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Controlling of Halophilic Isolates Using Thiocyanomethylthiobenzothiazole, Potassium Dimethyldithiocarbamate, and Benzyloxymethanol

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Abstract

The hides and skins are contaminated by bacteria that are found in the atmosphere, soil, water, dung, and slaughterhouses. Prior investigations have discovered antibiotic-resistant gam-positive and gram-negative bacteria in both the liquids used for soaking and the hides that were soaked, despite the use of several antimicrobial agents during the soaking process. Optimal concentration of the antimicrobial agent is essential to ensure the soaking process is carried out efficiently and effectively.

Hence, it is feasible to control bacterial contamination on the skin. This study aimed to investigate the Minimum Inhibitory Concentrations (MICs) of antimicrobial agents containing three different chemical materials: Thiocyanomethylthiobenzothiazole, potassium dimethyldithiocarbamate, and benzyloxymethanol. Each of these active ingredients was tested separately at different concentrations against gram-positive bacteria, including *Staphylococcus haemolyticus*, *Staphylococcus taiwanensis*, *Staphylococcus aureus*, S*taphylococcus epidermidis, Peribacillusfrigoritolerans, Bacillus haynesii, Bacillus piscis*, and *Corynebacterium aurimucosum*. Also against gram-negative bacteria *Pseudomonas iranica* and *Pseudomonas oryzihabitans*, those were previously obtained from leather industry. In addition, to assess the susceptibility of these isolates to eight different antibiotics:

Cefotaxime (5 µg), Amikacin (30 µg), Spectinomycin (100 µg), Tetracycline (30 µg), Rifampicin (5 µg), Cephalothin (30 µg), Sulbactam/ Ampicillin (10 µg), and Neomicin (30 µg). The Kirby-Bauer disc diffusion susceptibility test method was employed on Mueller Hinton agar. In addition, this investigation utilized 22 different doses of antimicrobial chemicals that had three different active components. The concentrations ranged from 2500 µg/ml to 0.001 µg/ml for thiocyanomethylthiobenzothiazole, 5000 µg/ml to 0.002 µg/ml for potassium dimethyldithiocarbamate, and 10000 µg/ml to 0.0047 µg/ml for benzyloxymethanol as the active ingredient. The results found that the growth of all tested isolates was inhibited after exposure to the concentrations of antimicrobial agents containing thiocyanomethylthiobenzothiazole as an active ingredients started from 2500 µg/ml to 39.06 µg/ml, 5000 µg/ml to 156.25 µg/ml containing potassium dimethyldithiocarbamate, and 1000 µg/ml to 312 µg/ml containing benzyloxymethanol. This investigation will be useful if it shares with industries.

Keywords: Bauer disk diffusion technique • Agar dilution method • Antibiotic resistance • Antimicrobial agent • MICs • Halophilic isolates

Introduction

The industry of leather has a significant contribution to the worldwide economic benefits, with an approximate annual worth over $100 billion [1]. Nevertheless, the leather industry is also a substantial contributor to ecological contamination, as effluent from tanneries has elevated concentrations of organic and inorganic contaminants [2]. Bacteria are a significant contributor to this contamination, and they can pose severe health risks to workers in the business [3]. Hence, it is necessary to devise novel techniques for regulating bacterial growth in the leather sector. Antimicrobial substances are also defined as physical compounds and chemical agents that may have ability to prevent the growth of microorganisms or destroy them after have been treated. Antimicrobial agents have been employed for the

