https://doi.org/10.1595/205651323X16426780863499 *Johnson Matthey Technol. Rev.*, 2023, **67**, (1), 85–96

JOHNSON MATTHEY
TECHNOLOGY REVIEW

technology.matthey.com

Evaluation of Antibacterial Potencies of Eight Lichen Extracts Against Gram-Positive Moderately Halophilic Bacteria

Ecological materials to control moderately halophilic bacteria for leather preservation

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PEER REVIEWED

Submitted 11th September 2021; Revised 24th December 2021; Accepted 20th January 2022; Online 20th January 2022

The leather sector has global economic importance. Overcoming microbiological problems, especially arising from halophilic bacteria, will greatly reduce product losses. In this study, lichen species including *Usnea* sp., *Platismatia glauca, Ramalina farinacea, Evernia divaricata, Bryoria capillaris, Hypogymnia tubulosa, Pseudevernia furfuracea* and *Lobaria pulmonaria* were examined for their antibacterial efficacies against *Staphylococcus saprophyticus* subsp. *saprophyticus* (TR5) and *Salinicoccus roseus* (KV3) which are proteolytic and lipolytic Gram-positive moderately halophilic bacteria. The extracts of *P. glauca, B. capillaris, P. furfuracea* and *L. pulmonaria* had no antibacterial efficacy against the test bacteria. On the other hand, the extracts of *H. tubulosa*, *R. farinacea*, *Usnea* sp. and *E. divaricata* had considerable antibacterial effect with varying percentages of inhibition. The maximum inhibition ratios at the tested concentrations of 15–240 µg ml^{-1} for lichen samples of *H. tubulosa, R. farinacea, Usnea* sp. and *E. divaricata* were detected as 94.72 ± 0.75%, 76.10 ± 1.85%, 99.36 ± 0.04%, 89.49 ± 2.26% for TR5 and 97.44 ± 0.14%, 95.92 ± 0.29%, 97.97 ± 0.39%, 97.58 ± 0.53% for KV3, respectively. The most remarkable suppression was obtained with *Usnea* sp. extracts against KV3. These results indicate the need for further studies investigating the applicability of these natural resources to control moderately halophilic bacteria in the preservation of raw hides and skins.

1. Introduction

Byproducts of various industries have long been used as raw materials. Demand for such natural resources is continuously increasing (1). Raw materials such as skins and hides are byproducts of the meat industry and are converted into valuable leather products such as bags, shoes, wallets, briefcases or backpacks in tanneries (2). The leather industry is of global economic importance. To obtain high-value leather products, the raw materials (hides and skins) must be properly preserved. Since raw hides or skins have high water and protein content, these raw materials become vulnerable to bacterial activity. To overcome

bacterial deterioration on hides and skins, raw hides and skins are traditionally cured with salt or brine after the animal is slaughtered (3). The undesirable bacterial population may consist of halophilic or non-halophilic bacteria which may come from the animal itself or from environmental contamination. Additionally, salt which is used in the salt curing process may contain halotolerant microorganisms, slightly halophilic bacteria, moderately halophilic bacteria, extremely halophilic archaea and fungi which can contaminate raw skins and hides (4–11).

Previous studies demonstrated that halophilic microorganisms on skins and hides are responsible for red heat, red discoloration, holes, problems on the grain surface and deterioration of hides and skins (12–15).

Among these bacterial groups, moderately halophilic bacteria include a wide variety of bacteria (16). These bacteria may be Gram-negative or Gram-positive, aerobic or facultative anaerobic. It has been reported that these bacteria may abundantly grow in saline systems such as saltern crystalliser ponds, saline soils, the Dead Sea and evaporated ponds. Moderately halophilic microorganisms may secrete different enzymes such as proteases, lipases, cellulases and chitinases (17–19). These microorganisms may grow under conditions including 3–15% sodium chloride concentration, 0–45°C and pH 5–10 (13, 20). In recent years, although the potential enzyme production profiles of halophilic bacteria have led to a focus on their industrial use, protease and lipase producing moderately halophilic bacteria are undesirable in the leather industry due to their potential for causing defects on the final product and possible economic losses.

There are some studies examining which species of moderately halophilic bacteria can grow on hides and skins (5–8, 15, 21–23). Molecular techniques and phenotypic characterisation methods allow new species belonging to the moderately halophilic bacteria to be identified more easily. For example, *Thalassobacillus pellis* sp. nov. and *Salimicrobium salexigens* sp. nov. were reported as newly identified moderately halophilic species from salted skin samples over the past decade (21, 22).

There are also studies focused on bacterial numbers of moderately halophilic microorganisms and their potential to cause defects on hides. The results of studies investigating the abundance of moderately halophilic bacteria on hide samples showed high numbers which could possibly cause hide and skin damage (6–8, 13). In a recent study, the correlation between bacterial population and their possible defects was examined. Salted skins with red and yellow areas, mucoid appearance, bad smell and hair slip were demonstrated to have 10^5–10^8 colony forming units (CFU) g^{-1} moderately halophilic bacteria (15). Moreover, in other studies the presence of Gram-positive moderately halophilic isolates was found to be higher than Gram-negative moderately halophilic isolates on salted sheep and goat skins (6, 7). It was reported that 41 isolates were Gram-positive and 36 isolates were Gram-negative out of 77 moderately halophilic bacteria isolated from salted sheep skins (6). In another study, 32 Gram-positive and 7 Gram-negative moderately halophilic bacteria were isolated from salted goat skin samples (7). These studies showed that Gram-positive moderately halophilic bacteria are abundant on salted skins.

It is of great importance for the leather industry to control moderately halophilic bacteria in order to gather maximum high-quality yield. A variety of methods against halophilic bacteria such as direct electric current, antimicrobial agents or bacterial toxins (7, 24–26) have been examined in the literature. The antibacterial efficacy of lichens is known, but there is a lack of literature on their activities against halophilic bacteria. Lichens are symbiotic organisms consisting of algae and fungus. These organisms produce some secondary metabolites with various biological activities. Recently, several lichen extracts have been examined against *Bacillus* species and *Enterococcus durans* isolated from soak liquor samples. A mixed culture of soak liquor and tank surface samples were tested with lichen extracts. These studies indicated that lichen extracts are successful for controlling bacterial growth (27–31).

