Physical and Antimicrobial Characteristics of *Aloe Vera* Treated Split Suede Leather

by

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Abstract

This study examined some characteristics of split suede leathers that were treated with a range of *Aloe vera* concentrations. Following the tanning process of split suede leathers, *Aloe vera* was applied at five different concentrations (2%, 4%, 6%, 8% and 10%). Leathers treated with *Aloe vera* took on a yellowish-brown color. Also, the moisture content and softness of the leathers increased according to the concentration of *Aloe vera* used, and the highest levels of both moisture content and softness were obtained at 8% and 10% application concentrations. The leathers that were treated with 6% or more *Aloe vera* had slightly higher tear load values when compared with control leathers. In addition, ethanol-based and water-based extracts of *Aloe vera* showed antimicrobial properties against some Gram (-) and Gram (+) bacteria and *C. albicans*.

Resumen

Este estudio examinó algunas de las características de cerrajes agamuzados que fueron tratados con un rango de concentraciones de Aloe Vera. Tras el proceso de curtido de cerrajes agamuzados, el Aloe Vera se aplicó a cinco diferentes concentraciones (2%, 4%, 6%, 8% y 10%). Los cueros tratados con Aloe Vera tomaron un color pardo amarillento. Además, el contenido de humedad y suavidad de los cueros aumentaron de acuerdo a la concentración de Aloe Vera utilizada, y los niveles más altos tanto por contenido de humedad y la resultante suavidad que se obtuvieron a concentraciones de aplicación del 8% y 10%. Los cueros que fueron tratados con un 6% o más de Aloe Vera tuvieron valores de desgarre ligeramente superiores en comparación con los cueros del control. Además, los extractos de Aloe Vera a base de etanol y a base de agua mostraron propiedades antimicrobianas en contra de algunas bacterias Gram (-) y Gram (+) y *C. albicans*.

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INTRODUCTION

Aloe vera is a semi-tropical plant the tree lily family. There are over 400 different species of *Aloe*, growing mainly in the dry regions of Africa, Asia, Europe and America. *Aloe barbadensis* Miller is the most common *Aloe* species and is the type of *Aloe* used in most commercially available products.[1, 2]

The *Aloe* plant contains between 99 and 99.5 percent water, with an average pH of 4.5. The *Aloe* leaf contains over 75 nutrients and 200 active compounds, including vitamins (A, B1, B2, B6, B12, C and E), enzymes (approximately 99 enzymes), sugars, anthraquinones, lignin, saponins, sterols, amino acids and salicylic acid.[3, 4] Anthranoids barbaloin and isobarbaloin are major components of *Aloe*. Barbaloin is used as an anti-inflammatory, a wound healer, an antimicrobial and an antioxidant agent.[5] This gives it a high commercial value. *Aloe vera* has effects such as skin protection, skin hydration, soothing and cooling.[6] Previous studies conducted on this topic have shown that *Aloe vera* based products are safe for use on the skin; they promote healing; increase the collagen content and crosslinks in granular tissues, help the healing of skin burns, refresh dry skin within a few weeks, and soften the skin.[7] Previous studies have examined the antimicrobial effects of *Aloe* extracts. Ferro *et al*. (2003) established the susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to the iner gel of *Aloe barbadensis* Miller or *A. vera*.[8] Similar studies have shown that *Aloe vera* has some antibacterial and antiviral effects.[9, 10] Thanks to these beneficial effects, *Aloe vera* has been widely used in recent years in many textile products ranging from socks to bed covers and provides these products with cooling antistress and antimicrobial characteristics.[11] *Aloe vera* is applied to fabrics via various methods and gives users a feeling of freshness when their skin contacts the fabric.[12, 13] In another study, West et al. (2003) used gloves containing *Aloe vera* to treat dry skin. Study participants wearing the treated gloves showed a considerable improvement in just 10 days when compared to those in the control group.[14] Studies continue to be conducted on the use of *Aloe vera* in various clothing and other new products. The present study examined some physical characteristics and antimicrobial properties of split suede leathers that were treated with *Aloe vera*.