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treatment and prevention of illnesses caused by bacteria, viruses, fungi, and parasites. Antimicrobial agents can be categorized into many groups, such as antibiotics, antiviral, antifungal, and antiparasitics. Antibiotics are employed for the treatment of bacterial infections, whereas antiviral are utilized for the treatment of viral sicknesses. Antifungals are employed for the purpose of treating fungal infections, while antiparasitics are utilized to address parasitic infections. The utilization of antimicrobial drugs has brought about a significant transformation in current medicine and has successfully protected many lives [4,5]. Antimicrobial drugs function by selectively attacking distinct elements of microorganisms, including cell walls, cell membranes, and metabolic processes. Administration can occur orally, topically, or intravenously, depending on the sickness type and disease severity [6-8]. Researchers have recently focused on utilizing the active components of natural and chemical materials as antimicrobial agents to control bacteria in tanneries. Some of the compounds used for this purpose include quaternary ammonium compounds, chitosan, silver nanoparticles [2,9-11], potassium salts [12], and sodium dimethyldithiocarbamate [12,13]. Nevertheless, further investigation is required to ascertain the effectiveness of these substances. Potassium dimethyldithiocarbamate is a biocide [14] employed in the treatment of wastewater. Its antibacterial action has been demonstrated to prevent the proliferation of bacteria and fungus [15]. Potassium dimethyldithiocarbamate inhibits bacterial adhesion to surfaces, potentially through its capacity to chemically bond with proteins and create a shielding film on metallic surfaces [16,17]. This drug also exerts inhibitory effects on intricate enzyme processes, specifically transfer events, which play a crucial role in microbial metabolism. The solution begins gradually breakdown, resulting in the formation of hydrogen sulfide and dimethylamine. This decomposition process is increased by acidity. It does not mix well with acids, peroxides, and acid halides [17,18]. Based on the data that published by [19]. Hammer, Katherine A, et al. The Lethal Dose (LD_{50}) of Potassium dimethyldithiocarbamate in mouse was 350 mg/kg. Benzothiazole derivatives are highly intriguing owing of their extensive spectrum of biological activity and therapeutic uses. The acknowledged data suggests that benzothiazole derivatives exhibit antibacterial activity by stopping various enzymes, including dihydroorotase, DNAgyrase, uridinediphosphate-n-acetyl enolpyruvyl glucosamine reductase (MurB), peptide deformylase, aldose reductase, casdihydrofolatereductase, enoyl acyl carrier protein reductase, dialkylglycine decarboxylase, dehydrosqualene synthase, dihydropteroate synthase, and tyrosine kinase [20]. The compound 2-(Thiocyanatomethylthio) benzothiazole has been demonstrated to interact with the ribosome during the Polymerase Chain Reaction (PCR) of cell nuclei. This binding affects the action of enzymes and inhibits the process of transcribing DNA into RNA. The medicine additionally prevents the proliferation of bacteria, thereby limiting the occurrence of infectious disorders, including tuberculosis. The compound 2-(Thiocyanatomethylthio) benzothiazole has demonstrated sub-lethal effects in mice, without any noticeable impact on body mass index or wastewater treatment. This is a synthetic organic chemical that has the potential to be utilized in the treatment of bacteria that are resistant to antibiotics. Thiocyanomethylthiobenzothiazole is a fungicide that finds application in the tanning industry. It is utilized in the pickling and tanning procedures at a concentration ranging from 0.02% to 0.3% based on the weight of the hide. It can be found in both organic and aqueous solutions. Benzyloxymethanol is a chemical which has been investigated for its ability to kill bacteria and prevent their growth. Benzyloxymethanol exhibits potent activity in enhancing DNA cleavage by selectively targeting and inhibiting bacterial topoisomerase IA. Recent study by Elmaidomyet al., in indicates that natural materials exhibit promising potential as powerful treatments against pathogenic microorganisms. In that study the researchers examine different potential mechanisms for addressing pathogens that are resistant to multiple drugs. It investigates the antimicrobial properties of terrestrial phytochemicals derived from plants, lichens, insects, animals, fungi, bacteria, mushrooms, and minerals, either on their own or in conjunction with traditional antibiotics. The authors indicate that this article may reveal potential medication candidates that could be utilized in future drug development efforts. According to the findings of Bhatet al., the study evaluated the antibacterial properties of plant extracts against bacteria that were isolated from leather industry. The study revealed that the extracts of *Cinnamomum zeylanicum*, *Syzygium aromaticum*, and *Allium sativum* had noteworthy antibacterial activity against the microorganisms that were examined. The authors recommend that these botanical extracts have the potential to serve as organic antibacterial agents within the leather industry. Kumar et al., conducted a study to assess the antibacterial effectiveness of silver nanoparticles against bacteria that were obtained from leather industry. The study revealed that the silver nanoparticles exhibited substantial antibacterial efficacy against the bacteria that were examined. The authors propose that employing silver nanoparticles may be a favorable strategy for creating antimicrobial agents to resist germs found in leather industries. All halophilic isolates that used in during study were isolated from leather industries and diagnosed as halophilic bacteria depending on the sequence of 16S rRNA gene in our previous study, also the ability of these isolates to produce proteolytic and lipolytic were tested. In addition to the effects of these isolates on sheep and goat skin were investigated. Eventually, the reference isolates were categorized into gram positive and gram negative halophilic bacteria. This study aims to investigate the antibacterial activity of three different antimicrobial agents against ten halophilic isolates which are including (*Staphylococcus haemolyticus, Staphylococcus taiwanensis, Staphylococcus aureus, Staphylococcus epidermidis, Peribacillus frigoritolerans, Bacillus haynesii, Bacillus piscis, Corynebacterium aurimucosum, Pseudomonas iranica*, and *Pseudomonas oryzihabitans*), and to compare the results to the effects of reference antibiotics used in this study, and to estimate the minimum inhibition concentration of thiocyanomethylthiobenzothiazole, potassium dimethyldithiocarbamate, and benzyloxymethanol against above mentioned isolates. The results acquired from this study will definitely contribute in identifying the optimal concentration of this antimicrobial agent against halophilic bacteria that cause harm effects on the skin during leather production, therefore preventing the need for excessive chemical usage.

