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Haapakorva, E

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Novel aqueous oil-in-water emulsions containing extracts of natural coniferous resins are strongly antimicrobial against enterobacteria, staphylococci and yeasts, as well as on bacterial biofilms

E. Haapakorva\textsuperscript{1,2}, T. Holmbom\textsuperscript{3,4} and A. von Wright\textsuperscript{1}

\textsuperscript{1} Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland
\textsuperscript{2} Kasve Ltd, Kuopio, Finland
\textsuperscript{3} Laboratory of Wood and Paper Chemistry, Åbo Akademi, Turku, Finland
\textsuperscript{4} Oy Separation Research Ab, Turku, Finland

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Correspondence
Atte von Wright, University of Eastern Finland, Faculty of Health Sciences, Institute of Public Health and Clinical Nutrition, Kuopio, Finland
E-mail: atte.vonwright@uef.fi

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Abstract

\textbf{Aims}: The aim of this study was to examine the antimicrobial properties of novel aqueous natural rapeseed oil/saline emulsions containing different soluble components of spruce resin.

\textbf{Methods and Results}: The composition of aqueous resin emulsions was analysed by GC-MS and their antimicrobial properties were studied with challenge tests and with turbidometric assays. The emulsions were strongly antimicrobial against common Gram-positive and Gram-negative bacteria (including MRSA) as well as common yeasts. Furthermore, they inhibited the biofilm formation and eradicated the microbial biofilms on tested microbes. Characteristic for the emulsions was the presence of oxidized resin acids. Other main components present in emulsions, such as lignans and coumaric acids, were not antimicrobial, when tested separately.

\textbf{Conclusions}: The results indicated that the oxidized resin acids were the antimicrobial components in the emulsions. Also, there appears to be a stoichiometric relationship between the number of resin acid molecules and the number microbe cells in the antimicrobial action.

\textbf{Significance and Impact of the Study}: The fact that these solutions do not contain abietic acid, which is the main allergenic compound in resins, suggests that these solutions would be suitable, well-tolerated antimicrobials for various medical applications. The aqueous formulation will also allow the expansion of the use of these emulsions in from medical applications to the food preservatives and disinfectants.

Introduction

Creams or salves based on natural resins collected from trunks (“callus” resin) of Norway spruce (\textit{Picea abies}), have been used as home-made remedies for skin wounds and infections for hundreds of years (Cowan 1999; Kalemba and Kunicka 2003; Mahady 2005; Rautio \textit{et al.} 2007, 2012; Sipponen and Laitinen 2011). The water-insoluble main resin acids (e.g. abietic acids or dehydroabietic acid) in coniferous resins have been shown to be antimicrobial and anti-fungal in several previous studies (Söderberg \textit{et al.} 1990; Savluchinske Feio \textit{et al.} 1999; Rautio \textit{et al.} 2007, 2012; Sipponen and Laitinen 2011). However, the poor solubility in water limits their applicability as microbicidal agents, or as modern liquid antiseptics (Peng and Roberts 2000; Keeling and Bohlmann 2006).

A novel method to disperse the partially soluble components of natural coniferous resins into water makes the manufacturing of aqueous resin emulsions possible. However, this might lead to the loss antimicrobial properties due to the exclusion of resin acids. In this study, we examined the retained antimicrobial activities of two...
aqueous resin emulsions that consisted of extracts of purified coniferous resin (RE) from Norway spruce, or of rosin (or colophony) (RO) obtained from industrial tall oil. Our intention was to find out, whether these emulsions still have any therapeutic potential.

Materials and methods

Preparation of oil-in-water emulsions of coniferous resin (RE-RY-SA) and rosin (RO-RY-SA)

The oil-in-water emulsion (stock) of coniferous callus resin (RE) was prepared with sc. Resol technology (patents pending: 2142005FI, 2142005PC). In short, extracts of natural “callus resin” (aged resin) collected from trunks of Norway spruce (Picea abies) were transferred to water or saline (later SA) with rapeseed oil (abbreviated as RY) to receive an oil-in-water emulsion (RE-RY-SA). This stock emulsion is stable and can be diluted with water without precipitations or stratifications.