MATERIALS AND METHODS

Materials

The present study used 25 wet-blue split calf leathers. Wet-blue split calf leathers were obtained from Cihan Deri San. Ltd. in the Istanbul Leather Industry Industrial Area. The *Aloe vera* extract used in the study was in dust form (LORAND Laboratories, USA); it was yellow-to-brown in color; it had a barbaloin value of >60% (HPLC method), and a moisture value of 0.20%.

Methods

Processing of Wet-Blue Split Calf Leathers

Split leathers were processed whole as far as the wet blue stage. For the *Aloe vera* experiments, the leathers were divided into two along the backbone line. One side of the leathers were processed with *Aloe vera* and those from the other side were processed as the control leather. Each level of *Aloe vera* was tested with five iterations. First, the wet-blue calf splits were shaved to a thickness of 1.6mm. They were then neutralized to fix their pH values at 5.0. Following the neutralization process, leather samples were treated with five different concentrations of *Aloe vera* (2%, 4%, 6%, 8, and 10%.) for 60 minutes. The leathers were lubricated, dried and then subjected to mechanical processes. The recipes used are given in Table I.

Some Physical Properties of the Leathers

The color of the leathers was determined according to CIE 1976 using a Minolta CM-508d model spectrophotometer. A loop softness test was conducted using a BLC SMS MT-LQ model texture analyzer.[15] Moisture ratio was measured with an Aqua-Boy Moisture Meters FEI,[16] and tear load was determined according to TS 4118-1 EN ISO 3377-1.[17]

Antimicrobial Properties of the Leathers Preparation of *Aloe vera* Ethanol Extract (AVEE) and *Aloe vera* Water Extract (AVWE)

10 grams of *A. vera* were dissolved in 100 ml of ethanol (96% v/v) or in 100 ml of distilled water. These solutions were incubated at 25° C for 24 hours and then centrifuged at 7000 rpm for 10 minutes. The supernatants were lyophilized to dryness and refrigerated at +4° C until use.

Determination of Minimum Inhibitory Concentration (MIC) of AVWE and AVEE

The microdilution broth method was used to determine the MIC of AVEE and AVWE.[18] Stock solutions of 100 mg/ml of AVEE and AVWE were prepared in Muller Hinton Broth (MHB). Further serial dilutions were prepared in the wells of a 96-well microplate in a range from 50.0 mg/ml to 1.0 mg/ml.

The microorganisms used for the test were *Staphylococcus aureus* ATCC 6538-P, *Bacillus subtilis* ATCC 6633, *Pseudomonas aureginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445, *Escherichia coli* ATCC 12228, *Proteus vulgaris* AATC 6897, *Klebsiella pneumoniae* CCM 2318 and the unicellular yeast *Candida albicans* ATCC 10239. All of the test microorganisms were grown in MHB at 37° C for 24 hours. Each microdilution well contained 100 µl of the microbial inoculum (1.1×10^4-2.3×10^5 colony forming units (CFU) per ml) and 100 µl of *A. vera* extracts. *A. vera* extract-free growth medium was included together with the sterility control, and *A. vera* extract-free growth medium containing the microorganisms was also used as a

TABLE I
Recipe Used on the Leather

Process	Chemical Additives	Temperature (°C)	Concentration (%)	Time (min)	Remarks
Weight					
Washing	Water	30	300		
	Nonionic surfactant		0.3	20	
Washing	Water	30	300	15	
Retanning	Water	30	100		
	Chrome–aluminium syntan		3	60	
	Sodium Formate		1	30	
	Sodium bicarbonate		0.3	30	pH:5.0
Washing	Water	30	300	15	
Fatliquoring	Water	50	100		
	Amphoteric syntan		4	20	
	Acrylic copolymer		4	20	
	Aloe vera extract		X	60	X: 2.0, 4.0, 6.0, 8.0 and 10
	Sulphited natural and syntetic oil		6		
	Syntetic sulphated and sulphonated oil		3	60	
	Formic acid		1	30	pH:4.0
Washing	Water		300	10	
Drying and process mechanics					

positive control. After incubation at 37° C for 24 hours, 10 µl of test culture from serial dilutions was spotted on to Muller Hinton Agar (MHA) and incubated under the same conditions. The MIC was considered as the lowest concentration of extract that inhibited microbial growth. Each extract was tested in triplicate and the experiment was repeated twice.