Materials and Methods

Preparation of stock antimicrobial agent solution containing thiocyanomethylthiobenzothiazole

A quantity of 50,000 mg of the antimicrobial agent, which includes thiocyanomethylthiobenzothiazole, was measured and then dissolved in 250 ml of sterile distilled water. The initial concentration was utilized as a stock solution to generate the subsequent concentrations. Specifically, 2 ml of the initial concentration was combined with distilled water, as shown in Table 1.

No	First concentration (µg/ml)	Stock (ml)	Distil water (ml)	Concentration before addition of ml of medium (µg/ml)	Final concentration (µg/ml) after addition of 19 ml of medium onto 1 ml of antimicrobial agent
1	50000	2	0	50000	2500
2	50000	2	2	25000	1250
3	50000	1	3	12500	625
4	6250	2	2	6250	312.5
5	6250	1	3	3125	156.25
6	6250	1	7	1562.5	78.125
7	781.25	2	2	781.25	39.06
8	781.25	1	3	390.625	19.53
9	781.25	1	7	195.31	9.76
10	97.66	2	2	97.66	4.88
11	97.66	1	3	48.83	2.44
12	97.66	1	7	24.41	1.22
13	12.66	2	2	12.21	0.61
14	12.66	1	3	6.1	0.3
15	12.66	1	7	3.05	0.15
16	1.52	2	2	1.52	0.075
17	1.52	1	3	0.76	0.037
18	1.52	1	7	0.38	0.018
19	0.190	2	2	0.19	0.009
20	0.190	1	3	0.09	0.004
21	0.190	1	7	0.04	0.002
22	0.023	2	2	0.02	0.001

Table 1. Preparation of antimicrobial agent with different concentrations containing thiocyanomethylthiobenzothiazole.

Preparation of stock antimicrobial agent solution containing potassium dimethyldithiocarbamate

A quantity of 200,000 mg of the antimicrobial agent, which includes potassium dimethyldithiocarbamate, was measured and then dissolved in 1000 ml of sterile distilled water. Subsequently, this first concentration was regarded as the primary stock solution from which the subsequent concentrations were prepared. This involved taking 2 ml from the initial concentration and diluting it with distilled water, as indicated in the Table 2.

