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A STUDY TO REDUCE SALT USAGE IN PRESERVATION OF SKINS AND HIDES WITH ALTERNATE USE OF PLANT EXTRACT

V. Sivabalan[1] and A. Jayanthi[2]
[1]P.G. and Research Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamilnadu, India
[2]School of Biotechnology, Chemical and Biomedical Engineering, VIT University, Vellore, Tamilnadu, India
E-Mail: jayanthi.abraham@gmail.com

ABSTRACT

Raw hides and skin are preserved before processed into leather. Salt preservation is the general and age old popular practice. Possibilities of using other methods of preservations have been analysed in order to reduce the pollution load in the soaking process of leather by many scientist. However, in the present study less salt preserving systems based on herbal extract have been investigated and evaluated at ambient temperature of 30-37ºC as alternative preservation system. The efficacy of this alternative system was assessed by parameters such as determination of bacterial count, isolation and characterization of microorganism from raw goat skin, antimicrobial activity by using herbal extraction with well diffusion method, percentage weight loss during storage of cured skin, determination of hydrothermal stability of the skin and physical properties of finished leather was conducted. 12 plants were screened for antimicrobial assay among which only 5 plants had antimicrobial activity *Weddilia chininsis, Cassia alatta, Clerodentron pholomides, Solanum trilobotum, Calotropis procera*. Hence these 5 plants were chosen for direct application on the skin along with reduced percentage of NaCl.

Keywords: skins, hides, preservation, plant extract, reduce pollution.

INTRODUCTION

Raw hides and skin are associated in most occasions with aerobic and facultative anaerobic organisms. Different components of skin harbor large number of microorganism especially bacteria, depending on the body location and amount of skin moisture, the number of bacteria on the skin surface may range from only about 1000 organisms per square centimeter.

The bacteria in the fresh skin are predominantly gram positive Micrococcus and Bacilli. But storage skin harbor mainly gram negative Enterobacteriaceae and Alcaligens. Bacteria, molds and yeast can be isolated from raw skins and finished leather. The skin flora is predominantly aerobic and hemolytic organism like Staphylococcus pyogens, Staphylococcus aureus, Hemolytic sarcinacutis, Bacillus, Anthracoides, Bacillus mesenteries and Torula sp. [1].

When the animal is slaughtered the nature's defense mechanism ceases to function and the invaded bacteria then become active which start decomposing the skin. The commonest bacteria on the clean skin belong to the group of gram positive cocci a single genus *Staphylococcus.* The S*taphylococcus* sp on the normal skin form white colonies on agar medium are name *Staphylococcus epidermis* another species forms golden or orange colored colonies of *Staphylococcus aureus* which are potentially pathogenic to skin. In moist skin areas gram negative coli form, bacilli and Diptheroids may be abundant [2].

Skin and hides processed into leather salt preservation is the popular practice in India and other tropical countries. Skins and hides are preserved by application of sodium chloride at a concentration of 40-50%. The dual function of common salt is dehydrating ability and bacteriostatic effects.

The sodium chloride curing system is most popular animal skin preservation method adopted globally the subsequent processing of salt preserved skins in tanneries results in the generation of quantities of total dissolved solids (TDS) one of the pollutants that are very difficult to treat consequently, tanners have been forced to search for better alternatives. Salt free and less salt preserving systems based on herbal extract have been investigated and evaluated at ambient temperature of 30-37ºC as alterative preservation system. The efficacy of these alternative system was assessed by parameters such as determination of bacterial count, isolation and characterization of microorganism from raw goats skin, antimicrobial activity by using herbal extraction with well diffusion method, percentage weight loss during storage of cured skin, Total dissolved solids, determination of hydrothermal stability of the skin and physical properties of finished leather preserved by the new system.

So in order to abate pollution an alternative preservation is undertaken in this study. The alternative preservative will reduce salt concentration and act as preservation for skin curing. In the present study herbal extracts act as preservative which will inhibit the microbial growth on the skin/hide and plants with antimicrobial activity are chosen.