The stock emulsion of rosin (RO) was prepared similarly as described as the RE emulsion, but instead of the callus resin, the rosin fraction of the industrial tall oil was used. This emulsion was the stock of the RO-RY-SA liquid.

The RY-SA emulsion without the resin or rosin components was used as a control emulsion in the experiments. Furthermore, solutions of purified pinoresinol and p-coumaric acid (trans-4-Hydroxycinnamic acid) were prepared in RE-SA at the same concentrations they occur in the RE-RY-SA solution.

Compositional analysis of the emulsions

The compositions of the emulsions were quantified with GC-FID (Shimadzu GC-2010, Shimadzu Corp., Kyoto, Japan) and the individual components were identified with GC-MS (Shimadzu GC-MS-QP2010Plus; Shimadzu Corp., Japan) as their TMS derivatives. The lignans were identified by comparing both their retention times and mass spectra to purified reference compounds. Quantification was done with GC-FID using betulinol as internal standard. The quantification of the oxidized resin acids was done as a group. Highly oxidized components of resin acids may not elute on GC. It is, therefore, likely that the total amount of oxidized components is higher and the result should be interpreted as being the minimum amount of oxidized resin acids in the emulsions.

Antimicrobial challenge tests

Antimicrobial challenge tests were performed according to guidelines of European Pharmacopeia (Efficacy of antimicrobial preservation, Ph. Eur.; Chapter 5.1.3) or ISO 11 930 in validated and certificated laboratories (Laboratory of Microbiology, Hjelt Institute, University of Helsinki, Finland; MikroLab Ab, Stockholm, Sweden or Ramboll Laboratories, Ramboll Finland Oy, Lahti, Finland). The Ph. Eur. challenge tests are standards in cosmetic and pharmaceutical industries in the EU countries and consist of a specific set of common micro-organisms, against which the antimicrobial action of the challenging liquid (resin emulsions in the present study) is studied. The tests were performed with the strains Staphylococcus aureus ATCC 6538, Esterichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Apergillus brasiliensis ATCC 16404 and Candida albicans ATCC 10231, as specified in Ph. Eur.

The density of the inoculation in the Ph. Eur. challenge test was in the range of $10^4$–$10^6$ CFU per ml (or CFU per g according to the Ph. Eur.). After a 24-h challenge, the micro-organisms were cultured on specific microbiological media, and a reduction in the number of microbial colony-forming units (CFU) in test samples as a function of time was used as a measure of the antimicrobial influence.

According to the Ph. Eur. Challenge test criteria, a reduction in bacteria by at least Log 2 CFU within 48 h and at least Log 3 within 7 days is considered to indicate a significant antibacterial activity. In the present analysis, the reduction by more than $10^3$ CFU (>Log 3) within 24 h was considered significant. Correspondingly, a reduction in moulds or yeasts of more than $10^3$ CFU (>Log 2) within 24 h was considered fungistatic.

Antimicrobial tests with Bioscreen

The automated turbidometric assay with Bioscreen C (Oy Growth Curves Ab Ltd., Raisio, Finland) was used for testing the Optical density (OD) of microbial cultures in microplate wells with specific culture broth for each micro-organism to be tested. The tested microbial strains representing different known pathogens, such staphylococci, enterobacteria and yeasts, are listed in Table 1.

For the standard assay, an overnight culture of each test strain was pelleted, washed, suspended in physiological saline and the concentration of each tested microbe was set to $1 \times 10^6$ CFU per ml. In another set of experiments, different bacterial inoculum sizes were tested with a constant amount of resin acid emulsions in order to get an indication, whether the antimicrobial effect shows a stoichiometric relationship.

