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ANTIMICROBIAL ACTIVITY OF TEXTILES TREATED WITH ROSEMARY AND ORANGE ESSENTIAL OILS AGAINST A SELECTION OF PATHOGENIC FUNGI

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Abstract

The aim of the research was the evaluation of antimicrobial activity of a textile fabric treated with essential oils extracted from Rosmarinus officinalis (rosemary) and Citrus sinensis (orange), against pathogenic strains of Aspergillus niger (IMI 45551), Candida albicans (ATCC 90028), Trichoderma viride (isolated from agricultural soil), Aspergillus flavus (isolated from agricultural soil) and Epidermophyton floccosum (CCM 8339). Plant extracts were obtained by steam distillation from rosemary vegetal mater and orange peel. GC-MS analysis, carried out in hexane and diethyl-ether, allowed identification of main compounds, with high quantities of eucalyptol, camphor and α – pinene, in rosemary oil, respectively limonene, limonene oxide, α – pinene and β-phellandrene in orange oil. A textile substrate (56% cotton/44% polyester) was treated with concentrations of 1%, 3% and 5% of each oil and antimicrobial activity was assessed against each strain. Bioassays registered various percentage reduction rates, depending on oil concentration and tested strain: on Aspergillus niger, rosemary treatment registered a maximum of 22.12%, whilst orange treatment reached 51.45%; on Candida albicans, both treatment yielded 100% reduction rates for all tested concentrations; on Trichoderma viride, textiles treated with rosemary oil reached a maximum of 76.48% reduction rates, and 100% on orange treatment; on Aspergillus flavus, maximum efficiency on rosemary treatment was of 18.3% and 60.57% on orange treated materials; on Epidermophyton floccosum dermatophyte, maximum reduction rate on rosemary treatment was of 56.99% whilst on orange treatment it registered a maximum of 92.48%. The obtained results promote textiles functionalized with rosemary and orange essential oils as efficient active antimicrobial barriers.

Key words: *plant extracts, fungi, antimicrobial, textiles.*

INTRODUCTION

Fungi are ubiquitous microorganisms, with representative species that pose highly pathogenic potential to human hosts, as some of them are significant infectious agents to immunocompromised individuals but also to immunocompetent ones. Pathogenicity represents the ability of a microorganism to damage a living host (Casadevall et al., 1999) by affecting the target homeostasis, triggering an immune response or mechanical action at tisular level (Arturo, 2007).

Essential oils derived from plants pose great potential as antimicrobial agents, against a wide range of pathogens (Friedman et al., 2002; Mimica-Dukić et al., 2004). The biocidal effect of plant extracts is caused by its constituent types, such as alcohols, ethers, phenols, aldehydes, ketones, which renders them highly efficient against a wide range of microbial strains (Kalemba et Kunicka, 2003). Public awareness of pathogenic effects caused by microorganisms lead to a continuously increasing demand for antimicrobial solutions. Textiles are constantly exposed to microorganisms, thus functionalization of these materials can prove to be an efficient method of obtaining antimicrobial active barriers. Actual global market promotes synthetic chemicals (metallic salts, triclosan, quaternary ammonium compounds, photocatalitic agents etc.)

(Ghoranneviss et Shahidi, 2013) as finishing agents for inducing antimicrobial properties, whilst plant derived chemicals can prove viable alternatives with similar efficiency and smaller footprint on the environment (Kalemba et Kunicka, 2003).

Most modern antimicrobial finished textiles are based on synthetic products, and current consumer demands must be correlated with obtaining environmental friendly final products. Conventional antimicrobial finishing includes treatment with quaternary ammonium compounds, triclosan, N-halamines, polybiguanides, nanoparticles of noble metals (nanosilver treatment) and metal oxides (Thilagavathi et Kannaian, 2010) but also treatment with titanium oxide doped with various elements for photocatalytic and antimicrobial induced properties. New trend in antimicrobial finishing promotes plant based dyes (Dumitrescu et al., 2012) over synthetic ones that can also act as antimicrobial agents (Lee et al., 2009).