No	First concentration (µg/ml)	Stock (ml)	Distil water (ml)	Concentration before addition of 19 ml of medium (µg/ml)	Final concentration (µg/ml) after addition of 19 ml of medium onto 1 ml of antimicrobial agent	Final concentration x 5% (µg/ml)
1	200	2	0	200	10000	5000
2	200	2	2	100	5000	2500
3	200	1	3	50	2500	1250
4	50	2	2	25	1250	625
5	50	1	3	12.5	625	312.5
6	50	1	7	6250	312.5	156.25
7	6250	2	2	3125	156.25	78.12
8	6250	1	3	1562.5	78.125	39.06
9	6250	1	7	781.25	39.06	19.53
10	781.25	2	2	390.63	19.53	9.76
11	781.25	1	3	195.31	9.76	4.88
12	781.25	1	7	97.66	4.88	2.44
13	97.66	2	2	48.83	2.44	1.22
14	97.66	1	3	24.41	1.22	0.61
15	97.66	1	7	12.21	0.61	0.3
16	12.21	2	2	6.1	0.3	1.15
17	12.21	1	3	3.05	0.15	0.07
18	12.21	1	7	1.53	0.07	0.03
19	1.51	2	2	0.19	0.038	0.01
20	1.51	1	3	0.09	0.019	0.009
21	1.51	1	7	0.04	0.009	0.004
22	0.76	2	2	0.02	0.0047	0.002

Table 1. Preparation of antimicrobial agent in different concentrations containing potassium dimethyldithiocarbamate.

Preparation of stock antimicrobial agent solution containing benzyloxymethanol

The antimicrobial agent with benzyloxymethanol was quantified at a weight of 200,000 mg and subsequently dissolved in 1000 ml of sterile distilled water. Subsequently, this initial concentration was regarded as the primary stock solution, from which 2 ml was extracted and combined with distilled water, as shown in Table 3, to prepare subsequent concentrations. After the stock solution has been prepared, it was mixed with distilled water in the proportions specified in Tables 1-3. Depending on the type of active ingredients that used in antimicrobial agent which are applied during this study the final concentration was calculated.

No	First concentration (µg/ml)	Stock (ml)	Distil water (ml)	Concentration before addition of 19 ml of medium (µg/ml)	Final concentration (µg/ml) after addition of 19 ml of medium onto 1 ml of antimicrobial agent
1	200	2	0	200	10000
2	200	2	2	100	5000
3	200	1	3	50	2500
4	50	2	2	25	1250
5	50	1	3	12.5	625
6	50	1	7	6250	312.5
7	6250	2	2	3125	156.25
8	6250	1	3	1562.5	78.125
9	6250	1	7	781.25	39.06
10	781.25	2	2	390.63	19.53
11	781.25	1	3	195.31	9.76
12	781.25	1	7	97.66	4.88
13	97.66	2	2	48.83	2.44
14	97.66	1	3	24.41	1.22
15	97.66	1	7	12.21	0.61
16	12.21	2	2	6.1	0.3
17	12.21	1	3	3.05	0.15
18	12.21	1	7	1.53	0.07
19	1.51	2	2	0.19	0.038
20	1.51	1	3	0.09	0.019
21	1.51	1	7	0.04	0.009
22	0.76	2	2	0.02	0.0047

Table 3. Preparation of antimicrobial agent in different concentrations containing benzyloxymethanol.

Preparation of reference bacterial strains

Pure colonies of the reference bacterial isolates were cultured separately in tubes containing Mueller Hinton Broth medium containing NaCl (3 M) and incubated at 37°C for 24 hours. After incubation period, the density of each test strain was adjusted to 10^8 CFU/ml (0.5 McFarland No. 3% NaCl).

Study the effects of antimicrobial agents against halophilic bacteria

By using different concentrations of antimicrobial substances containing thiocyanomethylthiobenzothiazole, potassium dimethyldithiocarbamate and benzyloxymethanolseparately tested against reference bacterial strainsand the Minimum Inhibitory Concentrations (MICs) were investigated by using the broth dilution method in a 96-well microtiter plate.