The turbidometric screens were performed in 100-well microtitre plates, which were then placed in the automated turbidometric system Bioscreen C. Each well contained 50 µl of 5× concentrated tryptone soy broth (TS,
Table 1 The microbial strains used in the Bioscreen experiments

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gram ±</th>
<th>Aerobic ±</th>
<th>Type</th>
<th>Routine growth medium</th>
<th>Cult. temp. (°C)</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>–</td>
<td>+</td>
<td>Rod</td>
<td>Blood agar or TS broth (Lab M Ltd., UK)</td>
<td>37</td>
<td>American Type Culture Collection, Manassas, Virginia, USA</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> 1987/2000 EELA</td>
<td>–</td>
<td>+</td>
<td>Rod</td>
<td>Blood agar or TS broth (Lab M Ltd., UK)</td>
<td>37</td>
<td>The Finnish Food And Safety Authority (EVIRA), Helsinki, FL</td>
</tr>
<tr>
<td><em>Salmonella enterica</em> ssp. enterica serovar infantis EELA 72</td>
<td>–</td>
<td>±</td>
<td>Rod</td>
<td>Blood agar or TS broth (Lab M Ltd., UK)</td>
<td>37</td>
<td>The Finnish Food And Safety Authority (EVIRA), Helsinki, FL</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> SLV/NFA 429</td>
<td>–</td>
<td>+</td>
<td>Rod</td>
<td>Blood agar or TS broth (Lab M Ltd., UK)</td>
<td>37</td>
<td>Livsmedelsverket/National Food Agency, SE</td>
</tr>
<tr>
<td><em>S. aureus</em> DSM 20231</td>
<td>+</td>
<td>±</td>
<td>Coccus</td>
<td>Blood agar or TS broth (Lab M Ltd., UK)</td>
<td>37</td>
<td>Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, DE</td>
</tr>
<tr>
<td><em>S. aureus</em> (MRSA) ATCC BAA–44</td>
<td>+</td>
<td>±</td>
<td>Coccus</td>
<td>TS agar or broth (Lab M Ltd., UK)</td>
<td>37</td>
<td>American Type Culture Collection, Manassas, Virginia, US</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> EELA 34 (NCTC 11145)</td>
<td>+</td>
<td>±</td>
<td>Rod</td>
<td>Blood agar or TS broth (Lab M Ltd., UK)</td>
<td>37</td>
<td>The Finnish Food And Safety Authority (EVIRA), Helsinki, FL</td>
</tr>
<tr>
<td><em>Candida albicans</em> EELA 188</td>
<td>NA</td>
<td>NA</td>
<td>Yeast</td>
<td>OQYE agar or broth (Lab M Ltd., UK)</td>
<td>30</td>
<td>The Finnish Food And Safety Authority (EVIRA), Helsinki, FL</td>
</tr>
<tr>
<td><em>Candida tropicalis</em> VALIO 4068</td>
<td>NA</td>
<td>NA</td>
<td>Yeast</td>
<td>OQYE agar or broth (Lab M Ltd., UK)</td>
<td>30</td>
<td>Valio Finnish Cooperative Dairies’ Association, Espoo, FI</td>
</tr>
</tbody>
</table>

Tests on microbial biofilms

The tests on in vitro biofilms were carried out as described in 2013 by Brackman et al. (Brackman et al., 2013), and were done in Laboratory of Pharmacological Microbiology, Gent University, Belgium. The tested bacteria were cutaneous staphylococci *S. aureus* (Mu50) and *S. epidermidis* (ET013). The strains were grown aerobically on Luria–Bertani (LB) agar (BD, Sparks, MD) at 37°C. The *S. aureus* Mu50 is a methicillin-resistant *S. aureus* strain.

Overnight cultures of the tested strains were pelleted, washed, suspended in physiological saline (SA) and diluted to 1 x 10⁶ CFU per ml. Medical grade silicone discs (Q7-4735; Dow Corning, Midland, MI, USA) were placed in the wells of a 24-well microtitre plate (TPP), and 10-µl aliquots of the cell suspension were spotted on each disc. One millilitre of biofilm medium (Bolton Broth with 50% plasma and 5% freeze–thaw leaked horse blood and 10 U per ml heparin) was subsequently added.