Plant extracts can be used as finishing agents during textile processes or can be encapsulated for inducing controlled release properties (acacia based capsule wall filled with herbal extracts (Lazko et al., 2004). Current researches regarding use of various plants extracts for the treatment of antimicrobial finished fabrics include functionalization of 100% cotton bed linen fabric with neem (*Azadirachta indica*) and Mexican daisy extracts (Tilagavathi et Bala, 2007), fabrics treated with turmeric rhizomes extract (*Curcuma longa*) pomegranate fruit rinds extract (*Punica granatum*), aloe vera extract (Jothi, 2009), tea oil, eucalyptus oil, tulsi leaves extract, with high antimicrobial efficiency (Joshi et al., 2009) against a series of fungi and Gram positive and Gram negative bacteria (Arsene et al., 2015). Beside extracts treatment, bioactive functionalisation of textile fibers include compounds such as phenolic and polyphenols, alkaloids, lectins, poypeptide, polyacetylene, terpenoids etc (Cown, 1999).

Beside antimicrobial properties, various plants extracts can also be used for inducing UV protection properties, such as annatto, ratanjot, manjistha, babool, grapefruit extract, honeysuckle extract etc (Latarzyna et Prezewozna, 2009).

Although the antimicrobial properties of various plants extracts has been thoroughly researched, the antimicrobial active functionalization of textile materials using plants extracts still require intensive documentation.

MATERIALS AND METHODS

Plant extracts. Essential oils were obtained by steam distillation from rosemary vegetal mater and orange peel. Steam distillation was preferred to direct extraction by heating, in order to avoid loss and denaturation of constituent chemicals. Textile materials composed of a mix of cotton (56%) and polyester (44%) (mass: 156 g/m^2; thickness: 0.392mm; density/10cm: 350 per warp and 290 per weft) were treated by impregnation with essential oils of rosemary and orange, diluted by 1%, 3% and 5%, in Cosmol mineral oil.

GC-MS analysis. Main constituents of selected oils were assessed by Gas Chromatography-Mass Spectrometry (GC-MS) on a Agilent 6890N Gas Chromatograph system, 5973N MS detector (70 eV), Agilent ChemStation data system, HP-5ms (5% phenyl-methylpolysiloxane), 0.25 μmx 30 m x 0.25 mm column. The GC operating conditions were as follows: 70 to 290°C at a heating rate of 5°C/min and then isothermally held for 10 min, injector temperature of 270°C, injected volume was of 1 μL of the oil solutions in diethyl ether and hexane (1:100), pulsed split mode, with flow rate -1.5 mL/min for 0.5 min and then 1.0 mL/min, split ratio 40:1 and He gas used as carrier gas, at 1mL/min. MS conditions were set to the following parameters: ionization voltage: 70 eV; ion source temperature: 280°C; mass range: 35-500 and scan time 0.32s. The identification of each oil constituents was made by comparison with their mass spectra from Wiley 6, NIST02, Mass Finder 2.3 software.

Antimicrobial activity. Antimicrobial activity of the functionalized textile materials was tested according to ISO 20743:2007, modified absorption method, which is an evaluation method where the microbial suspension is inoculated directly onto the treated samples. The standard is used in order to test the efficiency of antimicrobial finished textiles products, including nonwovens. Fresh cultures were obtained for each microbial strain, preceding the tests and serial dilutions of 10^{-4}

were made for filamentous strains and 10^{-3} for *Candida albicans.* Treated textile samples (surface area of ~ 1cm^2) were inoculated in, sterilized flasks, with 50mL of last dilution of each strain, and incubated (24h) at 28°C for filamentous strains and 37°C for *Candida albicans*. After incubation period, each sample was vortexed for approx. 20 seconds in 1mL of sterile deionized water, and plated on Petri dishes, with Czapek-Dox media used for filamentous strains and Sabouraud-Agar with chloramphenicol media for *Candida albicans*.
Colony plate count method was used as quantification method for treatment efficiency and enumeration of CFUs, following incubation period. Untreated textile fabric was used as control for validation of growth condition of tested strains.

RESULTS AND DISCUSSIONS

Composition and quantity of constituent components in a volatile oil can determine its efficiency against certain microbial strains. Assessment of chromatographic profiles of selected oils allowed identification of the main compounds, carried out in two solvents, namely hexane and diethyl ether (table 1-4).

