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An *in vitro* Assessment of The Effectiveness of Some Bactericides on Bacteria Isolated from Soaking Float

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In this study, the effectiveness of 5 commercial bactericides commonly used in leather processing was examined *in vitro*. Bacteria were isolated from soak water by means of proteolytic bacteria culture medium, plate count agar (PCA) and halotolerant bacteria medium containing 10 % NaCl and identified as Gram positive *Staphylacoccus* sp., *Diplococcus* sp., *Micrococcus* sp., *Corynobacterium* sp., *Bacillus* sp. and Gram negative bacterium. The effectiveness of bactericides was determined *in vitro* on these bacteria through disc diffusion method. Results were evaluated in comparison with antimicrobial activities of some standard antibiotics on the same microorganisms. It was observed that Derbio DB 99® (bactericide I) was effective on all types of bacteria. Biocide B-7® (bactericide II) was effective on *Staphylacoccus* sp., *Diplococcus* sp. and *Micrococcus* sp. It was also observed that Aracit KL® (bactericides III) and Preventol Z-L® (bactericides IV) were effective only against *Staphylacoccus* sp. and *Diplococcus* sp. On the other hand, Pluscide HP® (bactericides V) did not show enough effectiveness. As a result, it was found out that the most effective bactericide was Bactericide I.

Key Words: Leather industry, Soaking process, Sheep skin, Antimicrobial activity.

INTRODUCTION

Leather production includes some serial processes. Soaking, the first stage has favour conditions for microbial growth. It is not unusual to find millions of bacteria per mililiter of soak water within 4-6 h after the soak process begins[1,2]. Many kinds of bacteria have been isolated from the soak water such as *Staphylococcus*, *Micrococcus*, *Bacillus*, *Clostridium*, *Proteus*, *Escherichia*, *Corynobacterium*, *Pseudomonas*, *Sarcina*, *Chromobacter*, *Lactobacillus* and *Serretia* species[3,4]. Various investigations about the subject have been carried out and different results have been obtained.

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Orlita[5] indicated that 100 different strains of bacteria were isolated from salted hides. 36 of 100 strains of bacteria were identified as halophilic cocci and the remaining 64 as either Gram (-) or Gram (+) rod. The study showed that on a salted raw hide proliferation of halophilic bacteria resulted in production of pigments. *Micrococcus roseus, M. luteus* and *M. morrhuae* were identified frequently from the coloured spots. *Bacillus subtilis, B. megaterium, B. pumilus* and *Pseudomonas aeroginosa* were also identified from soaking water and putrefied spots on hides and skins. Linder and Neuber[6] have pointed out that the predominant bacteria found in soaking water are *Staphylococcus aureus, Enterobacter aerogenes, Bacillus mycoides, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris* and other gelatine liquifying bacteria. Two hundred twenty five hide and chrome tanned leather samples were examined at different stages of processing by Birbir and Ilgaz[7]. They isolated and identified wide variety of bacteria species such as *Bacillus, Micrococcus, Staphylococcus, Kurtia* and *Pseudomonas*.

Many serious or heavy damages such as putrefactive odour, hair slip, loosening and destruction of grain and loss of hide substance may occur if an effective bactericide is not added to soaking float[8,9]. To overcome these damage, addition of appropriate bactericide is especially required for longer soaking periods including slightly alkaline pH values and at a temperature above 16 ºC. It should be remembered that any destruction of collagen by bacteria prior to tanning cannot be compensated by the tanner's skill and experience[10]. There are several effects for bacteria. Bacteriostatic effect is observed when growth is inhibited, but no killing occurs. On the other hand bactericidal effects kill cells[11]. Russell[12] pointed out that bacteria show a wide divergence in their sensitivity to biocides and spores are more resistant to biocides than non-sporing forms. To control the bacteria in soaking float, it is important to select appropriate bactericide. Commonly used procedure for studying antimicrobial action is the disc diffusion method. In addition, this method is routinely used to test for antibiotic sensivity in pathogens[11].