MATERIALS AND METHODS

Skin sample

Raw buffalo hide was collected in clean sampling bags from Thanjavur slaughter house immediately after flaying the slaughtered animal and transported to the laboratory in ice bucket maintaining the temperature 2-4 °C. Freshly flayed Indian goat skin pieces weighing 5g per piece were taken and each piece was soaked in 50ml

distilled water and the skin sample was prepared by shaking in a shaker at 200rpm for 30 minutes [3].

Isolation and identification of microbes from raw hide

Raw hide pieces of average weight 5 g and the size of 2.5 cm × 2.5 cm were placed in sterile petri plates without application of any preservative. The petriplates were kept at ambient temperature (28 ± 2 °C) in a container wherein humidity was maintained. On each consecutive day, the hide pieces were transferred to conical flask containing 100 ml sterile saline (0.85%). The flasks were then kept on rotary shaker (100 rpm) at ambient temperature (28 ± 2 °C) for 180 min. The suspension formed was then serially diluted. Selected dilutions were then plated agar using spread plate technique on nutrient agar. The plates were incubated at 37 °C for 48 h. The total number of bacteria from pool of bacterial isolates for each sampling day was then obtained. Sabouraud's dextrose agar was used for the isolation of fungi. The inoculated plates were incubated for 24 hours at 37°C.

Subculture technique

The isolates of bacteria species were sub cultured on nutrient agar plates to check its purity and incubated at 37 °C for 24 hours. After completion of incubation period the plates were observed and the colony morphology was noted. Single colony was streaked on the sterile dry nutrient agar slant. This culture was subjected to further morphological and biochemical analysis.

Collection of plant material

The fresh and healthy leaves of Clerodentron pholmoides, Weddilia chininsis, Cassia alatta, Vitex nigundo.L, Eclipta Alba, Albezzia lebbek, Solanum trilobotum, Calotropis procera, Abrus precatorius, Abutilon indicum, Phyllanthus niruri, Thespesia populnea were collected from the herbal garden of Ponnaiyah Ramajayam, Thanjavur. Few leaves were taken and then sterilized with distilled water for the removal of dust particles. The identification of the plant specimen was confirmed by using standard floras [4] and standard reference [5, 6].

Extraction with methanol

The leaves of the plants were dried, ground to a fine texture and then soaked in methanol for extended periods. The slurry was then filtered and washed. The powder was dried which was obtained by filtering through a filter paper. The filtrate was centrifuged for about five times for clarification. When water was used for extraction plants were generally soaked in distilled water.

Extraction with hexane

The leaves of the plants were dried and then ground to a fine texture. The ground paste was soaked in hexane for extended periods. The slurry was the filtered and washed. The powder was dried under reduced pressure and re-dissolved in hexane respectively. The filtrate was obtained by filtering through a filter paper. The filtrate was centrifuged for about five times for clarification.

Antibacterial assay (well diffusion method)

The antibacterial activity of different plant species was evaluated by agar well diffusion method [7] for crude extract and solvent extract using Muller Hinton Agar No. 2 medium for the assay. The microorganism was activated by inoculating a loopful of the strain in the nutrient broth (25ml) and incubated at room temperature on a rotary shaker. Then 0.2ml of inoculum was inoculated into the molten Muller Hinton Agar media and after proper homogenization it was poured into 100 mm petri dishes. For the agar well diffusion, a well was made in the seeded plates with the help of a cup-borer (8.5mm). The test compound was introduced into the well and all the plates were incubated at 37ºC for 24 hours. The experiment was performed 3 times under strict aseptic conditions. Microbial growth was determined by the zone of inhibition. The antimicrobial activity was checked for 12 plants *Weddilia chininsis, Cassia alatta, Clerodentron pholomides, Vitex negundo, Eclipta alba, Albezzla lebbeck, Solanum trilobotum, Calotropis procera, Abrus precatorius, Abutilon indicum, Phyllanthus nirur* and *Thespesia populnea*. Among the 12 plants evaluated for antimicrobial assay only 5 plants had the antimicronial activity (*Weddilia chininsis, Cassia alatta, Clerodentron pholomides, Solanum trilobotum, Calotropis procera*). Hence these 5 plants were chosen for direct application on the on skin as an alternative for NaCl.