Table 1. Main components of rosemary oil extracted in hexane

Rosemary oil compounds	Percentage
Eucalyptol	42.05 %
α - Pinene	14.41 %
β - Pinene	5.59 %
Camphor	14.92 %
Camphene	7.96 %
Linalool	0.79 %
Limonene	4.70 %
Terpineol	2.45 %
2 - Thujene	0.66 %
Borneol	1.98 %
Bornyl acetate	1.20 %
Isoborneol	1.23 %
2 - Bornene	0.66 %
Caryophyllene	1.39 %

According to results from table 1, eucalyptol is the main component in rosemary oil, with 42.05%, followed by camphor (14.92%) and α–pinene (14.41%). The rest of the constituents are in small quantities, not more than 8%. Extraction of compounds was also carried out in diethyl ether solvent (table 2).
Results obtained on diethyl ether are similar with those obtained on hexane, for rosemary oil, with eucalyptol having the highest prevalence (37.92%), followed by camphor, with 15.76% and α-pinene, with 14.42%. The other compounds are in high quantity, with camphene having the highest concentration of under 10%. Moving forward, extraction of main compounds of orange oil was carried out in hexane solvent (table 3) and diethyl ether (table 4).

Table 2. Main components of rosemary oil extracted in diethyl ether

Rosemary oil compounds	Percentage
Eucalyptol	37.92 %
α - Pinene	14.42 %
β - Pinene	6.64 %
Camphor	15.76 %
Camphene	8.56 %
Linalool	0.88 %
Limonene	4.86 %
α - Terpineol	3.7 %
Terpinene	0.12 %
Borneol	1.28 %
Bornyl acetate	1.25 %
Verbenone	0.20 %
Caryophyllene oxide	0.54 %
Caryophyllene	1.60 %
α - Caryophyllene	0.20 %

Table 3. Main components of orange oil extracted in hexane

Orange oil compounds	Percentage
Limonene	97.75 %
Limonene oxide	0.48 %
β - Pinene	0.51 %
β - Phellandrene	1.26 %

Extraction of orange main components in hexane solvent allowed quantification of 4 compounds, with limonene having the highest prevalence (97.75%) followed by small traces of compounds in quantities of under 2%.

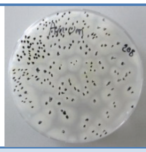
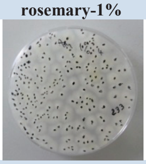
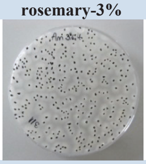
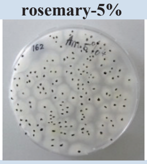
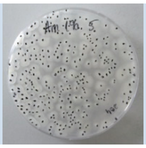
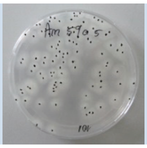
Table 4. Main components of orange oil extracted in diethyl ether

Orange oil compounds	Percentage
Limonene	94.07 %
Limonene oxide	0.63 %
α - Pinene	0.74 %
β-Phellandrene	0.34 %
2 - Thujene	0.27 %
Linalool	0.52 %
Carveol	0.21 %
Β - Myrcene	1.59 %
Decanal	0.16 %
cis - Carveol	0.19 %
p - Mentha-6,8-dien-2-one	0.27 %

Similar to the extraction in hexane, chromatographic profile carried out in diethyl ether allowed identification of the first 4 compounds as being limonene, limonene oxide, α–pinene and β-phellandrene. Extraction in diethyl ether also allowed identification and quantification of 7 more constituents, totaling approx. 3.21% of total compounds in orange oil.

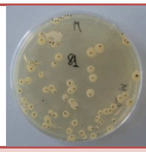
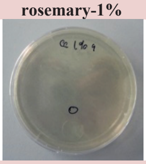
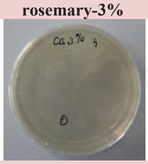
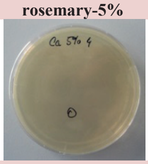
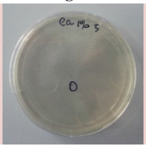
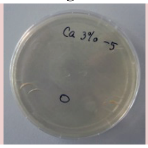
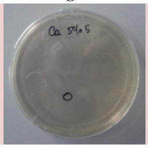
As can be noticed, for both oils matrixes, each constituent compound has different extraction percentage degree, depending on the solvent used. Antimicrobial assays (table 5-9) allowed screening of efficiency of selected plants, in concentrations of 1%, 3% and 5% when tested against four strains of filamentous fungi and one yeast strain.