Antimicrobial Activities of Leather Samples Prepared with AVWE and AVEE

Two methods were used to evaluate the antimicrobial activity of leather samples containing *A. vera* extracts. Firstly, the qualitative antimicrobial activities of leather samples prepared with AVWE and AVEE were determinated by using the AATCC test method 147-1993 referred to as the Parallel Streak Method.[19] Leathers samples 20 mm wide by 70 mm long were saturated with 5 ml of 20 mg/ml AVEE or 5 ml of 40 mg/ml AVWE in sterile Petri dishes and maintained at room temperature for 2 hours. These leather samples were then placed on MHA plates which had been previously streaked with an inoculum of the test microorganisms. After incubation at 37° C for 24 hours, the antibacterial properties of leather samples treated with AVEE and AVWE and untreated leather samples were examined by observing peripheral inhibition around them.

Secondly, AATCC Test Method 100-1993 was also used as the quantitative procedure for the evaluation of the degree of antimicrobial activity. The test was performed on both the experimental leather samples and the untreated control samples.[20, 21] The samples saturated with AVWE, AVEE and the control samples described above were placed in sterile 50 ml Erlenmeyer flasks and 15 ml of MHB inoculated with *S. aureus* or *K. pneumoniae* to the level of $1.4x10^4$-$2.3x10^5$ CFU/ml was added. The Erlenmeyer flasks were incubated at 37° C with 150 rpm agitation for 24 hours. Samples for viable counts were taken at time intervals of 4 hours. Samples were serially diluted in phosphate-buffered saline (0.1 M, pH 7.0, 0.85% NaCl) and the numbers of surviving colony-forming

units (CFU) per ml were determined by plating on MHA. Inhibition rates (%) were calculated as follows:

Inhibition rate % = (A-B) / A x 100

where *A* is the number of bacteria at time intervals of 4 hours in untreated leather and *B* is the number of bacteria at time intervals of 4 hours in treated leather. The tests were performed in triplicate.

RESULTS AND DISCUSSION

Some Physical Properties

Analysis of L^*, a^* and b^* values given in Table II shows that depending on the concentration of *A. vera* used, the leathers turned yellow-to-brown in color. This color tone is similar to the colors obtained when the leathers are treated with other plant-based tanning materials. This natural color produced by *A. vera* showed a homogenous distribution in every part of the leather and no irregular appearance was recorded. This means that unless otherwise specified by the customer, this natural-color leather can be marketed without the need for any further coloring process.

As shown in Table III, the moisture content of the leathers increased in parallel with the increase in the concentration of *A. vera* applied. The biggest moisture change was recorded at 8% and 10% application concentrations. This result explains the "moisture-retaining" and resulting "cooling" effect of *A. vera*.[2] Increased moisture content also improves the tactile characteristics of the *A. vera*- treated leathers.

The softness of the leathers was evaluated in terms of compression energy and decompression energy. It was found that both the compression and the decompression energy values decreased (in other words, the softness of the leathers increased) according to the application concentration of *A. vera* (Table IV).

Table V shows that the leathers that were treated with 6% or more *A. vera* had slightly higher tear load values when compared with the control leathers. The increase recorded in softness and the slight increase in tear load are thought to result from the higher moisture content of the samples treated with *A. vera*.