Preparation of plant extract applied on skin

The leaves of plants *Clerodentron pholmoides, Weddilia chininsis, Cassia alatta, Vitex nigundo.L, Eclipta alba, Albezzia lebbek, Solanum trilobotum, Calotropis procera,* where weighed and washed with sterilized distilled water and crushed in a pestle and mortar. About 5g leaves of each of the 5 plants selected and the preparation of crude extract was done separately for each plant. The extracted juice was filtered through a filter paper and sterilized for 15 minutes. The filtrate was cooled to room temperature. Freshly flayed goat skins obtained from local slaughterhouse. The control was applied with 30% salt. Similarly, the less-salt preservation approach was used with the preparation of 5ml plant extract and directly applied on skin with 3% salt. After the addition of preservatives the skin samples were stored up to 15 days for preservation process [8].

Determination of percentage (%) loss during storage of cured skin

The loss of moisture content was noted every day. As the moisture content reduces from the skin samples during preservation process.

Determination of bacterial count

Preserved skin pieces weighing 5g per piece were taken and each piece was soaked in the 50 ml sterile water and the skin extract was prepared by shaking in a shaker at 200 rpm for 30 min. Soaked liquor measuring 1 ml was

taken in 9 ml of sterile water and shaken well to get uniform suspension of the bacteria. A volume of 0.1 ml of the respective diluted solution was taken in sterile petri plates and molten nutrient agar at 40 °C was poured and shaken gently to get uniform distribution of the bacteria. The plates were incubated at 37 °C for 48 h. The number of colonies on the agar medium was counted.

Preparation of skin sample

NaCl at a concentration of 3% was applied on the skin. The preparation of 5ml plant extract was taken and directly applied on skin with 3% salt. The control skin was maintained with 30% salt. After the addition of preservatives total skins samples stored up to 15 days for preservation process. The skins were folded and stored at the ambient temperature of 32 °C. The skins were monitored periodically for physical changes like smell and hair slip, which are indications for putrefaction [9]. The experimental skin treated with plant extract and 3% salt showed no signs of putrefaction, smell or hair slip even after a storage period of two weeks. These two preservation systems were selected for analytical studies. The efficacy of the systems was systematically assessed for moisture content, total extractable nitrogen and bacterial count. The shrinkage temperature of the tanned leather and physical properties of finished leather processed from the skins preserved by the new systems were also determined and compared with the results of using the conventional preservation system [10].

Determination of hydrothermal stability of the skin

The thermal stability of collagen is an important property for the assessment of the quality of skin, as it indicates indirectly any structural destabilization of the skin matrix. The thermal stability of the skin stock is normally assessed by shrinkage temperature. A shrinkage meter was used to determine the shrinkage temperature of the preserved skin. For measuring the shrinkage temperature the test samples of dimension 20 x 3mm were taken and hooked in the meter. The samples were immersed in a glycerin- water solution (70:30). The temperature at which the specimen starts shrinking was noted as shrinkage temperature of the particular skin. The shrinkage temperature is a measurement of breakdown of stabilizing linkages existing in the collagen matrix. The main aim of this component of the study was to understand whether the new curing systems being tested have any effect on the destabilization of collagen matrix.

RESULTS AND DISCUSSIONS

Raw hides and skin are associated in most occasions with aerobic and facultative anaerobic organisms were also isolated. In this present study the organisms isolated were *Staphylococcus aureus, Pseudomonas sp, Klebsiella pneumoniae, Bacillus subtilis, Proteus vulgaris, Serratia marcescens, Salmonella sp, Shigella sp.* (Tables 1 and 2). The funguses isolated from the skin sample were *Aspergillus flavus, Aspergillus terrus, Aspergillus fumicatus, Penicillium* sp. Morphological test were done to confirm the strains. Among the 12 plants screened for antimicrobial analysis only only 5 plants had the antimicronial activity (*Weddilia chininsis, Cassia alatta, Clerodentron pholomides, Solanum trilobotum, Calotropis procera*). Hence these 5 plants where chosen for direct application of the extract on skin as an alternative for NaCl. The present test was done before the commencements of leather making process.