Investigation of susceptibility of reference bacterial strains to eight different antibiotics

In this study eight kinds of antibiotics were tested against the reference halophilic bacteria by using BauerKirby methods, which are including Cephalothin (30 µg), Cefotaxime (5 µg), Rifampicin (5 µg), Spectinomycin (100 µg), Sulbactam/Ampicillin (10 µg), Amikacin (30 µg), Neomicin (30 µg) and Tetracycline (30 µg) (Oxoid). The reference isolates were cultured separately in test tubes with Muller Hinton Broth and incubated overnight at 37℃. The turbidity of all isolates was adjusted to 10^8 CFU/ml (0.5 No McFarland). After that the bacterial growth was spread on Muller Hinton Agar and the antibiotic discs were put on the surface of petri plates, and then incubated at 37℃ for 24 h. The inhibition zones around colonies were estimated by mm.

Results and Discussion

22's different descending concentrations of antimicrobial agents (µg/ml) with thiocyanomethylthiobenzothiazole, potassium dimethyldithiocarbamate, and benzyloxymethanolwere tested against (*Staphylococcus haemolyticus*, *Staphylococcus taiwanensis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Peribacillus frigoritolerans*, *Bacillus haynesii, Bacillus piscis*, *Corynebacterium aurimucosum*, *Pseudomonas iranica*, and *Pseudomonas oryzihabitans*), The main objective of this experiment was to determine the MIC and to reduce consumption of antimicrobial agent in the leather industry. The results found that the growth of all tested isolates were inhibited after exposure to the concentrations of antimicrobial agents containing thiocyanomethylthiobenzothiazole as an active ingredients started from 10000 µg/ml to 78 µg/ml (Table 4).

Isolates	**Tested isolates**									
Antimicrobial agent Thiocyanomethylthiobenzothiazole µg/ml	***Corynebacter lumaurimucosum***	***Staphylococcus epidermidis***	***Peribacillus frigoritolerans***	**Pseudomonas oryzihabitans**	***Bacillus haynesii***	***Pseudomonas iranica***	***Bacillus piscis***	***Staphylococcus aureus***	***Staphylococcus taiwanensis***	***Staphylococcus haemolyticus***
2500	-	-	-	-	-	-	-	-	-	-
1250	-	-	-	-	-	-	-	-	-	-
625	-	-	-	-	-	-	-	-	-	-
312.5	-	-	-	-	-	-	-	-	-	-
156.25	-	-	-	-	-	-	-	-	-	-
78.125	-	-	-	-	-	-	-	-	-	-
39.06	-	-	-	-	-	-	-	-	-	-
19.53	+	-	+	-	+	+	+	-	-	-
9.76	+	-	+	-	+	+	+	+	+	+
4.88	+	+	+	+	+	+	+	+	+	+
2.44	+	+	+	+	+	+	+	+	+	+
1.22	+	+	+	+	+	+	+	+	+	+
0.61	+	+	+	+	+	+	+	+	+	+
0.3	+	+	+	+	+	+	+	+	+	+
0.15	+	+	+	+	+	+	+	+	+	+
0.075	+	+	+	+	+	+	+	+	+	+
0.037	+	+	+	+	+	+	+	+	+	+
0.018	+	+	+	+	+	+	+	+	+	+
0.009	+	+	+	+	+	+	+	+	+	+
0.004	+	+	+	+	+	+	+	+	+	+
0.002	+	+	+	+	+	+	+	+	+	+
0.001	+	+	+	+	+	+	+	+	+	+
P.C*	-	-	-	-	-	-	-	-	-	-
N.C**	+	+	+	+	+	+	+	+	+	+

Note: P.C*=Positive Control (only bacteria+media without bacteriocin), and N.C**=Negative Control (only medium without bacteria and bacteriocin).

Table 4. The MIC of antimicrobial agent thiocyanomethylthiobenzothiazoleagainst halophilic isolates.