Taking into consideration the potential harmful effects of proteolytic and lipolytic Gram-positive moderately halophilic bacteria, two species, *Staphylococcus saprophyticus* subsp. *saprophyticus* (TR5) and *Salinicoccus roseus* (KV3), which were isolated in a previous study (6), were selected as test bacteria. The main goal of this study is to examine the potential antibacterial efficacy of selected lichen species (*Usnea* sp., *Platismatia glauca, Ramalina farinacea, Evernia divaricata, Bryoria capillaris, Hypogymnia tubulosa, Pseudevernia furfuracea* and *Lobaria pulmonaria*) against these moderately halophilic bacteria.

2. Materials and Methods

2.1 Moderately Halophilic Test Bacteria

TR5 and KV3, which were stored in the culture collections of the Division of Plant Diseases and Microbiology, Biology Department, Faculty of Arts and Sciences, Marmara University, Turkey, were selected and used as test bacteria in the present study. These were isolated from two salted sheepskin samples imported from Turkey and Kuwait and identified with molecular methods in the study of Caglayan *et al*. (6).

2.2 Lichen Samples

P. glauca, R. farinacea, E. divaricata, B. capillaris, H. tubulosa, Usnea sp., *P. furfuracea* and *L. pulmonaria* were collected from Bursa Aladağ region (**Figure 1**). The classical taxonomic method *via* microscopic examination was utilised in the identification of lichen samples. Stereomicroscope and light microscope were used for morphological and anatomical features. Anatomical features such as colour, thickness, size and shape of structural units were evaluated. The identification of lichens was made according to the procedure described by Smith *et al*. (32).

2.3 Extraction of Lichen Samples

Following washing and drying, samples were kept in sterile bottles including acetone (ACS, ISO, Reag. Ph. Eur.) in a dark place for 24 h. 100 ml of acetone solvent was added onto 10 g of lichen sample. Then, samples were filtered through filter paper. The acetone was evaporated by a rotary evaporator. After the evaporation process, total yield quantities were calculated for the extracts of *Usnea* sp., *B. capillaris, E. divaricata, H. tubulosa, P. furfuracea, R. farinacea* and *P. glauca* as 18.82 mg, 17.37 mg, 14.27 mg, 10.36 mg, 9.14 mg, 6.18 mg and 4.64 mg, respectively. The acetone extracts were stored until use at +4°C.

2.4 Antibacterial Tests

The bacterial growth of TR5 and KV3 was ensured by Tryptic soy agar supplemented with salt (100 g l^{-1}) and yeast extract (2.5 g l^{-1}) at 37°C for 24 h. In the antibacterial tests, Tryptic soy broth containing salt and yeast extract and 96-well CELLSTAR®, F-bottom microplates with lid (Greiner Bio-One GmbH, Austria) were used. The experiments were designed in four groups as a blank group (only medium), control (untreated group, medium and bacteria), antibiotic treatment group and lichen extract treatment groups (medium, bacteria and lichen extracts). The medium (Tryptic soy broth including salt and yeast extract) was put into each well in 96-well microplates. Then the tested lichen extracts were added. To make serial dilution, two-fold dilution concentrations of the tested lichen extract were made in every subsequent well and then overnight bacterial cultures of KV3 and TR5 adjusted to 0.02 McFarland with an optical density (OD) 600 nm were added to the wells.

Firstly, the antibacterial efficacy of acetone extracts of all lichen samples were tested for five dilutions. However, some lichen sample extracts were found to be effective at the fifth dilution so, to evaluate the antibacterial efficacy for lower concentrations, dilutions were made up to 10 dilution for these samples. Therefore, acetone extracts were applied at the concentrations of: 240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1} and 15 µg ml^{-1}

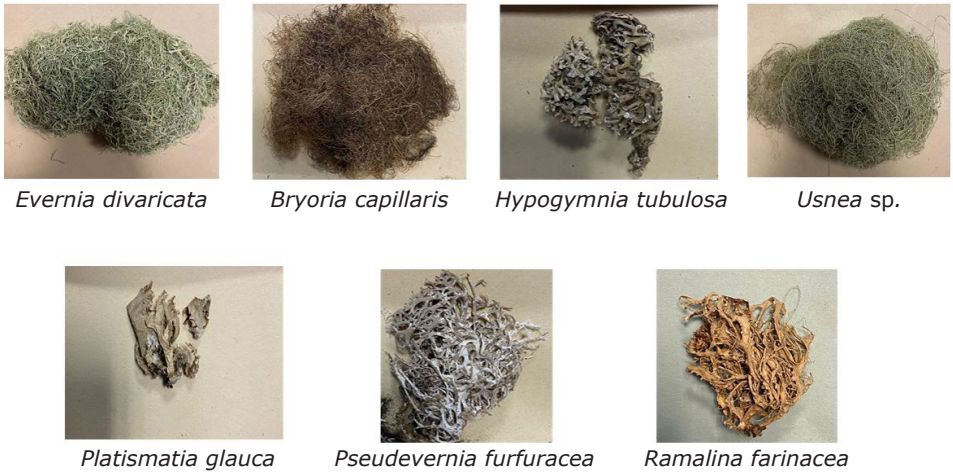

Fig. 1. The pictures of lichen samples. *P. glauca, R. farinacea, E. divaricata, B. capillaris, H. tubulosa, Usnea* sp. and *P. furfuracea*: Turkey, Bursa Aladağ province, N40^{0}06.397', E029^{0}17.494', G. Cobanoglu

(five dilutions) or 240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1}, 7.5 µg ml^{-1}, 3.75 µg ml^{-1}, 1.875 µg ml^{-1}, 0.9375 µg ml^{-1} and 0.46875 µg ml^{-1} (10 dilutions). In the antibiotic treatment groups, kanamycin, gentamicin, apramycin, vancomycin, chloramphenicol, erythromycin, tetracycline, penicillin, streptomycin and rifampicin were tested for screening with the disc diffusion method. The vancomycin and gentamycin were determined to be effective against both tested bacteria.