Table 5. Antimicrobial activity of selected plants against *Aspergillus niger*

Control 4.16x10^3 CFU/mL		
rosemary-1%	**rosemary-3%**	**rosemary-5%**
		
T_{24}= 4.66x10^3 CFU/mL % red.=-11%	T_{24}= 6.22x10^3 CFU/mL % red.=-33.2%	T_{24}= 3.24x10^3 CFU/mL % red.=22.12%
orange-1%	**orange-3%**	**orange-5%**
		
T_{24}= 5.88x10^3 CFU/mL % red.=-22.26%	T_{24}= 2.96x10^3 CFU/mL % red.=28.85%	T_{24}= 2.02x10^3 CFU/mL % red.=51.45%

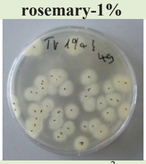
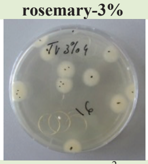
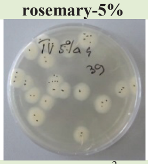
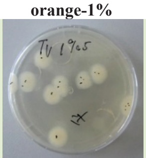
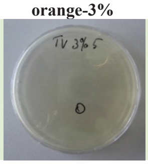
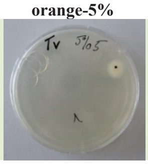
Results of efficiency of treated textiles when tested against *Aspergillus niger* revealed very mixed reduction rates, as only a few of the samples were able to percentually reduce the microbial population inoculated on the fabrics, with maximum of 51.45% for textiles treated with orange oil in 5% concentration, whilst for the materials treated with rosemary oil, the maximum was of only 22.12%, in 5% concentration. For three of the treated materials (two with rosemary oil and one with orange oil) the treatment not only that didn't reduce the strain concentration, but allowed cell proliferation (expressed here as negative percentage growth), underlining that the used concentrations were too low in order to properly induce efficient antimicrobial properties on the treated textiles. In terms of antimicrobial efficiency, orange oil proved to be more efficient against strain of *Aspergillus niger*.

Table 6. Antimicrobial activity of selected plants against *Candida albicans*

Control 1.62x10^3 CFU/mL		
rosemary-1%	**rosemary-3%**	**rosemary-5%**
		
T_{24}= 0 CFU/mL % red.= 100%	T_{24}= 0 CFU/mL % red.= 100%	T_{24}= 0 CFU/mL % red.= 100%
orange-1%	**orange-3%**	**orange-5%**
		
T_{24}= 0 CFU/mL % red.= 100%	T_{24}= 0 CFU/mL % red.= 100%	T_{24}= 0 CFU/mL % red.= 100%

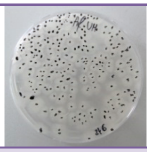
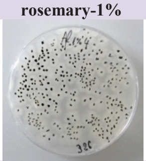
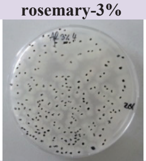
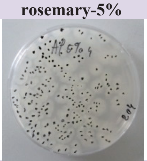
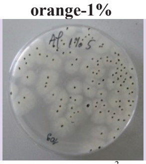
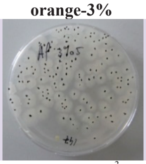
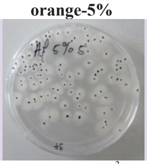
As can be seen on table 6, all textile materials treated with rosemary and orange oil reduced 100% of the strain inoculated on them, regardless of the oil and concentration used.

Table 7. Antimicrobial activity of selected plants against *Trichoderma viride*

Control 1.36x10^3 CFU/mL		
rosemary-1%	**rosemary-3%**	**rosemary-5%**
		
T_{24}= 9.8x10^2 CFU/mL % red.= 27.95%	T_{24}= 3.2x10^2 CFU/mL % red.= 76.48%	T_{24}= 7.8x10^2 CFU/mL % red.= 42.65%
orange-1%	**orange-3%**	**orange-5%**
		
T_{24}= 3.4x10^2 CFU/mL % red.= 75%	T_{24}= 0 CFU/mL % red.= 100%	T_{24}= 2x10^1 CFU/mL % red.= 98.53%

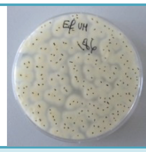
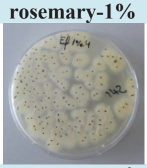
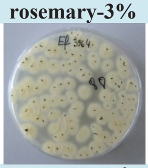
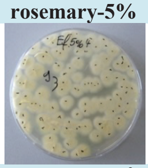
Antimicrobial efficiency assessment of textiles treated with rosemary and orange oil, tested against *Trichoderma viride* revealed better reduction rates of materials treated with orange oil when compared with the ones treated with rosemary oil, with average rate of 91.17% for orange oil and average rate of 49.02% for treatment with rosemary oil.