While the MICs of the *Staphylococcus aureus, Staphylococcus taiwanensis* and *Staphylococcus haemolyticus* was 19.53 µg/ml; the MICs of the *Corynebacterium aurimucosum*, *Peribacillus frigoritolerans*, *Bacillus haynesii*, *Pseudomonas iranica* and *Bacillus piscis* was 39.06 µg/ml. Also the growth of *Staphylococcus epidermidis* and *Pseudomonas oryzihabitans* was inhibited at the concentration 9.76 µg/ml. Previous study revealed that the multidrug resistance isolates which isolated from leather industries (soaked liquor) were inhibited at concentrations started from 500 to 4000 µg/ml of antimicrobial agent with sodium dimethyldithiocarbamate, whereas the MICs of *Staphylococcus aureus* 12228 and *Enterococcus faecalis* ATCC 29212 was 15.6 µg/ml, and the MICs of *Bacillus subtilis* ATCC6633 is 3.9 µg/ml and the of *Escherichia coli* ATCC25922 and *Bacillus cereus* ATCC11778 is 500 µg/ml.

Depending on the results provided by applying 22 different concentrations of the antimicrobial substance containing Potassium dimethyldithiocarbamate to halophilic bacteria, the lowest concentration value that inhibited the growth of *Corynebacterium aurimucosum*, *Bacillus piscis*, and *Staphylococcus aureus* was 78.12 µg/ml, *Staphylococcus epidermidis* and *Staphylococcus taiwanensis* was 39.06 µg/ml, and the MICs of *Pseudomonas oryzihabitans* was 19.53 µg/ml. *Bacillus haynesii, Pseudomonas iranica*, and *Staphylococcus haemolyticus* were more resistant than others and the MICs was 156.25 µg/ml (Table 5).

The minimum inhibitory of an antimicrobial agent with methylisothiazolinone used in leather industry against multidrug resistance isolates obtained from leather tanneries was investigated in earlier study conducted by Caglayan, the findings provided that the MIC value for *Escherichia coli* ATCC25922 was found to be 1250 µg/ml. The MIC value for *Micrococcus luteus* ATCC9341, *Staphylococcus aureus* ATCC29213, and *Pseudomonas aeruginosa* ATCC27853 was found to be 5000 µg/ml. While the MIC value for *Bacillus subtilis* ATCC6633 was calculated as 9.76 µg/ml; the MIC value for *Bacillus cereus* ATCC11778 was 156 µg/ml, it was found to be 39 µg/ml for *Staphylococcus epidermidis* ATCC12228 and *Enterococcus faecalis* ATCC29212.

	Tested isolates									
Antimicrobial agent Potassium dimethyldithiocarbamate. µg/ml	***Corynebacterium aurimucosum***	***Staphylococcus epidermidis***	***Peribacillus frigoritolerans***	***Pseudomonas oryzihabitans***	***Bacillus haynesii***	***Pseudomonas iranica***	***Bacillus piscis***	***Staphylococcus aureus***	***Staphylococcus taiwanensis***	***Staphylococcus haemolyticus***
5000	-	-	-	-	-	-	-	-	-	-
2500	-	-	-	-	-	-	-	-	-	-
1250	-	-	-	-	-	-	-	-	-	-
625	-	-	-	-	-	-	-	-	-	-
312.5	-	-	-	-	-	-	-	-	-	-
156.25	-	-	-	-	-	-	-	-	-	-
78.12	-	-	-	-	+	+	-	-	-	+
39.06	+	-	+	-	+	+	+	+	-	+
19.53	+	+	+	-	+	+	+	+	+	+
9.76	+	+	+	+	+	+	+	+	+	+
4.88	+	+	+	+	+	+	+	+	+	+
2.44	+	+	+	+	+	+	+	+	+	+
1.22	+	+	+	+	+	+	+	+	+	+
0.61	+	+	+	+	+	+	+	+	+	+
0.3	+	+	+	+	+	+	+	+	+	+
1.15	+	+	+	+	+	+	+	+	+	+
0.07	+	+	+	+	+	+	+	+	+	+
0.03	+	+	+	+	+	+	+	+	+	+
0.01	+	+	+	+	+	+	+	+	+	+
0.009	+	+	+	+	+	+	+	+	+	+

0.004	+	+	+	+	+	+	+	+	+	+
0.002	+	+	+	+	+	+	+	+	+	+
P.C*	+	+	+	+	+	+	+	+	+	+
N.C**	-	-	-	-	-	-	-	-	-	-

Note: P.C*=Positive control (only bacteria+media without bacteriocin), and N.C**=Negative control (only medium without bacteria and bacteriocin).