Experiments were done in triplicate. Three experiments were conducted for each screening experiment to determine both extract efficacy and effective concentrations. The bacterial growth was evaluated every 20 min for 24 h using Cytation™ 3 Multi-Mode microplate reader (BioTek Instruments Inc, USA), by measuring the absorbance. The results were given according to the difference between the optical densities of the bacterial suspensions with and without the extract treatment (untreated group) and the inhibition rates were calculated depending on the OD values of suspensions by subtracting OD values of the medium. The antibacterial effects of acetone extracts of lichen samples against the test samples were compared with the control samples.

2.5 Statistical Analysis

Statistical analyses were evaluated by IBM® SPSS® version 16.0 software program with one-way analysis of variance (ANOVA) (Tukey) to find significant differences between varying concentration groups of extract and untreated groups. A p value below 0.05 was accepted as significant. The same letters in the figures indicate that there is no significant difference between the concentrations, while different letters indicate a significant difference.

3. Results

In this study, acetone extracts of lichen samples identified as *P. glauca, R. farinacea, E. divaricata, B. capillaris, H. tubulosa, Usnea* sp., *P. furfuracea* and *L. pulmonaria* based on morphological and anatomical features were evaluated for their antibacterial activities against two moderately halophilic bacteria from salted sheepskin samples. The test concentrations applied in the experiments were 240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1} and 15 µg ml^{-1} (five dilutions) or 240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1}, 7.5 µg ml^{-1}, 3.75 µg ml^{-1}, 1.875 µg ml^{-1}, 0.9375 µg ml^{-1} and 0.46875 µg ml^{-1} (10 dilutions).

Before the experimental study design, some preliminary experiments were carried out to examine the presence of antibacterial efficacy. Then, samples having potential antibacterial efficacy were screened up to five or ten dilutions. Acetone was selected as the solvent in order to extract the most active compounds having potential antibacterial efficacy. Acetone is a preferred solvent due to its capability to dissolve both polar and nonpolar compounds.

No antibacterial effect for the acetone extracts of *L. pulmonaria, B. capillaris* and *P. furfuracea* was recorded for TR5 and KV3 during preliminary screening studies. For this reason, the figures belonging to the acetone extracts of *L. pulmonaria, B. capillaris, P. glauca* and *P. furfuracea* are not included in this paper. On the other hand, the extracts of *R. farinacea, Usnea* sp., *E. divaricata* and *H. tubulosa* had high antibacterial efficacies at certain concentrations against the test bacteria.

According to our results, acetone extracts of *H. tubulosa*, *R. farinacea*, *E. divaricata* and *Usnea* sp. were found to successfully suppress the bacterial growth of TR5. Extracts of *L. pulmonaria, B. capillaris, P. furfuracea* and *P. glauca* had no efficacy against TR5. Four tested concentrations (240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1} and 30 µg ml^{-1}) of the acetone extracts of *H. tubulosa* had high antibacterial efficacies against TR5 with inhibition percentages of 93.71 ± 2.68%, 94.05 ± 0.68%, 94.59 ± 0.27% and 94.72 ± 0.75%, respectively. The 15 µg ml^{-1} treatment group had no notable antibacterial activity against TR5 with an inhibition ratio of only 32.50 ± 6.84% (**Figure 2**). No statistically significant difference was detected among the 240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1} and 30 µg ml^{-1} groups. In addition, statistically significant differences were found when the control group and all treatment groups were compared (p<0.05) (**Figure 2**).

Antibacterial efficacy was lower for the extracts of *R. farinacea* against TR5. The inhibition percentages were 70.95 ± 9.75%, 76.10 ± 1.85%, 48.20 ± 4.39%, 23.86 ± 5.48% and 18.36 ± 0.64% for all tested concentrations, respectively. From these results, test concentrations of 240 µg ml^{-1} and 120 µg ml^{-1} may be evaluated as slightly effective for controlling growth of TR5. According to statistical analyses, there was no difference between 240 µg ml^{-1} and 120 µg ml^{-1} and also 30 µg ml^{-1} and 15 µg ml^{-1} treatment groups. All groups were statistically different in comparison to control group (p<0.05) (**Figure 3**).