Similar antimicrobial studies on medicinal plants have been performed by Nair [6]. Twelve medicinal plants were screened for potential antibacterial activity against 5 medically important bacterial strains namely *Bacillus subtilis* ATCC6633, *Staphylococcus epidermidis* ATCC23564, *Pseudomonas pseudoalcaligens* ATCC17440, *Proteus vulgaris* NCTC8313, *Salmonella typhimurium* ATCC23564. The antibacterial activity of aqueous and methanol extracts was determined by agar disk diffusion and agar well diffusion method. Chloroform, ethanol and aqueous extract of *Wrightia tinctoria* leaves were investigated for antimicrobial activity against *Staphylococcus, S. epidermidis, Bacillus subtilis, Klebsiella pneumoniae, Salmonella paratyphi A, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Aspergillus niger,Penicillium* sp., *Cryptococcus neoformans and Candida albicans*. Among the various extracts, maximum antibacterial activity was exhibited by chloroform extract (40, and 22 mm) followed by ethanol extract (40,20and 20 mm) and aqueous extract against *Staphylococcus aureus, S. epidermidis and Pseudomonas aeruginosa*, respectively there is no antifungal activity exhibited by all the three extracts against various fungi used in the study. The good bactericidal activity is due to the presence of tryptanthrin, which makes it an alternative medicine in treating infections caused by *Staphylococcus aureus, S. epidermidis and Pseudomonas aeruginosa*.

The antimicrobial activity of essential oils against Esherichia *coli, Salmonella enteritidis*, and *Salmonella typhimurium* was conducted by Pedro Penalver *et al.,* 2005. The result of this work confirms the antimicrobial activity of some essential oils, as well as their potential application in the treatment and prevention of disease caused by *Enterobacteriaceae* family. Methanol, ethyl acetate, and hexane extracts of *Bridelia ferruginea* leaves exhibited significant activity against *Pseudomonas fluorescens, Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Streptococcus faecalis* [10].

The results presented in Table-3 indicate the effect of various plant extracts on the preservation of skin. The physical evaluation such as hair loosening, putrefaction (by odour) are taken as a measure for assessing the efficacy of curing of 48 h duration. The plant extracts (salt 13% and 5gm) of *Cassia alatta, Solanum trilobotum, Calotropis procera* were able to preserve the goat skin with no hair loosening with no putrefaction odour when compared to other two plant extracts.

A gradual increase in the water loss in all the samples could be observed until the end of the study period. Among the five plants assayed for alternative salt preservative method *Calotropis procera* was found to be more effective in bringing the moisture content of the skin

to about 29.6% followed by *Cassia alatta* 29.07%, Weddilia chininisis 28.82% and Clerodentron pholmoides 27.78% (Tables 4 and 5). With combination of salt 13% and 5gm plant extract there was decrease in the weight of skin which showed good preservation established on skin and hides. Whereas the weight loss in the control skin sample was found to be 36.70%. In all the trials the skin was found to be well preserved. This could be attributed to the antimicrobial property of the herbal extracts against the microorganisms of the raw skin habitat and its contaminants.

Table-1. Morpholoical characterization of microorganism isolated from skin sample.

#	Organisms	Size µm diameter	Colour	Form	Margin	Elevation	Opacity
1.	*Staphylococcus aureus*	1-2	Golden Yellow	Circular	Lobate	Convex	Opaque
2.	*Pseudomonas sp*	1.5-3	Patches with metallic sheen	Irregular colonies	Entire	Raised	Opaque
3.	*Bacillus subtilis*	2-3	White	Circular	Curled	Flat	Transparent
4.	*Klebsiella sp*	1-2* 0.5-0.8	Colorless	Large dome	Mucoid	Umblicate	Translucent
5	*Proteus vulgaris*	1-3* 0.4-0.7	Colorless	Spindle	Entire	Raised	Transparent
6	*Serratia marcescens*	1-3*0.5	Pink	Circular	Isolate	Convex	Opaque
7	*Salmonella sp*	1.3*0.5	White	Circular	Entire	Convex	Translucent
8	*Shigella sp*	0.5*1-3	White	Circular	Entire	Convex	Translucent

Table-2. Morpholoical and biocehmical characterization of microorganism isolated from skin sample.

