of *C. tamala* essential oil showed the diameter of the zone of inhibition in the range of 31-76 mm against gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*).³⁷ MIC of *C. tamala* EO against bacteria *E.coli*, *S. aureus*, and *Bacillus cereus* ranged in between 50-500µl/ml. Against fungi, it was reported to be 5µl/ml for *Aspergillus niger*, 20 µl/ml for both *A. fumigatus* and *Candida albicans* and >20 µl/ml for *Saccharomyces cerevisiae*.³⁶ Anti-dermatophytic activity against *Microsporium audouinii* and *Trichophyton mentagrophytes* was confirmed by complete mycelial growth inhibition (MGI) by *C. tamala* EO (68.4 - 285 µg/ml).¹² Moreover, *C. tamala* essential oil and its components, cinnamaldehyde and linalool were showcased not only to be inhibiting the bacterial growth but these also inhibited the virulence factors of *P. aeruginosa*, hence retarding the biofilm formation.³⁸ Higher sensitivity of Gram-positive bacteria towards essential oils as compared to their counterpart, Gram-negative can be attributed to the difference in structural and permeability differences of the cell wall. As the microbial cells gets attached to the fibers of the fabrics, the essential oil or its component absorbs and may disrupt the cell membrane of the microorganisms through physical and ionic disruptions. Our study also infers the highest inhibition by EOs of *Trachyspermum ammi* and *Cinnamomum tamala*, both against *Bacillus subtilis*. The potential topical application of *C. tamala* and *T. ammi* EOs for the possible antiseptic of wounds has been confirmed qualitatively by the presence of a zone of inhibition around the spotted oil as performed by *in-vitro* dressing model. These pronounced findings are herewith speculated for the first time; in the future can be applied as topical antiseptic ointments or on surgical gauzes for wound management.

EOs when coated on fabrics and disposables imparts a fragrance that may alter the acceptability of the consumer; hence should be evaluated in a sensorial manner. The ordinal rating system showed better sensorial perception of *C. tamala* and *T. ammi* EOs. Lesser reduction in the odour intensity over the period can be explained due to retention of the chief components of *Cinnamomum tamala* (methyl eugenol, eugenol, trans-cinnamyl acetate³⁹ and *Trachyspermum ammi* (thymol, cymene, and terpinene).⁴⁰

Sudan Red is an azo dye which is lipophilic in nature,

has been utilized to check the absorption of essential oil by the treated fabric and gauze. The controlled release of EOs ensures the incorporation, retention, and subsequent deliverance which are prerequisites for aroma-therapeutic materials.²⁹ Retention of EO upon washing could be a challenge for its application in re-usable items. Washing EO-treated fabrics in the laboratory for different laundry cycles has reflected the retention of EOs upto 15 washes which is comparable to that of triclosan and silver-based antimicrobial agents.³¹ The present findings shows the perseverance of antimicrobial activity of *Cinnamomum tamala* and *Trachyspermum ammi* EOs in fabrics and surgical gauzes.

CONCLUSION

The present study investigated the application of five essential oils (EOs) in fabrics and surgical gauze as anti-microbials. EOs of *Cinnamomum tamala* and *Trachyspermum ammi* showed a high anti-bacterial and anti-dermatophytic activity as assessed by the disc diffusion method, micro-broth dilution studies, and *in-vitro* dressing models. Significant microbial reduction and laundering durability after washing cycles also advocates the possible application of both of these eco-friendly EOs in fabrics, and products used in clinical settings such as wound dressings, bandages, and hygiene items. Furthermore, the present incorporation of EOs by pad-dry method could be improved by fabricating

nanoparticles or microcapsules which can ensure the controlled release of these EOs.