To evaluate the biofilm inhibitory effect, gauzes with or without the test emulsion were placed over each disc immediately after inoculation. To assess the eradicating activity, the biofilm model was incubated first for 24 h at 37°C to allow biofilm formation on the discs. After this, the medium was removed, the biofilms were washed with SA, fresh medium was added and test emulsions in gauze were placed on top of the biofilms. In both types of experiments, plates were incubated at 37°C for 24 h. Each sample was evaluated in triplicate.

Subsequently, gauzes were removed and biofilms were rinsed with SA. The discs were placed into 10 ml SA, sessile cells were removed from the discs by three cycles of vortexing (30 s) and sonication (30 s; Branson 3510; Branson Ultrasonics Corp., Danbury, CT), and the number of CFU/discs was determined by plating the resulting suspensions on TSA. The data were analysed using two-tailed Student’s t-test. Statistical analysis was carried out using GraphPad Prism software, ver. 7 (GraphPad Software Inc, La Jolla, CA, USA).
Results

The chemical composition of the emulsions

The results of GC-FID and GC-MS analyses of the RE-RY-SA emulsion are shown in Fig. 1. The aqueous RE-RY-SA emulsion contains, in addition to the rapeseed oil and saline, p-coumaric acid, lignans and oxidized resin acids (Fig. 1). Lignans identified in the RE-RY-SA emulsions were: pinoresinol (most abundant), lariciresinol, isolariciresinol and secoisolariciresinol. The resin acids present were oxidized, weakly soluble resin acids in quantities of some hundreds of ppm ($\mu$g ml$^{-1}$). The main water-insoluble resin acids (abiatic acid, dehydroabiatic acid, etc.) were not detected.

Compositionally the aqueous RO-RY-SA emulsion was similar to the RE-RY-SA emulsion but contained only oxidized resin acids. Lignans, p-coumaric acid and the main resin acids were absent.

RE-RY-SA and RO-RY-SA emulsions are microbicidal in challenge tests

In 24-h challenge tests carried out by Ph. Eur. guidelines for antiseptics and cosmetics, both the RE-RY-SA and RE-RY-SA emulsions were microbicidal against Gram-positive and Gram-negative bacteria, and against C. albi
cans yeast, among others. The microbicidal effects were strong and significant (reduction in microbe count more than Log 3 CFUs in 24-h challenge) (Table 2). The dilutions of the stock emulsions of both RE-RY-SA and RO-RY-SA emulsions with saline up to 1:3 were still microbicidal and tested microbes resistant to antibiotics (e.g. MRSA) were also sensitive to both emulsions (data not shown).

The microbial growth inhibition by resin and rosin emulsions in turbidometric assays

In Bioscreen tests, both RE-RY-SA and RE-RY-SA emulsions inhibited significantly the growth of all tested micro-organisms (Fig. 2a–b, Table 3). No or only minor inhibition occurred in tests with purified coumaric acid or lignans, exemplified by p-coumaric acid and by pinoresinol which is the most abundant lignan in coniferous resins, indicating that the oxidized resin acids were the ingredients that are antimicrobial in the RE and RO emulsions.

In microbial cultures without RE or RO, there was a linear relationship between the turbidity index and the concentration of the initial inoculum in 48-h cultures (Fig. 2c–d). The presence of RE resulted, instead, in non-linear decrease in turbidity of the cultures with linearly decreasing concentrations of the microbe inoculum. The turbidity was proportionally (in percentage) and exponentially greater in the cultures in which the concentration of the inoculum (count of the microbes initially added to the microplate wells) was small as compared to the cultures in which concentration was high. As to the antimicrobial action mechanism of the resin emulsions, these observations were considered to indicate a stoichio-
metric quantitative relationship between the quantity of

Figure 1 GC/MS of coniferous “callus” resin in oil-in-water emulsion (RE-RY-SA) and the content (inserted table) of p-coumaric acid, resin acids, oxidized resin acids and lignans in the RE-RY-SA and RO-RY-SA emulsions. The analyses were performed on both short column GC and GC/MS. Component identification was based on retention times and mass spectra (GC/MS) and the quantification was made on short column GC with betulinol as standard.
biofilms formed by cutaneous staphylococci (2013), where the effects of RE-RY-SA and RO-SY-SA on in vitro cation of microbe biofilm and in a significant inhibition of microbe biofilm and the number of microbe cells in the culture.