Table 8. Antimicrobial activity of selected plants against *Aspergillus flavus*

Control 4.92×10^3 CFU/mL		
rosemary-1%	**rosemary-3%**	**rosemary-5%**
		
T_{24}= 6.52×10^3 CFU/mL % red.= -24.54%	T_{24}= 4.02×10^3 CFU/mL % red.= 18.3%	T_{24}= 4.08×10^3 CFU/mL % red.= 17.08%
orange-1%	**orange-3%**	**orange-5%**
		
T_{24}= 2.18×10^3 CFU/mL % red.= 55.7%	T_{24}= 2.94×10^3 CFU/mL % red.= 40.25%	T_{24}= 1.94×10^3 CFU/mL % red.= 60.57%

Results of tests against *Aspergillus flavus* showed poor efficiency against the strain on textiles treated with rosemary oil in concentration of 1%, allowing strain proliferation, whilst the other treatments didn't achieve reduction rates higher than 18.3%. On the other side, orange treatment yielded satisfactorily efficiency against the strain, with maximum percentage reduction rate of 60.57%, on the material treated with 5% orange oil concentration. However, when the results were compared with the ones obtained on testing against the other *Aspergillus* strain, the reduction rates were better, only the material treated with orange oil in concentration of 5% yielding similar reduction rates with the ones tested against *Aspergillus flavus* (of 51.45%).
Antimicrobial efficiency testing of treated fabrics against *Epidermophyton floccosum* dermatophyte strain revealed satisfactorily reduction rates on textiles treated with rosemary oil, with minimum of 23.66%, for 1% oil concentration, and maximum of 56.99%, for 3% oil concentration, while for materials treated with orange oil, the minimum was of 37.64%, for 1% oil concentration, whilst maximum of 92.48% was achieved for the fabric treated with 5% orange oil concentration.
As a general pattern, materials treated with orange oil extract presented higher percentage reduction rates, with total average of 66.08%, when compared to the ones treated with rosemary oil extract, with total average reduction rate of 37.76, thus promoting orange extract as better antimicrobial finishing agent.
Furthermore, comparative analysis focused on efficiency of each treatment, taking into consideration the oil concentration used and the tested strain (figure 1-2).

Table 9. Antimicrobial activity of selected plants against *Epidermophyton floccosum*

Control 3.72×10^3 CFU/mL		
rosemary-1%	**rosemary-3%**	**rosemary-5%**
		
T_{24}= 2.84×10^3 CFU/mL % red.= 23.66%	T_{24}= 1.6×10^3 CFU/mL % red.= 56.99%	T_{24}= 1.86×10^3 CFU/mL % red.= 50%
orange-1%	**orange-3%**	**orange-5%**
		
T_{24}= 2.32×10^3 CFU/mL % red.= 37.64%	T_{24}= 1×10^3 CFU/mL % red.= 73.12	T_{24}= 2.8×10^2 CFU/mL % red.= 92.48%

Results show high resistance of *Aspergillus niger* strain to rosemary treatment in all three concentrations, while textiles treated with orange oil yielded higher reduction rates, with significant reduction rates for concentrations of 3% and 5%. The textiles treated with 1% rosemary concentration did not present any reduction rate, allowing the cells to proliferate, when compared to control.

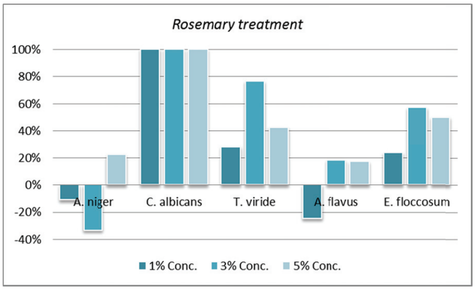


Figure 1. Rosemary treated textiles antimicrobial efficiency at different concentrations

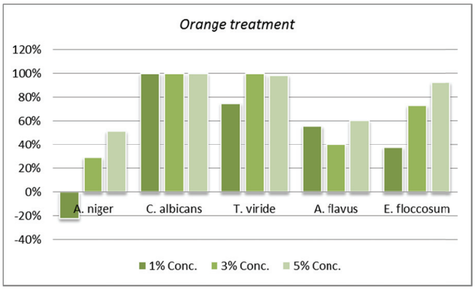


Figure 2. Orange treated textiles antimicrobial efficiency at different concentrations

When tested against *Candida albicans*, treated materials yielded maximum reduction rates for all concentrations and type of treatment, thus eliminating the need for concentration higher than 1%.