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REFERENCES

- Cooper, T. and Claxton, S. Garment failure causes and solutions: Slowing the cycles for circular fashion. *Journal of Cleaner Production*.2022;**351**:131394.
- Sanders D, Grunden A and Dunn RR. A review of clothing microbiology: The history of clothing and the role of microbes in textiles. *Biology Letters*.2021;**17**(1):20200700
- Mehta S, Sahni N, Singh VA, Bunger R, Garg T and Shinu P. Nosocomial wound infection amongst post operative patients and their antibiograms at Tertiary Care Hospital in India. *African Journal of Clinical and Experimental Microbiology*.2014;**15**(2):60.
- Munyeshyaka E, Cyuzuzo P,Yadufashije C and Karemera J. Contribution of Medical Wards Contamination to Wound Infection among Patients Attending Ruhengeri Referral Hospital. *International Journal of Microbiology*.2021:1-7.
- Tawiah B, Badoe W, Fu S. Advances in the development of antimicrobial agents for textiles: The Quest for natural products-Review. *Fibres and Textiles in Eastern Europe*.2016;**3**(117): 136–149.
- Zheng G, Webster TF and Salamova A. Quaternary ammonium compounds: Bioaccumulation potentials in humans and levels in blood before and during the covid-19 pandemic. *Environmental Science & Technology*.2021;**55**(21):14689–14698.
- Imran H, Sohail T, Rahman A , Iqbal W, Fatima N and Shakir M. Original article: A comparative study of four indigenous medicinal plants of Pakistan against some oral pathogens. *Bangladesh Journal of Medical Science*.2020;**19**(2):284-290.
- Malik T, Singh P, Pant S, Chauhan N, Lohani H, Kumar V, et al. Inhibition of swarming behaviour in proteus mirabilis by *Pelargonium graveolens* essential oil. *Bangladesh Journal of Medical Science*. 2015;**14**(4):384–388.
- Singh P, Malik T. Essence of plants: essential oils as antimicrobials. In Parihar P, Parihar L, eds. *Advances in Applied Microbiology*. Agrobios Publishers, Bikaner, India, 2008:137-148.
- Cerempei A, Muresan EI, Sandu I, Chirila L and Sandu IG. Textile Materials with Controlled Release of Rosemary Essential Oil. *REV.CHIM. (Bucharest)*.2014;**65**(10):1154–1157.
- Malik T, Singh P, Pant S, Chauhan N and Lohani H. Potentiation of antimicrobial activity of ciprofloxacin by *Pelargonium graveolens* essential oil against selected uropathogens. *Phytotherapy Research*. 2011;**8**(25):1225-1228.
- Sirohi S, Malik T, Pant S , Chauhan N and Lohani H. Anti-Dermatophytic Potential Of *Cinnamomum tamala* Leaf Essential Oil. *International Journal of Pharma and Bio Sciences*.2016;**7**(3):291–295.
- Mehta S and MacGillivray. Aromatherapy in textiles: A systematic review of studies examining textiles as potential carrier for therapeutic effects of essential oils. *Textiles* 2022;**2**:29-49.
- Saleh-e-In MM, Sultana N, Rahim MM, Ahsan MA, Bhuiyan MNH, Hossain MN, et al. Chemical composition and pharmacological significance of *Anethum sowa* L. Root. *BMC Complementary and Alternative Medicine*.2017;**17**(1):127.
- Bharadwaj A, Rashi A and Garg G. Evaluation of phytochemical and antibacterial properties of leaf extract of *Cinnamomum tamala* oil. *Journal of Experimental Biology and Agricultural Sciences*.2022;**10**(2):416–422.
- Cebi N and Erarslan A. Determination of the antifungal, antibacterial activity and volatile compound composition of *Citrus Bergamia* Peel Essential Oil. *Foods*.2023; **12**(1):203.
- Mahboubi M and Valian M. Anti-dermatophyte activity of *Pelargonium graveolens* essential oils against dermatophytes. *Clinical Phytoscience*.2019;**5**(1):25.
- Sadiki FZ, Idrissi ME, Sbiti M and Rahou A. Antibacterial properties of the essential oil of *Pelargonium graveolens* L'Hér. *RHAZES: Green and Applied Chemistry*.2019;**4**(4):17–23.
- Ribeiro SO, Fontaine V, Mathieu V, Abdesselam Z, Dominique B, Caroline S, et al. Antibacterial activities of homemade matrices miming essential oils compared to commercial ones. *Antibiotics*.2021;**10**(5):584.
- Gressier P, Smetc DD, Beharya N, Campagnea C, Vanneste M. Antibacterial polyester fabrics via diffusion process using active bio-based agents from Essential Oils. *Industrial Crops and Products*.2019;**136**:11–20.
- Ibrahim DA and Albadani RN. Evaluation of the potential nephroprotective and antimicrobial effect of *Camellia sinensis* leaves versus *Hibiscus sabdariffa* (in vivo and in vitro studies). *Advances in Pharmacological Sciences*.2014: 389834.
- Ahmed HAM, Rajendran R. and Balakumar C. Nanoherbal coating of Cotton Fabric to Enhance Antimicrobial Durability. *Elixir Appl. Chem*. 2012:7840–7843.
- Stular D, Jerman I, Mihelcic M, Simoncic B and Tomsic B. Antimicrobial activity of essential oils and their controlled release from the Smart PLA Fabric. *IOP Conference Series: Materials Science and Engineering*.2018;**460**:012011.
- Yimer S, Manoharan and Sahu O. Extraction of Essential oil from Eucalyptus Leaves as Antibacterial Application on Cotton Woven Fabric. *International Journal of bacteriology, Virology and Immunology*.2014;**1**(1): 001–007.
- Edwards-Jones V, Buck R, Shawcross SG, Dawson MM, Dunn K. The effect of essential oils on methicillin-