RE-RY-SA emulsion eradicates microbe biofilm and inhibits the biofilm formation

RE-RY-SA emulsion resulted both in a significant eradication of microbe biofilm and in a significant inhibition in the formation of biofilm in validated experimental in vitro tests when tested against S. aureus (Mu50) and S. epidermidis (ET013) (Fig. 3).

Discussion

All tests carried out in this investigation showed that the oil-in-water emulsions of natural coniferous resins, that is, both the RE-RY-SA and the RO-RY-SA emulsions, were strongly antimicrobial against bacteria and yeasts in broad scale, including the methicillin-resistant S. aureus strain. The emulsions further eradicated and inhibited microbial biofilms in validated tests (Brackman et al. 2013), where the effects of RE-RY-SA and RO-SY-SA on biofilms formed by cutaneous staphylococci S. aureus and S. epidermidis were compared to plane controls.

The RO-RY-SA emulsion contained only oxidized resin acids by GC/MS analysis. The RE-RY-SA emulsion, on the other hand, contains also p-coumaric acid and lignans, in addition to oxidized resin acids. Noteworthy, all resin acids in both emulsions were present in oxidized, partly soluble form. The so-called main resin acids (e.g. abietic acid, dehydroabietic acid or pimaric acid) were not present in detectable amounts in the solutions. The oxidized resin acids occurred in emulsions in low concentrations, in range of some hundreds of ppms at most. In earlier literature, the main resin acids, like abietic acid and dehydroabietic acid, are shown to be antimicrobial (Söderberg et al. 1990; Leandro et al. 2014; Gogulramnath et al. 2015). These main resin acids are not, however, water soluble and will not be transferred into aqueous emulsions or dispersions as the case is with the oxidized resin acids.

Taking into account the marked antimicrobial effects of the RE and RO emulsions in the present experiments, and the poor activity or inactivity of purified p-coumaric acid and pinoresinol (the most abundant lignan in coniferous resins) in Bioscreen tests, it can be concluded that the antimicrobial action of the resin emulsions is mediated mainly via oxidized resin acids. Although it has been recently shown that p-coumaric acid also has some antimicrobial properties at the same concentrations used in this study (Lou et al. 2012), the antimicrobial effect of p-coumaric acid in Bioscreen experiments was only seen on some strains and the effect being much weaker than those of RE or RO. Recent research of Zhao et al. (Zhao et al. 2015) also supports this finding. They found that p-coumaric acid exhibits low inhibitory activity against S. aureus, but no inhibitory effect on other tested bacteria, including E. coli, Listeria monocyctogenes and Staphylococcus typhimurium. However, the fact that RO solution contained approximately tenfold of oxidized resin acids compared to RE solution, and that RE solution has performed better in all experiments, may suggest that p-coumaric acid and oxidized resin acids have some synergistic co-effects.

In the present Bioscreen tests, the effective antimicrobial concentrations of oxidized resin acids were approximately thousand times lower than the resin content is in traditional oily resin salves and creams (Sipponen and Laitinen 2011; Sipponen 2013). Therefore, it seems that the oxidized resin acids are extremely potent natural antimicrobial and antiseptic compounds, and can be incorporated in emulsions as biologically favourable formulations. They can be extracted and transferred into aqueous emulsions or dispersions from crude resin in concentrations high enough to exert marked antimicrobial actions without any chemical modifications or heating.