Table 5. The MIC of antimicrobial agent containing potassium dimethyldithiocarbamate against halophilic isolates.

Based on the results that acquired by employing 22 different concentrations of the antimicrobial substance with benzyloxymethanolto salt-loving isolates, while the MIC value that prevents the growth of *Staphylococcus epidermidis*, *Bacillus piscis*, *Staphylococcus aureus*, and *Staphylococcus taiwanensis* was 156.25 µg/ml of benzyloxymethanol. Alsoresults revealed the minimum inhibitory of an antimicrobial agent with benzyloxymethanol for *Staphylococcus haemolyticus* and *Bacillus haynesii* was 78.125 µg/ml, and for *Pseudomonas oryzihabitans* was 39.06. Whereas, *Corynebacterium aurimucosum*, *Peribacillus frigoritolerans* and *Pseudomonas iranica* showed high resistance in contrast to other species, and the MIC value for these isolates was 312.5 µg/ml (Table 6).

	Tested isolates									
Antimicrobial agent (benzyloxymethanol)	***Corynebacter iumaurimucosum***	***Staphylococcus epidermidis***	***Peribacillus frigoritolerans***	***Pseudomonas oryzihabitans***	***Bacillus haynesii***	***Pseudomonas iranica***	***Bacillus piscis***	***Staphylococcus aureus***	***Staphylococcus taiwanensis***	***Staphylococcus haemolyticus***
10000	-	-	-	-	-	-	-	-	-	-
5000	-	-	-	-	-	-	-	-	-	-
2500	-	-	-	-	-	-	-	-	-	-
1250	-	-	-	-	-	-	-	-	-	-
625	-	-	-	-	-	-	-	-	-	-
312.5	-	-	-	-	-	-	-	-	-	-
156.25	+	-	+	-	-	+	-	-	-	-
78.125	+	+	+	-	-	+	+	+	+	-
39.06	+	+	+	-	+	+	+	+	+	+
19.53	+	+	+	+	+	+	+	+	+	+
9.76	+	+	+	+	+	+	+	+	+	+
4.88	+	+	+	+	+	+	+	+	+	+
2.44	+	+	+	+	+	+	+	+	+	+
1.22	+	+	+	+	+	+	+	+	+	+
0.61	+	+	+	+	+	+	+	+	+	+
0.3	+	+	+	+	+	+	+	+	+	+
0.15	+	+	+	+	+	+	+	+	+	+
0.07	+	+	+	+	+	+	+	+	+	+
0.038	+	+	+	+	+	+	+	+	+	+
0.019	+	+	+	+	+	+	+	+	+	+
0.009	+	+	+	+	+	+	+	+	+	+

0.0047	+	+	+	+	+	+	+	+	+	+
P.C*	+	+	+	+	+	+	+	+	+	+
N.C**	-	-	-	-	-	-	-	-	-	-

Note: P.C*=Positive control (only bacteria+media without bacteriocin), and N.C**=Negative control (only medium without bacteria and bacteriocin).

Table 6. The MIC of antimicrobial agent containing benzyloxymethanol against halophilic isolates.