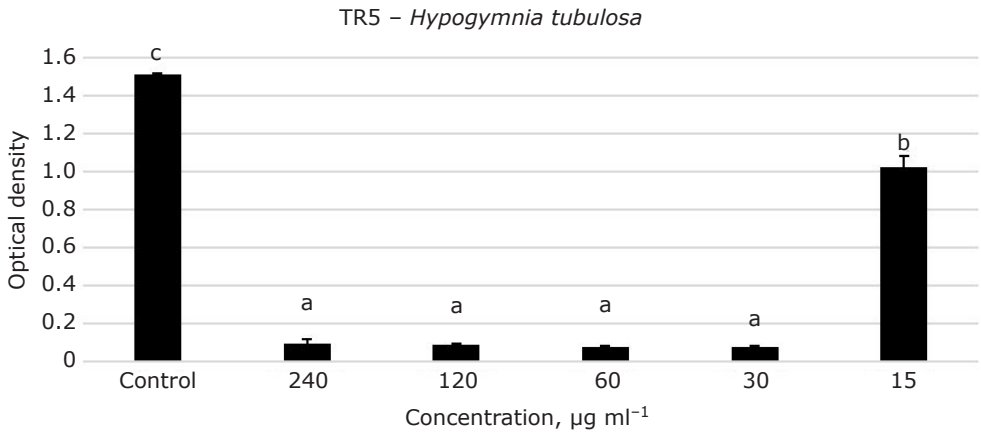

Fig. 2. The antibacterial effects of the extracts of *H. tubulosa* against TR5

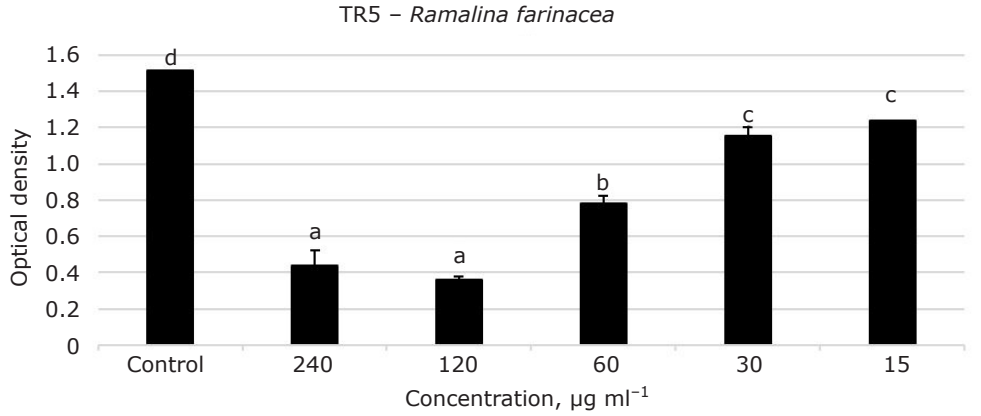

Fig. 3. The antibacterial effects of the extracts of *R. farinacea* against TR5

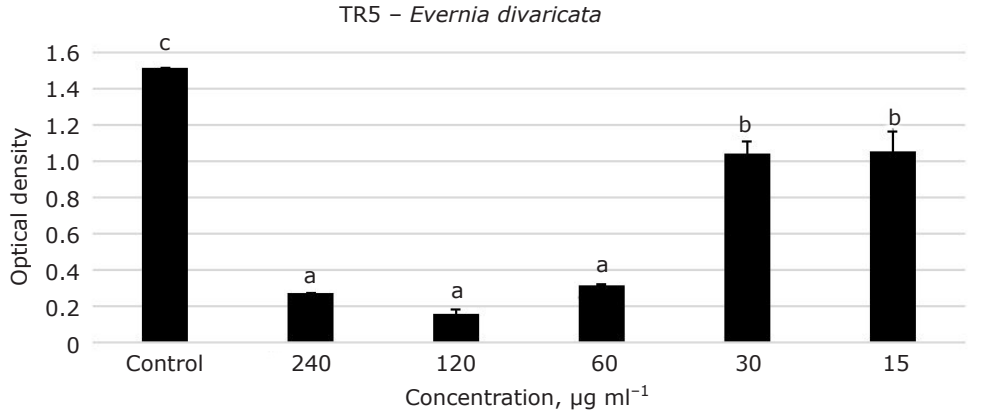

Fig. 4. The antibacterial effects of the extracts of *E. divaricata* against TR5

The acetone extracts of *E. divaricata* at concentrations of 240 µg ml^{-1}, 120 µg ml^{-1} and 60 µg ml^{-1} inhibited bacterial growth of TR5 with inhibition ratios of 82.02 ± 0.14%, 89.49 ± 2.26% and 79.42 ± 1.14%. At lower concentrations, little suppression was detected on the growth (31.38% and 30.53%, respectively). Statistical analyses revealed that there was no significant difference among 240 µg ml^{-1}, 120 µg ml^{-1} and 60 µg ml^{-1} and also between 30 µg ml^{-1} and 15 µg ml^{-1} treatment groups. All groups were significantly different when compared to controls (p<0.05) (**Figure 4**).

The extracts belonging to *Usnea* sp. from 240 µg ml^{-1} to 7.5 µg ml^{-1} showed great inhibitory effect against TR5. The inhibition percentages for the tested concentrations of 240 µg ml^{-1} to 7.5 µg ml^{-1} were respectively: 99.36 ± 0.04%, 85.16 ± 2.75%, 97.81 ± 0.78%, 98.25 ± 0.26%, 98.12 ± 0.23% and 97.19 ± 0.54%. Inhibition ratios were observed to be below 50% for 3.75 µg ml^{-1},

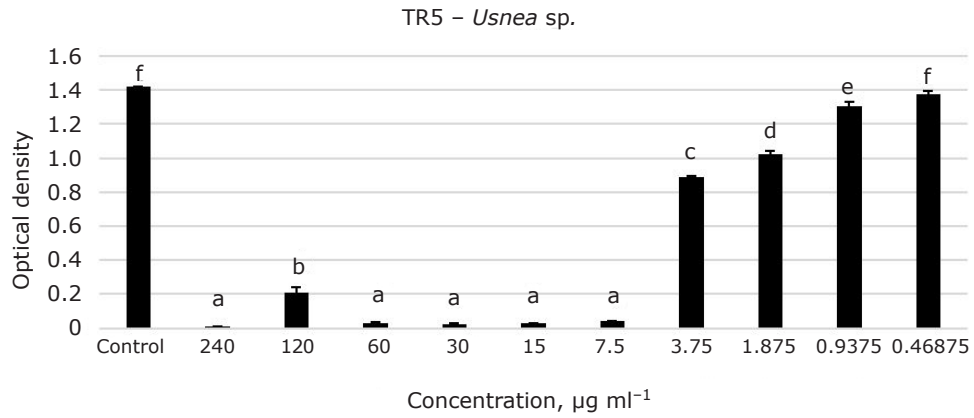

Fig. 5. The antibacterial effects of the extracts of *Usnea* sp. against TR5

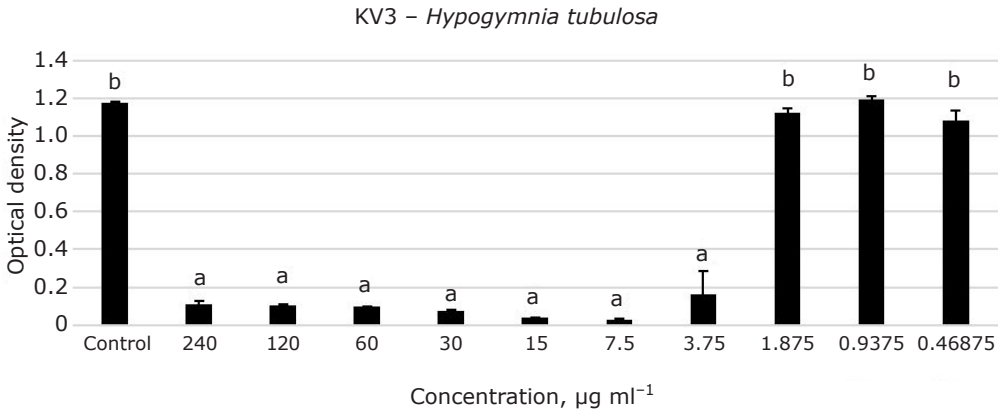

Fig. 6. The antibacterial effects of the extracts of *H. tubulosa* against KV3

1.875 µg ml^{-1}, 0.9375 µg ml^{-1} and 0.46875 µg ml^{-1} concentrations (37.56 ± 0.49%, 28.08 ± 1.86%, 8.07 ± 2.82% and 3.15 ± 2.70%, respectively). When compared to the control group, all treatment groups were found to be significantly different except 0.46875 µg ml^{-1} (p<0.05). In group comparisons, there was no statistically significant difference among 240 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1} and 7.5 µg ml^{-1} (**Figure 5**).