The broad-range antimicrobial activity of the present resin emulsions indicates that the action mechanisms of the resin emulsions against the microbes are general, antiseptic like and differ from antibiotics in this respect. The growth of the microbial inocula in the microplate wells, as measured by turbidity of the cultures in Bioscreen experiments, were markedly reduced in 24 h in the presence of the RE and RO emulsions compared to control cultures. However, this reduction was inversely related to the count of microbes in the initial inoculum: the higher the initial count was in CFUs, the smaller was the reduction in the proportional microbe count after

Table 2 Test results from the 24-h challenge tests (Efficacy of antimicrobial preservation, Ph. Eur. Curr. Ed. 5:1-3) of the RE-RY-SA emulsion against bacteria, yeast and mould. The bacterial growth was measured at time points 0 h and 24 h and the logarithmic reduction was calculated. The spore-forming fungi Aspergillus brasiliensis showed resistance to the RE and RO emulsions in these experiments

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gram ±</th>
<th>0 h</th>
<th>24 h</th>
<th>Log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus ATCC 6538</td>
<td>+</td>
<td>8-66</td>
<td>0-00</td>
<td>More than 8</td>
</tr>
<tr>
<td>E. coli ATCC 8739</td>
<td>–</td>
<td>8-29</td>
<td>2-34</td>
<td>More than 5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 9027</td>
<td>–</td>
<td>8-95</td>
<td>0-00</td>
<td>More than 8</td>
</tr>
<tr>
<td>Apergillus brasiliensis ATCC 16404</td>
<td>NA</td>
<td>4-34</td>
<td>3-73</td>
<td>Less than 1</td>
</tr>
<tr>
<td>Candida albicans ATCC 10231</td>
<td>NA</td>
<td>7-30</td>
<td>0-00</td>
<td>More than 7</td>
</tr>
</tbody>
</table>

*Note: All tests carried out in this investigation showed that the oil-in-water emulsions of natural coniferous resins, that is, both the RE-RY-SA and the RO-RY-SA emulsions, were strongly antimicrobial against bacteria and yeasts in broad scale, including the methicillin-resistant S. aureus strain.*
This inverse relationship indicates that the antimicrobial efficacy and the action mechanisms of resin emulsions are based on a stoichiometric relation between the number of exposed micro-organisms and the amount of oxidized resin acids present in the culture. The antimicrobial, or bacteriostatic, mechanism of resin acids has previously shown to occur due to the accumulation of lipophilic hydrocarbons in the lipid bilayer of the cell membrane. As a result of accumulated molecules, the membrane loses its integrity leading to structural and functional disruption of the membrane and thus influencing the replication, transcription and gene expression of microbial cells (Sikkema et al. 1995; Sipponen et al. 2009).

All components in the resin emulsions are natural and not chemically manipulated. The resinous components, oxidized resin acids with or without p-coumaric acid and lignans, appear in emulsions only in tiny amounts. The tests carried out with emulsions for safety aspects, such as skin irritation and skin sensitization tests (according to ISO 10993-10:2010), and in vitro cytotoxicity (according to ISO 10993-5:2009), have all been negative so far suggesting that the aqueous resin solutions (aqueous coniferous resin emulsions and dispersions) are potential safe...
natural antiseptics, or constituents for disinfectants or cosmetics (data not shown).

The fact that these solutions do not contain abietic acid, which is the main allergenic compound in resins (Karlberg 1988; Downs and Sansom 1999), suggest that these solutions, although as antimicrobial as traditional resin salves, would be better tolerated in different medical applications, such as in treatment of severe wounds (Sipponen et al. 2007, 2012). The aqueous formulation will also allow for a wider range of applications than strictly medical ones (e.g. as disinfectants).