Antimicrobial Properties

The efficiency of antimicrobial textiles may be estimated through standard test methods. These methods are also applied to finished leathers which are treated with antimicrobial agents.[22] The present study measured MIC values as an indication of the growth inhibition effect of AVWE and AVEE against the experimental microorganisms. The MIC values of AVWE and AVEE for the test microorganisms are given in Table VI. The MICs obtained

TABLE II
L^*, a^*, b^* Results

% *Aloe vera* added	Coordinates					
	Control			*Aloe vera*		
	L^*	a^*	b^*	L^*	a^*	b^*
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
2	65,843± 1,410	-5,121± 0,125	2,318± 0,179	46,564± 1,312	7,281± 0,243	21,298± 1,250
4	66,849± 1,835	-4,975± 0,290	2,310± 0,191	44,616± 1,645	8,723± 0,208	24,476± 0,998
6	67,276± 1,462	-4,589± 0,291	2,147± 0,189	41,789± 1,589	9,041± 0,203	28,368± 1,325
8	66,442± 1,630	-4,775± 0,266	2,471± 0,188	38,686± 1,207	9,908± 0,220	31,071± 1,273
10	68,768± 1,761	-4,766± 0,280	2,466± 0,267	37,789± 1,225	10,421± 0,215	32,841± 1,100

TABLE III
Moisture Results

	% *Aloe vera* added				
	2	4	6	8	10
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control (%)	13,00±0,35	13,00±0,18	12,75±0,31	13,25±0,31	13,00±0,25
Aloe vera (%)	13,75±0,25	14,25±0,25	14,75±0,35	15,75±0,18	15,50±0,31

TABLE IV
Compression Energy and Decompression Energy Results

		% *Aloe vera* added				
		2	4	6	8	10
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Compression Energy (kg/mm)	Control	1,241± 0,056	1,653± 0,093	1,082± 0,067	1,018± 0,048	1,322± 0,064
	Aloe vera	1,195± 0,042	1,569± 0,079	0,963± 0,047	0,857± 0,031	1,084± 0,074
Decompression Energy (kg/mm)	Control	0, 779± 0,022	0,905± 0,044	0,737± 0,027	0,787± 0,028	0,859± 0,035
	Aloe vera	0,742± 0,033	0,835± 0,042	0,675± 0,025	0,667± 0,016	0,691± 0,013

TABLE V
Tear Load Results

	% *Aloe vera* added				
	2	4	6	8	10
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control N (mm)	8,8976±0,527 (1,66±0,080)	8,9820±0,749 (1,65±0,066)	9,8816±0,516 (1,75±0,057)	8,7575±0,671 (1,64±0,068)	8,8838±0,554 (1,65±0,054)
Aloe vera N (mm)	8,7680±0,644 (1,64±0,087)	9,2741±0,690 (1,67±0,093)	10,3701±0,899 (1,77±0,094)	9,7677±0,677 (1,67±0,081)	9,8997±0,710 (1,68±0,084)

for AVWE ranged from 40 mg/ml for *B. subtilis* and *E. coli* to 10 mg/ml for *S. aureus*. AVWE did not show any antimicrobial activity against the remaining 4 test microorganisms. The MICs obtained for AVEE were in the range of 20 to 5 mg/ml. MICs of 5 mg/ml were determined for *S. aureus* and *C. albicans*. Comparison of MI data for AVWE and AVEE shows that AVEE was more effective than AVWE.

In the quantitative procedure and the parallel streak test method, the concentrations of *A. vera* extract used on leather samples were chosen with regard to the MIC end points which they showed against the test organisms. AVEE applied to leather samples (20mm x 70mm) at concentrations of 5 ml of 20mg/ml showed a good growth inhibition effect for *S. aureus* and *K. pneumoniae:* as much as 88% and 76.1%, respectively. Leather samples treated with AVWE at a

TABLE VI

Table VI. *In vitro* susceptibility of Test Microorganisms to AVWE and AVEE as Determined by the Broth Microdilution Method and the Parallel Streak Method.