The aim of the study was to determine the effectiveness of 5 commercial bactericides used in Turkish leather industry against bacteria isolated from soak liquor.

EXPERIMENTAL

In this research, dry-salted domestic sheep skins were used as material.

Plate Count Agar[13] (PCA), proteolytic bacteria culture medium and halotolerant bacteria culture medium were used for isolation of bacteria. Proteolytic bacteria culture medium contained 23.0 g nutrient agar, 5.0 g gelatin in 1.0 L of distilled water. Halotolerant bacteria culture medium contained 23.0 g nutrient agar, 5.0 g gelatin and 10 % NaCl in 1.0 L of distilled water[4]. Antimicrobial studies and bacterial culture activation were carried out *in vitro* by using Muller Hinton Agar and Muller Hinton Broth, respectively.

Bactericides: The bactericides used *in vitro* assessment were as follows. Derbio DB 99® (bactericide I): the formulation of quaternized compounds, Biocide B-7®

(bactericide II): free of the pentachlorophenate and similar toxic products, Aracit KL® (bactericide III): organic sulphur compounds, Preventol Z-L® (bactericide IV): sodium salt of dithiocarbamates, Pluscide HP® (bactericide V): Synergistic composition of organic compounds.

Standard antibiotic discs: Sulbactam-Cefoperazona (S-C) (30 mcg-75 µg), Sulbactam-Ampicillin (S-A) (10-10 mcg), Vancomycin (V) (30 mcg), Levofloxacin (L) (5 µg), Cefixime (C) (5 mcg), Gentamicin (G) (10 mcg) and Ampicillin (A) (10 mcg) were used for assessment and comparision of the examined bactericides.

Skins were soaked using conventional production method suggested by Thorstensen[14]. 10 g of samples were aseptically cut from sheep skin. After preparing appropriate serial dilution of samples, they were plated onto 3 types of the media (PCA, proteolytic bacteria culture medium and halotolerant bacteria culture medium containing 10 % NaCl) by the spread plating method[15]. PCA plates were incubated at 37 ºC for 48 h. Proteolytic and halotolerant bacteria culture medium plates were incubated at 41 ºC for 72 h[4].

After the incubation, different bacterial colonies were picked and evaluated to Gram reaction and some biochemical tests. Isolated bacteria were identified as Gram (+) *Staphylacoccus* sp., Gram (+) *Diplococcus* sp., Gram (+) *Micrococcus* sp., Gram (+) *Corynobacterium* sp., Gram (+) *Bacillus* sp. and Gram (-) bacterium[16].

The effectiveness of 5 bactericides commonly used in Turkish leather industry has been screened *in vitro* against these bacteria by disc diffusion method[15,17]. *In vitro* performances of the bactericides were determined by using maximum concentration as suggested by their information datasheets. Empty sterilized antibiotic discs having a diameter of 6 mm were impregnated with 30 µL of bactertericides solution. Bacteria were incubated at 37 ºC for 24 h by inoculation into Muller Hinton Broth (Oxoid). An inoculum containing 10^6 bacterial cells/mL was spread on Muller Hinton Agar (Oxoid). The discs injected with bactericides solutions were placed on the inoculated agar by pressing slightly and incubated at 37 and 41 ºC.

Standard antibiotic discs such as S-C, S-A, V, L, C, G, A were used for control. Experiments were repeated 3 times and the results were expressed as average values. The results obtained from bactericides used in the study were compared with the results of various standard antibiotics[18].

RESULTS AND DISCUSSION

Table-1 shows antibacterial activities of 5 bactericides *in vitro*. Bactericide I exhibited high *in vitro* activity against *Staphylococcus* sp., *Diplococcus* sp., *Micrococcus* sp. and *Corynobacterium* sp. It also showed moderatly active *versus* Gram (+) *Bacillus* sp. and Gram (-) bacterium. Bactericides II, III and IV were found to be highly active *versus Staphylococcus* sp. and *Diplococcus* sp. having an inhibition zone of 26-29 mm. However, Bactericide V revealed moderatly active against the same bacteria compared to other bactericides. No activity was found in Bactericides II, III, IV, V against *Corynobacterium* sp., Gram (+) *Bacillus* sp. and Gram (-) bacterium.

