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

Assessment of potential antibacterial efficiency of acetone extracts from eight lichen species against Gram-positive moderately halophilic bacteria which were isolated from salted sheep skin samples

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ABSTRACT

Considering the global economic importance of the leather sector in world trade, overcoming the microbiological problems, especially arise 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 efficiency against 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 240-15 µg/ml 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/skins.

Keywords: Antibacterial, lichen extract, moderately halophilic bacteria, skins, leather, *Staphylococcus saprophyticus* subsp. *saprophyticus*, *Salinicoccus roseus.*

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1. Introduction

By-product of various industries have been used as a natural raw materials for many years. The demand for natural resources is continuously increased in the world (1). The raw materials such as skins and hides are by-products of meat industry and are converted into valuable leather products such as bag, shoes, wallet, briefcases, backpacks in tanneries (2). Due to the global economic importance of leather industry, high quality leather products should be produced by processing by-products from meat industry for leather. To obtain huge value in leather products, raw materials (hides and skins) should be properly preserved. Since raw hides or skins have high water and protein content, these raw materials become vulnerable to bacterial activities. To overcome this bacterial deterioration on hides/skins, raw hides/skins are traditionally cured with salt or brine after animal is slaughtered (3). These undesirable bacterial population may consist of halophilic or non-halophilic ones which may come from animal itself or various environments as contaminants. Additionally, salt which is used in salt curing process may contain halotolerant microorganisms, slightly halophilic bacteria, moderately halophilic bacteria, extremely halophilic archaea and fungi which can contaminate raw skins/hides (4-11).

Previous studies demonstrated that halophilic microorganisms on skins and hides are responsible from red heat, red discolorations, holes, problems on grain surface and deterioration on hides/skins (12-15).

Amongst these bacterial groups, moderately halophilic bacteria include a wide variety of bacteria (16). These bacteria may be Gram-negative or Gram-positive, aerobic or

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facultative anaerobic. It has been reported that these bacteria may abundantly grow in saline systems such as saltern crystallizer ponds, saline soils, Dead Sea, evaporated ponds. Moderately halophilic microorganisms may secrete different enzymes such as proteases, lipases, cellulases, chitinases etc. (17-19). These microorganisms may grow under conditions at 3%-15% NaCl concentrations, 0-45°C and 5-10 pH values (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 producer moderately halophilic bacterial population are undesirable in the leather industry due to their potential defects on the final product and possible economic losses.

There are some studies examining which species of moderately halophilic bacteria can grow on the hides/skins (5-8, 15, 21-23). With detailed studies, new species belonging to the moderately halophilic bacteria can be identified more easily with the advantages of molecular techniques and phenotypic characterization methods. 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). On the other hand, there are also studies focused on bacterial numbers of moderately halophilic microorganisms and their potential defects on hides. In the studies investigating abundancy of moderately halophilic bacteria on hide samples, the results showed that they were considerably in high numbers and such a large population could possibly cause hide/skin damages (6-8, 13). In a recent study, the correlation between this bacterial population and their possible defects was examined. The salted skins with red and yellow areas, mucoid appearance, bad smell,

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hair slips were demonstrated to have 10^5-10^8 CFU/g moderately halophilic bacteria (15). Moreover, in other studies the presence of Gram-positive moderately halophilic isolates was found higher than Gram-negative moderately halophilic isolates on the salted sheep skins and goat skins (6, 7). It was reported that 41 isolates were Gram-positive, and 36 isolates were Gram-negative of 77 moderately halophilic bacteria isolated from salted sheep skins (6). In addition, 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. In this respect, it is of great importance for the leather industry to control moderately halophilic bacteria in order to gather maximum and high-quality yield.

A variety of methods such as direct electric current, antimicrobial agents or bacterial toxins (7, 24-26) were examined against halophilic bacteria in the literature. Nowadays, antibacterial efficacy of lichens is known, but there is a lack of literature on their activities on halophilic bacteria. Lichens are symbiotic organisms consisting of algae and fungus. These organisms produce some secondary metabolites with various biological activities. More recently, several lichen extracts have been examined against some *Bacillus* species and *Enterococcus durans*, which were isolated from soak liquor samples. Furthermore, mixed culture of soak liquor and tank surface samples were tested with some lichen extracts. These studies indicated that lichen extracts are successful for controlling bacterial growth (27-31).