Antimicrobial assays carried out against *Trichoderma viride* strain indicated poor antimicrobial activity of fabrics treated with rosemary oil for concentrations 1% and 5%, and good percentage reduction rate for materials treated with 3% oil concentration. On the other side, the orange treatment proved to be highly efficient against this strain, with good reduction rate for 1% oil concentration and above 98% for concentrations of 3% and 5%. Following *Candida albicans* results, the bioassays carried out against *Trichoderma viride* yielded the second bets set of results, in terms of antimicrobial treatment efficiency.

Similar to results of *Aspergillus niger* testing, the antimicrobial activity of rosemary treated textiles was very poor when tested against *Aspergillus flavus*, with additional growth on material treated with 1%, when compared to control, and poor reduction rates of 3% and 5% oil concentration treatments (not exceeding 20% in reduction rates). Orange treatment proved to be far more efficient against this strain, with reduction rates above 40%, reaching its maximum on materials treated with 5% concentration, of 60.75%.

Bioassays carried out against dermatophyte strain of *Epidermophyton floccosum* indicate poor efficiency against textiles treated with 1% rosemary oil concentration, and medium reduction rates for the other two treatment concentration, with maximum of 56.99%. When tested against the same strain, textiles treated with orange oil showed improved efficiency with increasing of concentration, with minimum of 37.64%, for 1% concentration, to 73.12%, for 3% concentration and 92.48%, for 5% orange oil concentration treated fabrics.

Even though there are numerous studies regarding antimicrobial efficiency of plants extracts against several fungal and bacterial strains (Liolious et al., 2007) (Pereira, 2007) (Rodriguez et al., 2007), very few studies treat the antimicrobial efficiency of plants extracts functionalized fabrics against fungi strains.

Eucalyptus oil exhibits significant antimicrobial activity against both fungi (*Aspergillus niger, Candida albicans* etc.) and bacteria strains (*Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Staphylococcus epidermidis, Proteus vulgaris, Shigella dysenteriae, Salmonella paratyphi* etc.) (Safaei-Ghomi et Abbasi Ahd, 2010). Cotton and wool fabrics were treated with *Eucalyptus odorata* and *Eucalyptus cinerea* extracts for antimicrobial effectiveness against *Staphylococcus aureus* and *Escherichia coli* bacterial strains. Antibacterial efficiency in terms of bacterial reduction percentage for directly applied neem extract on fabric samples (scoured and bleached 100% cotton bed linen fabric) against *Staphyloccocus aureus* (100% reduction rate) and against *Escherichia coli* (78.44% reduction rate) (Thilagavathi et Kannaian, 2010). Quantitative tests carried out on 100% cotton fabric treated with similar concentrations used in the present study (3% and 5%) of turmeric, pomegranate and neem extracts against strains of *Bacillus cereus* and *Escherichia coli* revealed the following percentage effectiveness of plants extracts coated fabrics: for pomegranate treated fabrics, the yielded antimicrobial efficiency was of

62.83% for 3% extract and 82.42% for 5% extract against *Escherichia coli* and 36.39% for 3% extract and 46.049% for 5% extract against *Bacillus cereus*; for neem treated fabrics, the yielded antimicrobial efficiency was of 30.66% for 3% extract and 39.77% for 5% extract against *Escherichia coli* and 31.55% for 3% extract and 41.89% for 5% extract against *Bacillus cereus*; for turmeric treated fabrics, the yielded antimicrobial efficiency was of 37.48% for 3% extract and 46.65% for 5% extract against *Escherichia coli* and 22.89% for 3% extract and 28.76% for 5% extract against *Bacillus cereus* (Mahesh et al., 2011).

CONCLUSIONS

The results show great potential of rosemary and orange oils in functionalization of textile substrates for obtaining highly efficient antimicrobial textiles. Efficiency of treatment is highly dependent on type of oil, concentration and strain type. General pattern dictates higher concentrations to be used in order to induce maximum of efficiency, nevertheless, presented data shows good antimicrobial efficiency of rosemary and especially orange treated fabrics, at relatively small concentrations, thus promoting them as efficient bioactive barriers.

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