Similar to the results obtained in TR5, there was considerable inhibition by the extracts of *H. tubulosa*, *R. farinacea*, *E. divaricata* and *Usnea* sp. against KV3, especially by the acetone extracts of *H. tubulosa.* Significant inhibition was achieved even at very low concentrations including the seventh dilution (3.75 µg ml^{-1}). The inhibition ratios were recorded as: 90.59 ± 2.68%, 90.99 ± 0.41%, 91.56 ± 0.18%, 93.57 ± 0.27%, 96.86 ± 0.32%, 97.44 ± 0.14% and 86.26 ± 18.25%, respectively. The lower concentrations had no inhibition against KV3. In statistical analyses, there were significant differences in 240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1}, 7.5 µg ml^{-1} and 3.75 µg ml^{-1} treatment groups when compared to control groups (p<0.05). There was no significant difference between treatment groups of 240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1}, 7.5 µg ml^{-1} and 3.75 µg ml^{-1}. Likewise, no statistically significant difference was detected among 1.875 µg ml^{-1}, 0.9375 µg ml^{-1}, 0.46875 µg ml^{-1} and control groups (**Figure 6**).

Acetone extracts of *R. farinacea* showed noticeable inhibition for the first four of the concentrations (240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1} and 30 µg ml^{-1}). The inhibition percentages for these concentrations were respectively recorded as: 92.73 ± 3.86%, 92.17 ± 1.26%, 95.92 ± 0.29% and 93.79 ± 1.10%. The inhibition rates for the concentrations 240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1} and 30 µg ml^{-1} were not statistically different, indicating dose-independent inhibition. However, these treatment groups and the 15 µg ml^{-1} group were statistically different when compared to the control group (p<0.05). Lower concentrations below 30 µg ml^{-1} had no appreciable efficacy against KV3 (inhibition ranging from 19.86 ± 5.28% to 1.91 ± 2.57%). No significant difference was found for the sixth dilution and below (7.5 µg ml^{-1}, 3.75 µg ml^{-1}, 1.875 µg ml^{-1}, 0.9375 µg ml^{-1} and 0.46875 µg ml^{-1}) in comparison to the control group (**Figure 7**).

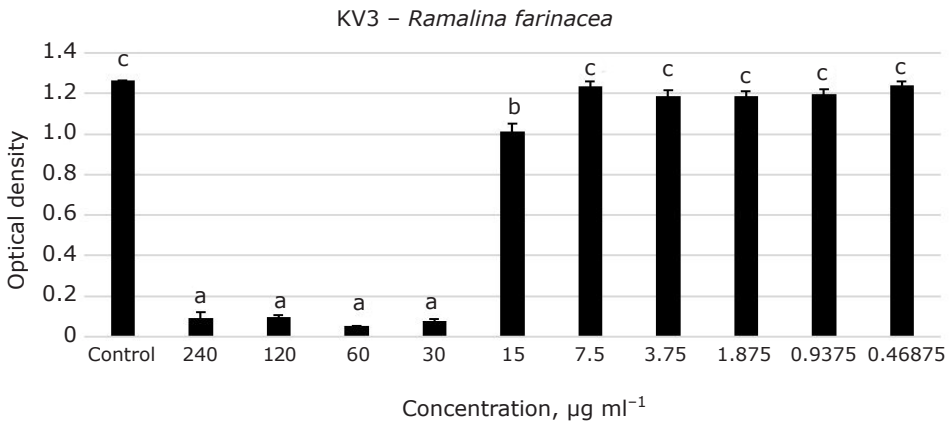

Fig. 7. The antibacterial effects of the extracts of *R. farinacea* against KV3

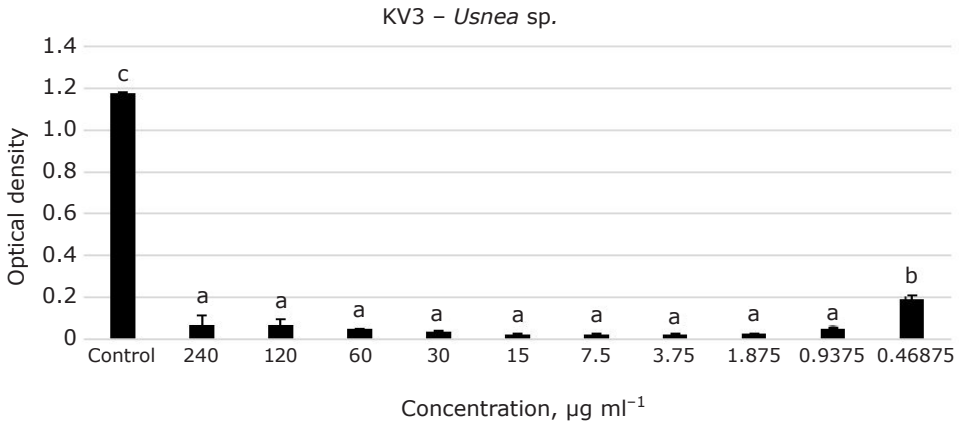

Fig. 8. The antibacterial effects of the extracts of *Usnea* sp. against KV3

The most remarkable results of the present study were obtained with *Usnea* sp. against KV3. As seen in **Figure 8**, all tested concentrations were highly effective to suppress the growth of KV3. Even the tenth dilution (0.46875 µg ml^{-1}) of the tested lichen extract had noticeable inhibition rates. The inhibition percentages were: 94.25 ± 6.98%, 94.02 ± 3.65%, 95.75 ± 0.30%, 96.88 ± 0.38%, 97.88 ± 0.22%, 97.97 ± 0.39%, 97.85 ± 0.29%, 97.57 ± 0.06%, 95.57 ± 0.49% and 83.55 ± 2.71% for all tested concentrations, respectively. There was no statistically significant difference between the first nine concentrations (240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1}, 7.5 µg ml^{-1}, 3.75 µg ml^{-1}, 1.875 µg ml^{-1} and 0.9375 µg ml^{-1}), but they were found to be significantly different compared to controls (p<0.05). The 0.46875 µg ml^{-1} treatment group also had statistically significant difference when compared to controls (p<0.05) (**Figure 8**).