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Taken into consideration of 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 the previous study of (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

Staphylococcus saprophyticus subsp. *saprophyticus* (TR5) and *Salinicoccus roseus* (KV3), which were stored in the culture collections of 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 isolates were isolated from two salted sheepskin samples imported from Turkey and Kuwait and identified with molecular methods in the study of Caglayan et al. (2017) (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. The classical taxonomic method via microscopic examination was utilized in the identification of lichen samples. In the determination of lichen samples, stereomicroscope and light microscope were used for morphological, and anatomical features. In anatomical examinations, features

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such as color, thickness, size and shape of structural units were evaluated. The identification of lichens was made according to procedure described by Smith et al. (2009). (32)

P. glauca, R. farinacea, E. divaricata, B. capillaris, H. tubulosa, Usnea sp., *P. furfuracea* and *L. pulmonaria*: Turkey, Bursa Aladağ province, N40^{0}06.397', E029^{0}17.494', G. Cobanoglu.

Figure 1. The pictures of lichen samples.

2.3 Extraction of Lichen Samples

Following washing and drying steps of samples, they were kept in sterile bottles including acetone (ACS, ISO, Reag. Ph Eur) solvent in a dark place for 24 hours. 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*

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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 *Staphylococcus saprophyticus* subsp. *saprophyticus* (TR5) and *Salinicoccus roseus* (KV3) was ensured by Tryptic Soy Agar supplemented with salt (100g/L) and yeast extract (2.5 g/L) at 37°C for 24 h. In the antibacterial tests, Tryptic Soy Broth containing salt and yeast extract, and 96-well microplates (Greiner Bio-One, CellStar, F-bottom, with lid) were used. The experiments were designed in four groups as a blank group (only medium), control (untreated group, medium, and bacteria), antibiotic treatment groupand 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 culture of KV3 and TR5, which was adjusted to 0.02 Mc Farland with an optical density (OD) 600 nm were added to the wells. Firstly, the antibacterial efficacy of acetone extracts of all lichen samples was tested for 5 dilutions. However, some extracts of lichen samples were detected to be effective at the 5^{th} dilution and to evaluate the antibacterial efficacy also for lower concentrations, the dilutions were made up to 10 dilution for these samples. Therefore, the acetone extracts were applied at the concentrations of 240-120-60-30-15 µg/mL (5 dilutions) or 240-120-60-30-15-7,5-3,75-1,875-0,9375-0.46875 (10 dilutions) µg/mL. In the antibiotic treatment groups, kanamycin, gentamicin, apramycin, vancomycin, chloramphenicol, erythromycin, tetracycline,

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penicillin, streptomycin, and rifampicin were tested for screening with the disc diffusion method. The vancomycin and gentamycin were determined to be efficient 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 multimode microplate reader (Biotek), 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 ones.

2.5 Statistical Analysis

Statistical analyses were evaluated by SPSS version 16.0 software program with One-way 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, the lichen samples which were 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 of them were evaluated for their

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antibacterial activities against two moderately halophilic bacteria from salted sheepskin samples. The potential antibacterial efficacy for the acetone extracts of *P. glauca*, *H. tubulosa*, *R. farinacea*, *Usnea* sp., *E. divaricata*, *L. pulmonaria*, *B. capillaris*, and *P. furfuracea* was tested against both test bacteria. The test concentrations applied in the experiments were 240-120-60-30-15 µg/mL (5 dilutions) or 240-120-60-30-15-7.5-3.75-1.875-0.9375-0.46875 (10 dilutions) µg/mL. Before the experimental study design built, some preliminary experiments were carried out to examine the presence of antibacterial efficacy. Then, the samples having potential antibacterial efficiency, were screened up to 5 dilutions. Although some of the extracts have antibacterial potency up to 5 dilutions, others had efficacy up to 10 dilutions. The reason for the selection of acetone solvent is to get most of the active compounds having antibacterial efficacy with the extraction method. As known, 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 *S. saprophyticus* subsp. *saprophyticus* (TR5) and *S. roseus* (KV3) due to preliminary screening studies. For this purpose, the figures belonging to the acetone extracts of *L. pulmonaria, B. capillaris, P. glauca* and *P. furfuracea* are not included in the paper. On the other hand, the extracts of *R. farinacea, Usnea* sp.*, E. divaricata* and *H. tubulosa* had considerably high antibacterial efficacies at certain concentrations against test bacteria.