- resistant *Staphylococcus aureus* using a dressing model. *Burns*.2004;**30**(8):772–777.
26. Muthaiyan A, Biswas D, Crandall PG, Wilkinson BJ and Ricke SC. Application of orange essential oil as an antistaphylococcal agent in a dressing model. *BMC Complementary and Alternative Medicine*.2012;**12**(1):125.
 27. Malik T and Singh P. Antimicrobial Activity Of Aroma Chemicals Against Uropathogens. *Journal of Environmental and Applied Bioresearch*.2015;3(2):86–91.
 28. Farouk A, Sharaf S, Refaie R and Abd El-Hady MM. Highly durable antibacterial properties of cellulosic fabric via β -cyclodextrin/Essential Oils Inclusion Complex. *Polymers*.2022;**14**(22):4899.
 29. Muresan A, Cerempei A, Dunca S, Muresan R and Butnaru R. Aromatherapeutic Characteristics of Cotton Fabrics Treated with Rosemary Essential Oil. *Cellulose Chemistry and Technology*.2009:435–442.
 30. Vajpayee M , Singh M, Ledwani L, Prakash R and Nema SK. Investigation of antimicrobial activity of DBD air plasma-treated banana fabric coated with natural leaf extracts. *ACS Omega*.2020;**5**(30):19034–19049.
 31. Dhiman G and Chakraborty JN. Antimicrobial performance of Cotton finished with Triclosan, silver and Chitosan. *Fashion and Textiles*.2015;**2**(1):1-14.
 32. Dadpe MV, Dhore SV, Dahake PT, Kale YJ, Kendre SB, Siddiqui AG. Evaluation of antimicrobial efficacy of *Trachyspermum ammi* (Ajwain) oil and chlorhexidine against oral bacteria: An *in-vitro* study. *Journal of Indian Society of Pedodontics and Preventive Dentistry*.2018;**36**(4):357-363.
 33. Gharajalar SN, Hassanzadeh M, Mahmoudi R, Emamverdizadeh P. Antibacterial activity of *Trachyspermum ammi* essential oil against *Streptococcus mutans* isolated from human dental plaques. *Journal of Medical Microbiology and Infectious Diseases*.2022;**10**(4): 192–198
 34. Das BS, Sarangi A, Sahoo A, Jena B, Patnaik G and Rout SS. Studies on phytoconstituents, antioxidant and antimicrobial activity of *Trachyspermum ammi* seed oil extract with reference to specific foodborne pathogens. *Journal of Essential Oil Bearing Plants*.2022;**25**(5):1012–1028.
 35. Javan AJ, Salimiraad S and Khorshidpour B. Combined effect of *Trachyspermum ammi* essential oil and propolis ethanolic extract on some foodborne pathogenic bacteria. *Veterinary Research Forum*.2019;**10**(3):235–240.
 36. Munda S, Dutta S, Pandey SK, Sarma N and Lal M. Antimicrobial activity of essential oils of medicinal and aromatic plants of the North East India: A biodiversity hot spot. *Journal of Essential Oil Bearing Plants*.2019;**22**(1):105–119.
 37. Kapoor I, Singh B, Singh G, Isidorov V and Szczepaniak L. Chemistry, antimicrobial and antioxidant potentials of *Cinnamomum tamala* Nees & Eberm. (Tejpat) essential oil and oleoresins. *Natural Product Radiance*. 2009. **8**: 106-116.
 38. Banu SF, Rubini D, Rakshitaa S, Chandrasekar K, Murugan R, Wilson A, *et al*. Antivirulent properties of underexplored *Cinnamomum tamala* essential oil and its synergistic effects with DNase against *Pseudomonas aeruginosa* biofilms – an *in vitro* study. *Frontiers in Microbiology*. 2017; **8**:1-14.
 39. Kumar S, Sharma S and Vasudeva N. Chemical compositions of *Cinnamomum tamala* oil from two different regions of India. *Asian Pacific Journal of Tropical Disease*.2012;**2**:S761–S764.
 40. Chahal KK, Dhaiwal K, Kumar A, Kataria D and Singla N. Chemical composition of *Trachyspermum ammi* L. and its biological properties: A review. *Journal of Pharmacognosy and Phytochemistry*.2017;**6**(3):131–140.