Microorganisms	MIC value (mg/ml)		Peripheral inhibition	
	AVWE	AVEE	AVWE	AVEE
Klebsiella pneumoniae CCM 2318	—	20	absent	present
Bacillus subtilis ATCC 6633	40	10	present	present
Salmonella thyphimirum CCM 5445	—	—	absent	absent
Staphylococcus aureus ATCC 6538-P	10	5	present	present
Pseudomonas aureginosa ATCC 27853	—	10	absent	present
Escherichia coli ATCC 12228	40	—	present	absent
Candida albicans ATCC 10239	—	5	absent	present

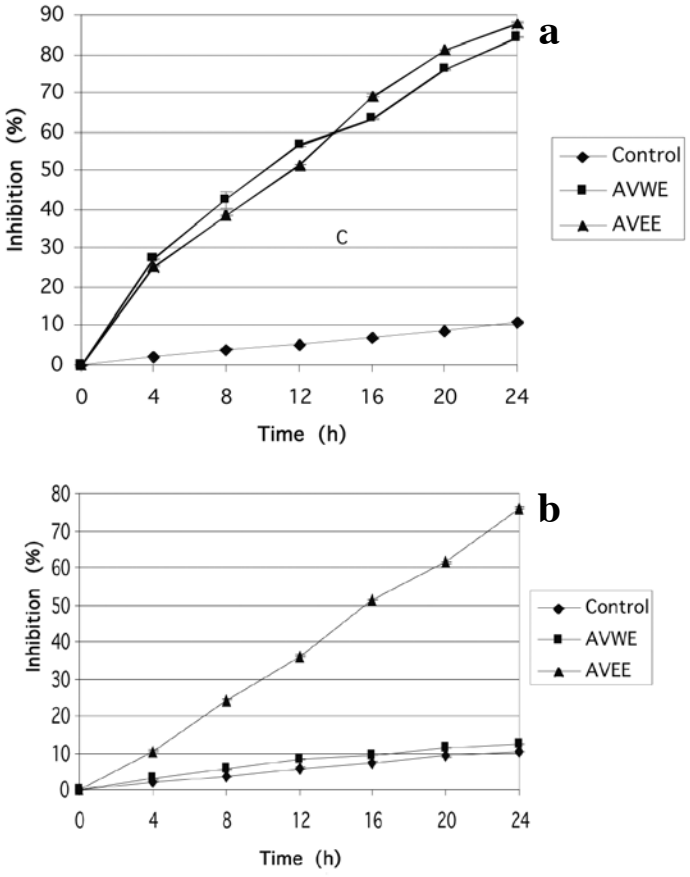

Figure 1. – Qualitative Antimicrobial Activities against Test Microorganisms of Leather Samples with AVWE and AWEE Against Test Microorganisms; a) *S. aureus* b) *K. pneumoniae*.

concentration 5 ml of 40 mg/ml also exhibited a good growth inhibition effect for *S. aureus* (84.3%) but not for *K. pneumoniae* after 24 hours' incubation. In the control leather samples, there was a 10.4-11.2% growth inhibition rate (Figure I). This may be due to the heavy metal content and some leather tanning materials in the leather used in this study. *A. vera* extracts showing antimicrobial activity by micro-broth dilution assay were further tested by the parallel streak test method. In the parallel streak method (see Figure I), peripheral inhibition zones were observed in leather samples treated with AVEE and AVWE which were in accordance with MIC end points. AVEE showed more significant antimicrobial ability than AVWE.

Previous studies have reported that growth of *K. pneumoniae, P. aureginosa and C. albicans* was inhibited by AWEE, but that these organisms were not affected by AVWE.[9] This may be due to the fact that these active plant compounds are highly soluble in ethanol. There are some conflicting reports of the antimicrobial activity of *A. vera* extracts. Major differences are described in the pre-experimental treatment of the plants, such as storage conditions, the use of fresh or dried components, variation of extraction method, the use of different parts of the plant, growing conditions, and the age of plants at harvest.[23, 24]

CONCLUSION

In recent years, innovative applications have gained importance in the production of textiles and similar products. The trend is to present innovations that are beneficial to human health and that provide comfort for users. Consumers prefer the use of natural materials in the provision of such benefits. Within this field, *Aloe vera* is one of the most commonly-used natural materials.

The present study showed that *Aloe vera* improved some of the performance characteristics of leather and that it provided some antimicrobial protection. The results suggest that *Aloe*

vera can be used in leather processing. However, further studies should be conducted to develop new *Aloe vera*-based calming leather products.

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