In this study eight antibiotic discs which are including Rifampicin (5 µg), Cephalothin (30 µg), Cefotaxime (5 µg), Tetracycline (30 µg), Spectinomycin (100 µg), Amikacin (30 µg), Sulbactam/Ampicillin (10 µg), and Neomicin (30 µg) were used as reference against halophilic isolates by using Kirby-Bauer disc diffusion method in order to compare the results of antibiotic susceptibility with antimicrobial agents results and the results found that the inhibition zones around colonies of *Corynebacterium aurimucosum* (8-31 mm), *Staphylococcus epidermidis* (15-31 mm), *Peribacillus frigoritolerans* (7-25 mm), *Pseudomonas* oryzihabitans (19-30 mm), *Bacillus haynesii* (12-20 mm), *Pseudomonas iranica* (13-40 mm), *Bacillus piscis* (11-30 mm), *Staphylococcus aureus* (13-36 mm), *Staphylococcus taiwanensis* (15-25 mm), and *Staphylococcus haemolyticus* (7-25 mm). While, Rifampicin (5 µg), Tetracycline (30 µg), Neomicin (30 µg), and Amikacin (30 µg) showed no resistance by all tested isolates; *Pseudomonas oryzihabitans* were resistant to Sulbactam/Ampicillin (10 µg), Cefotaxime (5 µg), and Cephalothin (30 µg). *Bacillus haynesii* showed resistance against Cephalothin (30 µg) and Cefotaxime (5 µg). Also Cefotaxime (5 µg) and Sulbactam/ Ampicillin (10 µg) were resisted by *Staphylococcus taiwanensis* (Table 7).

Antibiotics	*Corynebacter iumaurimucosum*	*Staphylococcus epidermidis*	*Peribacillus frigoritolerans*	*Pseudomonas oryzihabitans*	*Bacillus haynesii*	*Pseudomonas iranica*	*Bacillus piscis*	*Staphylococcus aureus*	*Staphylococcus taiwanensis*	*Staphylococcus haemolyticus*
	Inhibition zones (mm)									
Cephalothin	20	23	8	0	0	18	15	29	13	13
Cefotaxime	8	19	0	0	12	15	16	13	0	0
Rifampicin	31	31	18	19	16	40	29	36	25	25
Spectinomycin	15	16	18	28	15	0	15	15	9	6
Sulbactam/ Ampicillin	10	27	7	0	0	19	11	0	0	6
Amikacin	22	15	25	30	20	20	20	15	14	15
Neomicin	24	23	20	30	20	13	30	24	21	18
Tetracycline	23	30	20	24	18	29	21	19	21	16

Table 7. The antibiotics and inhibition zones around halophilic colonies on Muller Hinton agar.

Conclusion

The leather sector plays a crucial role in the world economy, although it also poses a substantial threat to the environment through its large contribution to pollution. Bacteria are a major contributor to this pollution, and they can pose significant health risks for workers in the industry. This study aimed to evaluate the efficacy of antimicrobial treatments in controlling bacterial strains obtained from leather industry. We have found a number of chemicals that have demonstrated encouraging outcomes when tested in a controlled laboratory environment. These compounds include thiocyanomethylthiobenzothiazole, potassium dimethyldithiocarbamate, and benzyloxymethanol. Our research shows that these substances have the ability to effectively eliminate the bacteria found in leather industries, therefore providing a way to reduce the industry's ecological impact. Current research has been conducted on the utilization of antimicrobial chemicals for the purpose of managing halophilic bacteria. Halophilic bacteria are microorganisms that thrive in environments with high salt concentrations and are known for producing challenges in various industries, including food, leather, oil, and gas. Research has been achieved to explore the possibility of using antimicrobial compounds to manage halophilic bacteria. The leather industry is a major contributor to environmental pollution as a result of the discharge of hazardous chemicals and heavy metals. The wastewater produced by these industries contains a significant amount of organic and inorganic contaminants, including microorganisms. The existence of bacteria in the sewage is a notable be concerned because to its potential to induce severe health complications. Hence, the imperative significance resides in the improvement of antimicrobial compounds targeting bacteria obtained from leather industries. Furthermore, the data acquired from the present investigation show significance in terms of regulating the proliferation of bacteria that were previously identified in the leather industry and share the same species as halophilic test strains in this study. This data also aids in preventing the potential harm they may cause on the quality of the skin.

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Author Contributions

KBM: Writing-original draft preparation.
AHS: Methodology.

Availability of Data and Materials

All data are available in the main text or supplementary material.

Declarations

Ethics approval and consent to participate.

Written informed consent is obtained from all the participants prior to the publication of this study.

Consent for Publication

The Author confirms that the work described has not been published before.

Competing Interests

Both of authors declare that there are no competing interests.

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