According to our results, the acetone extracts of *H. tubulosa*, *R. farinacea*, *E. divaricata* and *Usnea* sp. were found to be successful to suppress the bacterial growth of *S. saprophyticus* subsp. *saprophyticus* (TR5) amongst tested eight lichen species. On the

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other hand, the extracts of *L. pulmonaria, B. capillaris, P. furfuracea* and *P. glauca* had no efficacy against TR5. Four tested concentrations (240-30 µg/mL) of the acetone extracts of *H. tubulosa* had considerably high antibacterial efficacies against *S. saprophyticus* subsp. *saprophyticus* (TR5) at the inhibition percentages of 93.71±2.68, 94.05±0.68, 94.59±0.27, and 94.72±0.75, respectively. On the other hand, 15 µg/mL treatment group had no efficient antibacterial activity against TR5 with the inhibition ratio of 32.50±6.84% (Figure 1). No statistically significant difference was detected among 240, 120, 60, and 30 µg/mL groups. In addition, statistically significant differences were found when the control group and all treatment groups were compared ($p<0.05$) (Figure 1).

On the other hand, same antibacterial efficacy was not recorded for the extracts of *R. farinacea* against TR5. The inhibition percentages were detected as 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. In this meaning, test concentrations of 240 and 120 µg/mL may be evaluated as slightly efficient for controlling growth of *S. saprophyticus* subsp. *saprophyticus*. According to statistical analyses, there was no difference between 240 and 120 µg/mL and also 30 and 15 µg/mL treatment groups. All groups were statistically different in comparison to control group ($p<0.05$) (Figure 2).

The acetone extracts of *E. divaricata* at the concentrations of 240-60 µg/mL inhibited bacterial growth of *S. saprophyticus* subsp. *saprophyticus* (TR5) with the 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

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analyses revealed that there was no significant difference among 240, 120 and 60 µg/mL and also between 30 and 15 µg/mL treatment groups. All groups were significantly different when compared to controls ($p<0.05$) (Figure 3).

From 240 µg/mL to 3.75 µg/mL, a great inhibitory effect of the extracts belonging to *Usnea* sp. was observed against *S. saprophyticus* subsp. *saprophyticus* (TR5). The inhibition percentages for the tested concentrations of 240-3.75 µg/mL were respectively detected as 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. On the other hand, inhibition ratios were observed to be below 50% for 3.75-0.46875 µg/mL concentrations (37.56±0.49, 28.08±1.86, 8.07±2.82, 3.15±2.70%, respectively). When compared to control group, all treatment groups were found to be significantly different except 0.46875 µg/mL ($p<0.05$). In group comparisons, there was no statistically significant difference among 240-7.5 µg/mL except 120 µg/mL (Figure 4).

Similar to the results obtained in *S. saprophyticus* subsp*. saprophyticus* (TR5), there was considerably inhibition by the extracts of *H. tubulosa*, *R. farinacea*, *E. divaricata* and *Usnea* sp. against *S. roseus* (KV3). Considerably high inhibition for the growth of *S. roseus* was provided by the acetone extracts of *H. tubulosa.* A significant inhibition was achieved even at very low concentrations, including the 7th dilution (3.75 µg/mL) with the extracts. 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 *S. roseus.* In statistical analyses, there were significant differences in 240, 120, 60, 30, 15, 7.5 and 3.75

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µg/mL treatment groups when compared to control groups ($p<0.05$). On the other hand, there was no significant difference between treatment groups of 240, 120, 60, 30, 15, 7.5 and 3.75 µg/mL. Likewise, no statistically significant difference was detected among 1.875, 0.9375, 0.46875 and control groups (Figure 5).

According to the results obtained with the acetone extracts of *R. farinacea*, a noticeable inhibition was noted for the first four concentrations (240-30 µg/mL). 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. For concentrations of 240-30 µg/mL, these inhibition rates were not statistically different between them, indicating dose-independent inhibition. However, these treatment groups and also 15 µg/mL group were statistically different when compared to control group ($p<0.05$). On the other hand, lower concentrations tested below 30 µg/mL had no appreciable efficacy against *S. roseus* (inhibition ranging from 19.86±5.28-1.91±2.57). No significant difference was found for the 6^{th} dilution and below (7.5-0.46875 µg/mL) in comparison to control group (Figure 6).