The acetone extracts of *E. divaricata* also gave remarkable results, although not as much as *Usnea* sp. The bacterial growth of KV3 was suppressed by these extracts up to the sixth dilution. At the first six concentrations tested (240 µg ml^{-1}, 120 µg ml^{-1}, 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1} and 7.5 µg ml^{-1}), the inhibition percentages were recorded between 97.58 ± 0.53% and 72.72 ± 6.01%. The tested concentrations below 3.75 µg ml^{-1} had no considerable inhibition ratios for KV3. In statistical analyses, there were significant differences among 240 µg ml^{-1} *vs*. control, 120 µg ml^{-1} *vs*. control, 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1}, 7.5 µg ml^{-1} treatment groups *vs*. control, and 3.75 µg ml^{-1}, 1.875 µg ml^{-1}, 0.9375 µg ml^{-1}, 0.46875 µg ml^{-1} *vs*. control (p<0.05). On the other hand, no statistical differences were observed among the 60 µg ml^{-1}, 30 µg ml^{-1}, 15 µg ml^{-1} and 7.5 µg ml^{-1} treatment groups. The same situation was recorded among the treatment groups of 3.75 µg ml^{-1}, 1.875 µg ml^{-1}, 0.9375 µg ml^{-1} and 0.46875 µg ml^{-1} (**Figure 9**).

4. Discussion

It is known that moderately halophilic bacteria, mostly from salt, can be found in high numbers on hides or skins. Due to their ability to produce enzymes such as protease and lipase that have devastating effects on hides and skins, more attention should be paid to these bacterial populations in leather production processes and methods should be applied to prevent their

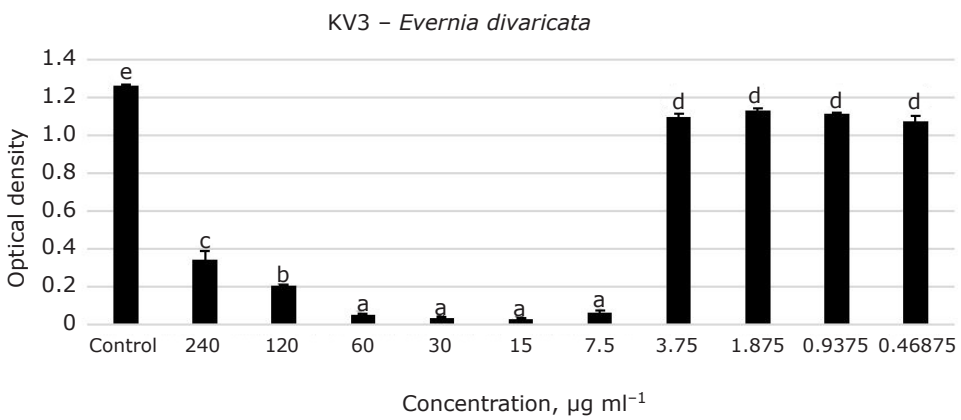

Fig. 9. The antibacterial effects of the extracts of *E. divaricata* against KV3

excessive growth. Given the high population and possibly high degradative effects of halophilic bacteria, salt curing does not seem to be the only solution to control them. Alternative strategies have to be investigated in order to obtain high-quality leather for economic benefit.

For this purpose, many researchers have tested various possible antimicrobial substances against halophilic bacteria isolated from the leather industry. Halocins, which are antimicrobial peptides produced by halophilic archaea, have been evaluated to control halophilic archaea with possibly degradative properties on leather and the potential efficacy of halocins against extremely halophilic bacteria has been reported (33, 34). Vreeland *et al*. indicated the antimicrobial effect of bile salt solution (0.025 g 100 ml^{-1}) against *Haloarcula hispanica, Haloferax gibbonsii* and *Haloferax mediterranei* and showed the potential protective effect of bile salt solutions on cured hides up to 45 days (35). Gehring *et al*. evaluated porcine bile in brine-curing solution against halophilic archaeal strains with positive results (36).

The potential suppressive effect of electric current application has been reported against extremely halophilic archaea and moderately halophilic bacteria (5, 25). Caglayan *et al*. reported the antibacterial efficiency of different electric current applications on moderately halophilic *Staphylococcus saprophyticus, Bacillus pumilus, Bacillus licheniformis, Gracilibacillus dipsosauri* and *Idiomarina loihiensis* isolated and identified from salted skins (37). In another study, different levels of direct and alternating electric current treatments were examined for preventive effects against skin deterioration and the inactivation of the growth of a mixed moderately halophilic bacterial culture including *Chromohalobacter israelensis, Chromohalobacter canadensis, Halomonas halodenitrificans, Staphylococcus nepalensis* and *Halomonas halmophila* isolated from salted sheep and goat skin samples (38).

In the literature, there are also studies investigating plant materials as eco-benign materials for their possible antibacterial effects. Sivakumar *et al*. reported the antimicrobial activity of myrobalan (*Terminalia chebula* Retz.) application along with salt utilisation on the short-term preservation of raw hides and skins (39). It is well known that lichen substances have potential antibacterial activities. To the best of our knowledge, the antibacterial efficacy of the acetone extracts of lichen species against moderately halophilic bacteria has not been studied in the literature.