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Conflict of Interest

The antimicrobial challenge tests and the tests on microbial biofilms were sponsored by and the test articles were

Table 3 A summary of Bioscreen test results. RE-RY-SA and RO-RY-SA inhibited significantly the growth of all tested microbes in 24-h experiment. Data shown as relative areas under the curves (AUC) ± SD. *P ≤ 0.001 vs native control

<table>
<thead>
<tr>
<th>Strain</th>
<th>Control</th>
<th>RE-RY-SA</th>
<th>RO-RY-SA</th>
<th>pinoresinol</th>
<th>p-coumaric acid</th>
<th>RY-SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus DSM 20231</td>
<td>100 ± 6.5</td>
<td>5.1 ± 4.4*</td>
<td>29.9 ± 3.1*</td>
<td>90.2 ± 7.2</td>
<td>74.1 ± 3.1*</td>
<td>82.9 ± 6.4</td>
</tr>
<tr>
<td>Staphylococcus aureus (MRS) ATCC BAA-44</td>
<td>100 ± 2.3</td>
<td>9.2 ± 8.4*</td>
<td>17.7 ± 7.8*</td>
<td>103.7 ± 2.2</td>
<td>92.7 ± 15.7</td>
<td>92.6 ± 11.7</td>
</tr>
<tr>
<td>Bacillus cereus EELA 34 (NCTC 11145)</td>
<td>100 ± 4.6</td>
<td>4.1 ± 1.7*</td>
<td>4.9 ± 1.5*</td>
<td>89.2 ± 2.1</td>
<td>103.0 ± 4.9</td>
<td>101.1 ± 2.9</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa SLV/NFA 429</td>
<td>100 ± 1.8</td>
<td>2.5 ± 0.5*</td>
<td>2.3 ± 1.8*</td>
<td>93.1 ± 3.4</td>
<td>76.4 ± 2.8*</td>
<td>79.2 ± 3.7*</td>
</tr>
<tr>
<td>Staphylococcus infantis EELA 72</td>
<td>100 ± 3.3</td>
<td>16.2 ± 0.4*</td>
<td>12.2 ± 8.5*</td>
<td>98.1 ± 3.3</td>
<td>93.4 ± 2.1</td>
<td>88.0 ± 15.5</td>
</tr>
<tr>
<td>Klebsiella pneumoniae 1987/2000 EELA</td>
<td>100 ± 5.9</td>
<td>24.3 ± 0.1*</td>
<td>32.9 ± 2.2*</td>
<td>106.5 ± 3.6</td>
<td>107.2 ± 2.9</td>
<td>100.4 ± 0.1</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>100 ± 3.1</td>
<td>21.8 ± 2.5*</td>
<td>22.3 ± 1.2*</td>
<td>98.6 ± 7.0</td>
<td>82.3 ± 3.1</td>
<td>84.8 ± 7.7</td>
</tr>
<tr>
<td>Candida tropicalis VALIO 4068</td>
<td>100 ± 6.7</td>
<td>1.2 ± 0.8*</td>
<td>1.4 ± 1.2*</td>
<td>83.7 ± 9.1</td>
<td>67.0 ± 9.3</td>
<td>78.1 ± 23.7</td>
</tr>
<tr>
<td>Candida albicans EELA 188</td>
<td>100 ± 16.4</td>
<td>0.5 ± 0.2*</td>
<td>0.4 ± 0.1*</td>
<td>127.7 ± 26.4</td>
<td>119.6 ± 18.1</td>
<td>116.9 ± 17.4</td>
</tr>
</tbody>
</table>

Figure 3 Biofilm inhibitory (a) and eradication (b) effects of RE-RY-SA emulsion against biofilm forming bacteria Staphylococcus aureus Mu50 and Staphylococcus epidermidis ET013. Data shown as relative log CFU. Values represent means of three replicates ± SD. RE-RY-SA emulsion (grey bars) resulted in a significant reduction both in biofilm eradication and in biofilm formation compared to the untreated controls (black bars). *P ≤ 0.05 vs untreated control.
Antimicrobial resin solutions

provided by Repolar Pharmaceuticals Ltd. The research may lead to the development of new products, in which the authors have no business and/or financial interest.

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