The most remarkable results of the present study were obtained with *Usnea* sp. against *S. roseus*. As seen in Figure 7, all tested concentrations were highly effective for the suppression of the growth of *S. roseus*. Even the 10^{th} dilution (0.46875 µg/mL) of the tested lichen extract had noticeable inhibition rates. The inhibition percentages were noted as 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

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the first nine concentrations (240-0.9375 µg/mL), but they were found to be significantly different compared to controls ($p<0.05$). Also, 0.46875 µg/mL treatment group had also statistically significant difference when compared to controls ($p<0.05$) (Figure 7).

The acetone extracts of *E. divaricata* also gave remarkable results, although not as much as *Usnea* sp. The bacterial growth of *S. roseus* (KV3) suppressed by these extracts up to the 6th dilution. At the first six concentrations tested (240-7.5 µg/mL), the inhibition percentages were recorded between 97.58±0.53-72.72±6.01. The tested concentrations below 3.75 µg/mL, had no considerable inhibition ratios for KV3. In statistical analyses, there were significant differences among 240 µg/mL vs control, 120 µg/mL vs control, 60, 30, 15, 7.5 µg/mL treatment groups vs control, and 3.75, 1.875, 0.9375, 0.46875 µg/mL vs control group ($p<0.05$). On the other hand, no statistical differences were observed among 60, 30, 15 and 7.5 µg/mL treatment groups. Similarly, the same situation was recorded among the treatment groups of 3.75, 1.875, 0.9375, and 0.46875 µg/mL (Figure 8).

4. Discussion

As known, moderately halophilic bacteria, mostly come from salt, can be found in high numbers on the hides or skins. Due to their ability to produce enzymes such as protease and lipase that have devastating effects on hides/skins, more attention should be paid to these bacterial populations in leather production processes and some prevention methods should be applied for their excessive growth. Given the high population and possibly high degradative effects of halophilic bacteria, salt curing does

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not seem to be the only solution to control them. Alternative strategies have to be investigated in order to obtain high-quality leather, which is also effective on the economy of the countries. For this purpose, many researchers tested various possible antimicrobial substances against halophilic bacteria, which were isolated from the leather industry. Halocins, which are antimicrobial peptides produced by halophilic archaea, were evaluated to control halophilic archaea with possibly degradative properties on leather and the potential efficacy of halocins against extremely halophilic bacteria was reported (33, 34). Vreeland et al. (1999) indicated the antimicrobial effect of bile salt solution (0.025 g/100 mL) against *Haloarcula hispanica, Haloferax gibbonsii* and *Haloferax mediterranei* and showed the potential protective effect of bile salt solutions on the cured hides up to 45 days (35). Gehring et al. (2003) evaluated the porcine bile in brine-curing solution against halophilic archaeal strains and they gathered positive results (36). Moreover, the potential suppressive effect of electric current application was reported against extremely archaea and moderately halophilic bacteria (5, 25). Caglayan et al., (2014) reported the antibacterial efficiency of different electric current applications on moderately halophilic *Staphylococcus saprophyticus, Bacillus pumilus, Bacillus licheniformis, Gracilibacillus dipsosauri* and *Idiomarina loihiensis,* which were isolated and identified from salted skins (37). In another study, different levels of direct and alternating electric current treatments were examined for preventive effects of skin deterioration, inactivation of the growth of mixed moderately halophilic bacterial culture including *Chromohalobacter israelensis, Chromohalobacter canadensis, Halomonas halodenitrificans, Staphylococcus nepalensis* and *Halomonas halmophila* which were isolated from salted sheep and goat skin samples (38). In the literature, there are also studies investigating

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plant materials as eco-benign materials for their possible antibacterial effects. Sivakumar et al. (2016) reported the antimicrobial activity of myrobalan (*Terminalia chebula* Retz.) application along with salt utilization on the short-term preservation of raw hides/skins (39). Nowadays, it is well known that lichen substances have potential antibacterial activities. To our best of 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, the 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. Amongst them, especially *Usnea* sp. acetone extracts were observed to have most prominent antibacterial activity among tested lichen species against *S. roseus* (KV3) even at the lowest concentration tested. In the literature there are many studies about the antibacterial efficacy of *H. tubulosa*, *R. farinacea*, *E. divaricata* and *Usnea* sp. against several bacteria especially Gram-positive ones. On the other hand, some Gram-negative bacteria are reported to be resistant to *Usnea* sp. Similar to this information, we also determined antibacterial effect of tested lichens on Gram-positive test bacteria in the present study. More detailed studies reported many chemical compounds for various lichen species. For example, *Usnea* sp. has usnic acid, thamnolic acid, atranorin, barbatic acid etc. and *R. farinacea* has evernic acid, atranorin, usnic acid, chloroatranorin etc. (40, 41). However, *P. furfuracea* was reported to have evernic acid, atranorin, usnic acid, physodalic acid, chloroatranorin etc. Overall, some compounds from lichens seem to be same such as evernic acid, atranorin, chloroatranorin etc. (40, 42, 43). For example, atranorin is generally found in most lichen species including our tested lichen species. On the other hand, based