In this study, acetone extracts of *L. pulmonaria, B. capillaris* and *P. furfuracea* had no efficacy against our test bacteria. However, *H. tubulosa*, *R. farinacea*, *E. divaricata* and *Usnea* sp. extracts have noteworthy suppressive effects. *Usnea* sp. acetone extracts were observed to have the highest antibacterial activity against KV3 among the selected lichen species even at the lowest concentration tested. In the literature there are many studies of the antibacterial efficacy of *H. tubulosa*, *R. farinacea*, *E. divaricata* and *Usnea* sp. against various bacteria, especially Gram-positive ones. However, some Gram-negative bacteria are reported to be resistant to *Usnea* sp. Here, we determined the antibacterial effect of lichens on Gram-positive test bacteria.

More detailed studies report many chemical compounds for various lichen species. For example, *Usnea* sp. has usnic acid, thamnolic acid, atranorin and barbatic acid; *R. farinacea* has evernic acid, atranorin, usnic acid and chloroatranorin (40, 41); *P. furfuracea* was reported to have evernic acid, atranorin, usnic acid, physodalic acid and chloroatranorin. Some compounds seem to be common to many lichens, such as evernic acid, atranorin and chloroatranorin

(40, 42, 43). Atranorin is generally found in most lichen species including our tested lichen species. However, based on our results, atranorin does not appear to have selectivity for antibacterial activity against the tested bacterial strains since our screening experiments showed that not all of the tested lichen species were effective against KV3 and TR5. The presence of usnic acid has been indicated in the literature in some lichen species such as *Usnea* sp., *P. furfuracea, E. divaricata* and *R. farinacea.* Nevertheless, in our study *P. furfuracea* had no efficacy against our test bacteria which points to a lack of antibacterial efficacy of usnic acid.

The most successful lichen in our study was *Usnea* sp. In the literature, *Usnea* sp. has been reported to have other lichen metabolites such as thamnolic acid, barbatic acid, diffractic acid, evernic acid and squamatic acid. Its antibacterial efficacy may be due to one or more of the aforementioned metabolites, or the metabolites may have synergistic effect when applied together. Another possible scenario could be the difference in the amounts of lichen metabolites in different lichen species.

As seen in this study, extracts gathered from lichen species may exhibit varying efficacies against bacteria depending on the concentrations. Future studies to answer the question of which metabolite is effective on which bacteria may open up horizons. It can be difficult to predict what the effects on a mixed culture microorganism population might be. For example, in the mixed culture study performed by Berber *et al*. (27) when *Usnea* sp. acetone extracts were tested on the total bacterial population obtained from soaking liquors, successful results were obtained in some samples, but not in others.

5. Conclusions

The need for potent new antibacterial agents is emphasised in the literature. The presence of high populations of moderately halophilic bacteria on hides and skins inevitably leads to defects on finished products despite salt- or brine-curing methods applied to raw hides and skins. Many lichen species have been used for years for various purposes including their antibacterial potential. This study demonstrated that acetone extracts of *H. tubulosa*, *R. farinacea*, *E. divaricata* and *Usnea* sp. have antibacterial effects against certain moderately halophilic bacteria. Noteworthy antibacterial activity was detected in *Usnea* sp. acetone extracts against KV3 even at the lowest concentration tested. These results suggest that proteolytic and lipolytic moderately halophilic bacteria may be controlled by these ecological materials. Chemical analyses will be needed to determine which compound(s) are responsible for the antibacterial efficacy of the lichen species. These compounds could be applied onto hides and skins in microencapsulated or sprayed forms along with salt or brine during the storage period of raw stock.

Acknowledgements

We are thankful to Professor Gulsah Cobanoglu (Marmara University, Turkey) for identification of lichen species. Also, we would like to thank Arhun Ali Balkan (Marmara University, Turkey) for his help during the experiments.

References

1. M. A. Moktadir, H. B. Ahmadi, R. Sultana, F.-T. Zohra, J. J. H. Liou and J. Rezaei, *J. Clean. Prod.*, 2020, **251**, 119737
2. J. Wu, L. Zhao, X. Liu, W. Chen and H. Gu, *J. Clean. Prod.*, 2017, **148**, 158
3. P. S. Vankar and A. K. Dwivedi, *Desalination*, 2009, **249**, (1), 158
4. O. Ozyaral and M. Birbir, *J. Soc. Leather Technol. Chem*., 2005, **89**, (6), 237
5. Y. Birbir, S. Anik, M. Birbir and P. Caglayan, *Johnson Matthey Technol. Rev.*, 2015, **59**, (2), 109
6. P. Caglayan, M. Birbir, C. Sánchez-Porro and A. Ventosa, *J. Am. Leather Chem. Assoc.,* 2017, **112**, (6), 207
7. P. Caglayan, M. Birbir, C. Sánchez-Porro and A. Ventosa, *Turk. J. Biochem*., 2018, **43**, (3), 312
8. P. Caglayan, M. Birbir and A. Ventosa, 'A Survey Study to Detect Problems on Salted Hides and Skins', ICAMS 2018: 7th International Conference on Advanced Materials and Systems, Bucharest, Romania, 18th–20th October, 2018, pp. 409–414
9. E. Yilmaz and M. Birbir, *J. Am. Leather Chem. Assoc.,* 2019, **114**, (4), 118
10. M. Birbir, P. Caglayan and Y. Birbir, *Johnson Matthey Technol. Rev.*, 2020, **64**, (4), 489
11. M. Birbir, K. Kizilkaya and P. Caglayan, *J. Soc. Leather Technol. Chem.*, 2021, **105**, (5), 237
12. W. E. Kallenberger and R. M. Lollar, *J. Am. Leather Chem. Assoc.,* 1986, **81**, (8), 248
13. D. G. Bailey and M. Birbir, *J. Am. Leather Chem. Assoc.,* 1993, **88**, (8), 291
14. M. Birbir, *J. Turk. Microbiol. Soc.*, 1997, **27**, 68