TABLE-1
ANTIMICROBIAL ACTIVITIES OF VARIOUS BACTERICIDES AGAINST ISOLATED BACTERIA

Bacteria	Inhibition zone (mm)*				
	I	II	III	IV	V
Gram (+) *Staphylococcus* sp.	27	29	26	28	9
Gram (+) *Diplococcus* sp.	14	29	26	28	9
Gram (+) *Micrococcus* sp.	15	8	–	–	–
Gram (+) *Corynobacterium* sp.	14	–	–	–	–
Gram (+) *Bacillus* sp.	13	–	–	–	–
Gram (–) bacterium	12	–	–	–	–

*Includes diameter of disc (6 mm).
– = Inactive, 7-13 mm = Moderately active, >13 mm = Highly active.

In vitro antibacterial activities of standard antibiotics against isolated bacteria are shown in Table-2. Most of the standard antibiotics were highly active *versus* isolated bacteria. Antibiotic C was moderatly active against Gram (+) *Diplococcus* sp., Gram (+) *Micrococcus* sp., Gram (+) *Bacillus* sp. and Gram (-) bacterium, but it was inactive against Gram (+) *Corynobacterium* sp. When the results obtained from bactericides were compared with those of standard antibiotics (Tables 1 and Table 2), it was determined that *Staphylococcus* sp. was susceptible and *Diplococcus* sp., *Micrococcus* sp., *Corynobacterium* sp., Gram (+) *Bacillus* sp., Gram (-) bacterium were mid-susceptible to bactericide I. *Staphylococcus* sp. and *Diplococcus* sp. were more suspectible to bactericides II, III, IV than bactericide V. On the other hand, *Corynobacterium* sp., Gram (+) *Bacillus* sp. and Gram (-) bacterium were resistant to bactericides II, III, IV, V.

TABLE–2
ANTIMICROBIAL ACTIVITIES OF SOME STANDARD ANTIBIOTICS

Bacteria	Inhibition zone (mm)*						
	S-C	S-A	V	L	C	G	A
Gram (+) *Staphylococcus* sp.	35	30	32	35	25	30	33
Gram (+) *Diplococcus* sp.	28	26	17	29	11	22	28
Gram (+) *Micrococcus* sp.	30	25	18	30	7	27	29
Gram (+) *Corynobacterium* sp.	27	30	20	30	–	16	23
Gram (+) *Bacillus* sp.	30	20	18	23	7	15	7
Gram (–) bacterium	27	22	20	35	11	15	19

*Includes diameter of disc (6 mm)
– = Inactive, 7-13 mm = Moderatly active, >13 mm = Highly active.

In previous study[19], the same commercial bactericides were used at their recomended concentration on tannery scale. Effectiveness of bactericides was assessed against total aerobic mesophilic bacteria, proteolytic bacteria, halotolerant bacteria and aerob spore-forming bacteria. In that investigation, it has been observed that bactericides I and II affected against total aerobic mesophilic bacteria, proteolytic

bacteria and halotolerant bacteria. On the other hand, bactericide I was more effective against aerobic spore-forming bacteria than the other bactericides examined. Because of insufficent effectiveness of most bactericides against wide variety of bacteria especially aerobic spore-forming bacteria we need to determine the effectiveness of the same bactericides *in vitro*.

In present findings, both *in vitro* and in tannery scale (the previous study) gave rise to similar results. It was found out that the effectiveness of bactericides used in the studies was different and bactericide I was found out to be the most effective.

In leather industry, different compositions of bactericides are used to prevent bacterial damage which causes quality loss in finished leathers. In conclusion the overall data obtained from the study showed that antibacterial activity of bactericides are various, depending on their chemical compositions. So, it is of great importance for determining the most appropriate bactericide in soaking process. Thus, *in vitro* antimicrobial activity should be periodically tested *versus* bacteria and aerobic spore-forming bacteria isolated from soaking water.

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