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on our results, atranorin does not appear to have selectivity for antibacterial activity against tested bacterial strains since our screening experiments showed that all tested lichen species does not effective onto KV3 and TR5. Although the other metabolite usnic acid may also have antibacterial efficacy since the presence of this metabolite 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 efficiency against our test bacteria which points to non-effectivity of usnic acid. The most successful lichen was detected as *Usnea* sp. in our study. In the literature, *Usnea* sp. has been also reported to have some other lichen metabolites such as thamnolic acid, barbatic acid, diffractic acid, evernic acid, squamatic acid etc. This antibacterial efficacy may be due to these aforementioned metabolites, or it may be suggested that the lichen metabolites may have together synergistic effect. Another possible scenario could be the difference in the amounts of lichen metabolites in lichen species. From this point of view, it is necessary to give an answer to this question, what provides this selectivity. As seen in this study, extracts gathered from lichen species may exhibit varying efficacies against bacteria and depending on the concentrations. In future studies, a study on which metabolite is effective on which bacteria may open up horizons. Of course, it can be difficult to predict what effect it might have on a microorganism population as a mixed culture. In the mixed culture study performed by Berber et al. (2020) (27) when *Usnea* sp. acetone extracts examined on the total bacterial population obtained from the soaking liquors, successful results were obtained in some samples, but not in others.

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5. Conclusions

The need for new antibacterial agents with prominent potencies is emphasized in the literature. Based on the knowledge of high moderately halophilic bacterial population on hides/skins, possible defects seem to be inevitable on finished product despite salt-curing or brine-curing methods applied to raw hides/skins. Most of lichen species have been used for years for various purposes including antibacterial potential. Taken into consideration of this efficacy of lichens as natural resources, it is also important in terms of the approach towards the natural in the world. This study demonstrated that the acetone extracts of *H. tubulosa*, *R. farinacea*, *E. divaricata* and *Usnea* sp. extracts have antibacterial effects against tested moderately halophilic bacteria. The noteworthy antibacterial activity was detected in *Usnea* sp. acetone extracts against *S. roseus* (KV3) even at the lowest concentration tested. From this respect, proteolytic and lipolytic moderately halophilic bacteria may be taken under control by these ecological materials. The chemical analyses have to be performed to decide which compound/compounds are responsible for the antibacterial efficacy of lichen species. These compounds may be applied onto hides/skins in microencapsulated or sprayed forms. It can be suggested that these materials may be utilized along with salt or brine during the storage period of raw stock.

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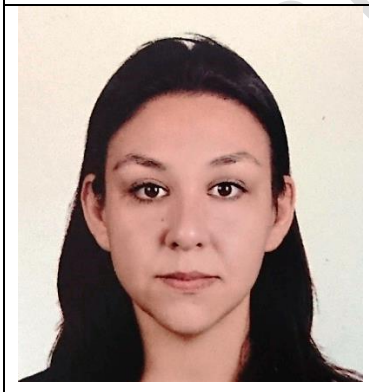

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Figure captions

Fig 1. The antibacterial effects of the extracts of *H. tubulosa* against *S. saprophyticus* subsp. *saprophyticus* (TR5).

Fig 2. The antibacterial effects of the extracts of *R. farinacea* against *S. saprophyticus* subsp. *saprophyticus* (TR5).

Fig 3. The antibacterial effects of the extracts of *E. divaricata* against *S. saprophyticus* subsp. *saprophyticus* (TR5).

Fig 4. The antibacterial effects of the extracts of *Usnea* sp. against *S. saprophyticus* subsp. *saprophyticus* (TR5).

Fig 5. The antibacterial effects of the extracts of *H. tubulosa* against *S. roseus* (KV3).

Fig 6. The antibacterial effects of the extracts of *R. farinacea* against *S. roseus* (KV3).

Fig 7. The antibacterial effects of the extracts of *Usnea* sp. against *S. roseus* (KV3).

Fig 8. The antibacterial effects of the extracts of *E. divaricata* against *S. roseus* (KV3).

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