15. C. Akpolat, A. Ventosa, M. Birbir, C. Sánchez-Porro and P. Caglayan, *J. Am. Leather Chem. Assoc.*, 2015, **110**, (07), 211
16. A. Ramos-Cormenzana, 'Ecology of Moderately Halophilic Bacteria', in "The Biology of Halophilic Bacteria", eds. R. H. Vreeland and L. I. Hochstein, Ch. 3, CRC Press Inc, Boca Raton, USA, 1993, 32 pp
17. F. Mohammadipanah, J. Hamedi and M. Dehhaghi, 'Halophilic Bacteria: Potentials and Applications in Biotechnology', in "Halophiles: Biodiversity and Sustainable Exploitation", eds. D. K. Maheshwari and M. Saraf, Ch. 11, Springer International Publishing, Dordrecht, Switzerland, 2015, pp. 277–321
18. C. Sanchez-Porro, S. Martin, E. Mellado and A. Ventosa, *J. Appl. Microbiol.*, 2003, **94**, (2), 295
19. R. Rohban, M. A. Amoozegar and A. Ventosa, *J. Ind. Microbiol. Biotechnol.*, 2009, **36**, (3), 333
20. A. Ventosa, J. J. Nieto and A. Oren, *Microbiol. Mol. Biol. Rev.*, 1998, **62**, (2)
21. R. R. de la Haba, P. Yilmaz, C. Sánchez-Porro, M. Birbir and A. Ventosa, *Syst. Appl. Microbiol.*, 2011, **34**, (6), 435
22. C. Sánchez-Porro, P. Yilmaz, R. R. de la Haba, M. Birbir and A. Ventosa, *Int. J. Syst. Evol. Microbiol.*, 2011, **61**, (5), 1206
23. P. Caglayan, C. Sánchez-Porro, A. Ventosa and M. Birbir, *J. Soc. Leather Technol. Chem.,* 2015, **99**, (5), 250
24. Y. Birbir and M. Birbir, *J. Electrostat.*, 2006, **64**, (12), 791
25. P. Caglayan, M. Birbir, A. Ogan, A. Ventosa, C. Sánchez-Porro and Y. Birbir, *J. Soc. Leather Technol. Chem.*, 2019, **100**, (5), 307
26. S. A. Hussain, M. I. Sarker and H. O. Yosief, *Lett. Appl. Microbiol.*, 2020, **70**, (3), 159
27. D. Berber, İ. Türkmenoğlu and N. C. Sesal, *J. Am. Leather Chem. Assoc.*, 2020, **115**, (10), 365
28. D. Berber, İ. Türkmenoğlu and N. C. Sesal, *Johnson Matthey Technol. Rev.*, 2020, **64**, (4), 480
29. D. Berber, *J. Am. Leather Chem. Assoc.*, 2020, **115**, (03), 96
30. D. Berber, *Int. J. Adv. Eng. Pure Sci.,* 2020, **32**, (3), 251
31. D. Berber, İ. Türkmenoğlu, M. Birbir and N. C. Sesal, *J. Am. Leather Chem. Assoc.*, 2020, **115**, (6), 222
32. "The Lichens of Great Britain and Ireland", eds. C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James and P. A. Wolseley, The British Lichen Society, London, UK, 2009, 1046 pp
33. M. Birbir, S. Eryilmaz and A. Ogan*, J. Soc. Leather Technol. Chem.,* 2004, **88**, (3), 99
34. M. Birbir and S. Eryilmaz, *J. Soc. Leather Technol. Chem.*, 2007, **91**, (2), 69
35. R. H. Vreeland, D. G. Bailey and R. W. Claunch, The United States of America as represented by the Secretary of Agriculture, Washington, DC, USA and Southeast Applied Research, New Orleans, USA, US Patent 5,945,027; 1999
36. A. G. Gehring, D. G. Bailey, R. F. Caveng and R. H. Vreeland, *J. Liq. Chromatogr. Relat. Technol.*, 2003, **26**, (7), 1041
37. P. Caglayan, Y. Birbir, M. Birbir, C. Sánchez-Porro and A. Ventosa, 'PP 13: Inactivation of Antibiotic-Resistant Species Belonging to Staphylococcus, Bacillus, Gracilibacillus and Idiomarina via Electric Current', Fourth International Meeting on Pharmacy and Pharmaceutical Sciences, Istanbul, Turkey, 18th–21st September, 2014, Marmara University, Faculty of Pharmacy, Istanbul, Turkey, 2014
38. P. Caglayan, M. Birbir, C. Sánchez-Porro, A. Ventosa and Y. Birbir, *J. Am. Leather Chem. Assoc.,* 2018, **113**, (2), 41
39. V. Sivakumar, R. Mohan, T. Rangasamy and C. Muralidharan, *Indian J. Nat. Prod. Resour*., 2016, **7**, (1), 65
40. N. Aoussar, F. E. Laasri, M. Bourhia, N. Manoljovic, R. A. Mhand, N. Rhallabi, R. Ullah, A. A. Shahat, O. M. Noman, F. A. Nasr, O. M. Almarfadi, M. El Mzibri, P. Vasiljević, L. Benbacer and F. Mellouki, *Evidence-Based Complement. Altern. Med.*, 2020, 8104538
41. A. Sepahvand, E. Studzińska-Sroka, P. Ramak and V. Karimian, *J. Ethnopharmacol.*, 2021, **268**, 113656
42. N. Aoussar, R. Manzali, I. Nattah, N. Rhallabi, P. Vasiljevic, M. Bouksaim, A. Douira, N. Manojlović and F. Mellouki, *J. Mater. Environ. Sci.*, 2017, **8**, (6), 1968
43. N. Aoussar, M. Achmit, Y. Es-sadeqy, P. Vasiljević, N. Rhallabi, R. A. Mhand, K. Zerouali, N. Manojlović and F. Mellouki, *Arch. Microbiol.*, 2021, **203**, (6), 2887

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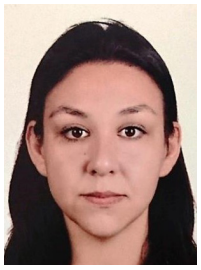

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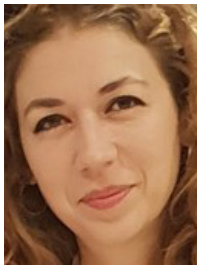

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