Organisms	Morphological test			Biochemical test						
	Gram's staining	Capsular Staining	Motility Test	Catalase Test	Oxidase Test	IMViC				TSI
						Indole	MR	VP	Citrate	
Staphylococcus aureus	(+)cocci	+	-	+	-	-	+	+	-	A/A
Pseudomonas Sp	(-)rod	-	+	-	+	-	-	-	+	K/K
Klebsiella pneumoniae	(-)rod	-	-	+	-	-	-	+	+	A/A
Bacillus subtilis	(-)rod	+	+	-	-	-	+	+	+	A/K
Proteus vulgaris	(-)rod	-	+	+	-	+	+	-	+/-	A/A
Serratia marcescens	(-)rod	-	+	+	-	-	+/-	+	+	K/A
Salmonella Sp	(-)rod	-	+	+	-	-	+	-	+	K/A
Shigella Sp	(-)rod	-	-	-	-	+/-	+	-	-	K/A

Note: (+)-positive, (-)-Negative, A/A-Acid slant/Acid butt, K/A-alkaline slant/Acid butt, A/K- acid slant/ alkaline butt, K/K-alkaline slant/ alkaline butt

Table-3. Quantitative evaluation of the preserved skins.

Plant extract sample	Hair loosening	Putrefaction
Control	No	No
Weddilia chininisis	Around the edges	Mild odour
Cassia alatta	No	No
Clerodentron pholmoides	Around the edges	Mild odour
Solanum trilobotum	No	Mild odour
Calotropis procera	No	No

Table-4. Percentage (%) weight loss during storage of cured skin after application of plant extract.

Plant extract sample	Wt of Fresh skin	10% Wt of salt added	Wt after 1 hr.	% loss in Wt.	Day 1 (30% salt added)	Day 1 (30% salt in Wt)	Day 1 (% loss Wt)	Day 1 (3% salt added + 5g extract)	Day 1 (3% salt + 5g extract)	Day 1 (% loss in Wt)
Control	128	12.8	127	9.80	146	48.8	22.95	-	-	-
Weddilia chininisis	96	9.6	94	10.9	-	-	-	92	7.76	19.15
Cassia alatta	108	10.8	107	9.93	-	-	-	103	8.09	18.82
Clerodentron pholmoides	104	10.4	102	10.83	-	-	-	100	8	18.30
Solanum trilobotum	91	9.1	86	14.08	-	-	-	83	7.49	22.85
Calotropis procera	119	11.9	117	10.61	-	-	-	113	8.39	18.87

Table-5. Percentage (%) weight loss during storage of cured skin after application of plant extract.

# *	Day 6 (30 % salt wt)	Day 6 (% loss in wt)	Day 6 (3% salt + 5g extract)	Day 6 (% loss in wt)	Day 9 (30% salt Wt)	Day 9 (% loss in wt)	Day 9 (3% salt + 5g extract)	Day 9 (% loss in wt)	Day 12 (30% salt in wt)	Day 12 (% loss in wt)	Day 12 (3% salt + 5g extract)	Day 12 (% loss in wt)	Day 15 (30% salt in wt)	Day 15 (% loss in wt)	Day 15 (3% salt + 5g extract)	Day 15 (% loss in wt)
1	135	28.79	-	-	134	29.32	-	-	126	33.54	-	-	120	36.70	-	-
2	-	-	86	24.42	-	-	85	25.30	-	-	82	27.94	-	-	81	28.82
3	-	-	95	25.20	-	-	94	25.92	-	-	91	28.28	-	-	90	29.07
4	-	-	94	23.20	-	-	93	24.01	-	-	90	26.47	-	-	89	27.78
5	-	-	77	28.4	-	-	75	30.3	-	-	73	32.1	-	-	71	34
6	-	-	104	25.3	-	-	103	26.0	-	-	99	28.9	-	-	98	29.6

* Plant extracts: 1. Control; 2. *Weddilia chininisis*; 3. *Cassia alatta*; 4. *Clerodentronpholmoides*; 5. *Solanum trilobotum* 6. *Calotropis procera*

CONCLUSIONS

It can be concluded that plant extract have the capacity to preserve skin by antimicrobial activity present in their extracts. The curing efficiency of any substance greatly reflects on inhibiting the microbial growth, especially the proteolytic bacteria which are found to colonize the skin.

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