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RESEARCH ARTICLE

An eco-friendly method for short term preservation of skins/hides using *Semecarpus anacardium* nut extract

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Abstract Preservation or curing of hides and skins is performed as the primary step of leather processing. Common salt is employed as the conventional agent for curing purpose. Use of salt enhances the pollution load of tannery effluent which becomes highly contaminated with increased total dissolved solids and chlorides. To overcome this hurdle, researchers are in constant search of alternative preservation techniques which are either totally void of salt or use only a meager amount of salt. In the present study, we had explored the possibility of using *Semecarpus anacardium* nut extract as an alternative to salt for the curing process by assessing different parameters like hair slip, putrefaction odor, volatile nitrogen content, moisture content, bacterial count, and shrinkage temperature in comparison to the salt curing method. The antibacterial property of the plant extract was also investigated. The results obtained substantiated that the nut extract of *S. anacardium* effectively could preserve the skins for more than a month, by its antibacterial activity along with the dehydrating property of acetone.

Keywords *Semecarpus anacardium* · Skins · Preservation · Hydrothermal stability · Collagen

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Introduction

The Indian leather industry, which contributes to an export revenue of Rs.125.46 billion (Euro 2.225 billion) with a global share of 3 %, is known to be one of the most promising and vibrant sectors of the country's economy. Moreover, India is known to produce superior quality of hides and skins.

It is well known that every production process is a major source of pollution, which holds good for the leather industry too. Leather production consists of four main processes namely beam house, tanning, post-tanning, and finishing, generating huge volumes of liquid and solid matter containing a variety of chemical and biological wastes. A major share of pollution load contributed by the leather industry is mainly generated from the pre-tanning process (Ayoub et al. 2011). Tannery effluent exhibits high values of the environment polluting parameters like biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS) and total dissolved solids (TDS) (Joseph and Nithya 2009). Common effluent treatment plants, which have been established to treat the effluent discharged by tanneries, have effectively removed SS and also brought down COD and BOD within safe limits (El-Sheikh et al. 2011; Tare et al. 2003). However, TDS remains high even in the treated effluent. The major portion of TDS is contributed by common salt used in preservation of

hides and skins (Sarkar 1981). The increased TDS affects the fertility and quality of the soil when used for irrigation.

Different methods of curing like chemical preservation methods using boric acid (Hughes 1974; Kanagaraj et al. 2005a), potassium chloride (Bailey and Joseph 1996), sodium metabisulfite (Kanagaraj et al. 2005b), silica gel (Kanagaraj et al. 2001), sulfates (Vankar and Dwivedi 2009), and soda ash (Davis and Scroggie 1973); biocidal methods like use of aureomycin, and terramycin (Berwick et al. 1990); and physical methods like drying (Rai et al. 2009) and irradiation with gamma rays (Bailey 1997) have been used to preserve the skins and hides before they are processed into leather. Recently, Murugan et al. (2013) have developed a desorption isothermal model for controlled drying depending on the relationship between equilibrium moisture content and water activity. Preservation of skins and hides by chilling has also been demonstrated (Chandrababu et al. 2012a, b). However, even today, the most common method for preservation is salting using 40–50 % sodium chloride, which is very economical but not eco-friendly.

Solution to this problem lies in developing green technologies to keep a check on pollution levels. The need of the hour is to implement cleaner production strategies which are necessary to achieve sustainable development of any country's economy.

Many plant-based formulations have been reported for preserving skins and hides (Kanth et al. 2009; Sivabalan and Jayanthi 2009; Vijayalakshmi et al. 2009). Exploitation of *Azadirachta indica* (neem) extract for the preservation of skins has also been reported (Preethi et al. 2006). Bronopol, a synthetic bactericide, has also been found useful in preserving skins (Muthusubramanian and Mitra 2006).

Short term preservation of skins/hides using *Semecarpus anacardium* nut extract: a new initiative

India is a country blessed with an enormous resource of medicinal and herbal plants. *Semecarpus anacardium* (Family: Anacardiaceae), known as bhallatak in India, is a deciduous tree found in the outer Himalayas and has been exploited by rural people for the treatment of variety of ailments. It has been reported to possess antiasthmatic (Choudhari and Deshmukh 2008), antidiabetic (Aseervatham et al. 2010), and anti-inflammatory (Satyavati et al. 1969), and cardio protective properties (Choudhari and Deshmukh 2008). In Ayurveda, the fruit of this plant is considered a *rasayana* for longevity and rejuvenation and is processed before use, as it is toxic in nature (Puri 2003). The nut extract is used for inflammatory diseases like arthritis (Ramprasath et al. 2005, 2006).

The most significant components of the *S. anacardium* are bhilwanols, phenolic compounds, sterols and glycosides (Rao et al. 1973), and biflavonoids (Ishatulla et al. 1977). Bhilwanol from fruits was shown to be a mixture of cis- and transisomers of ursuhenol; this compound consists mainly of 1,2,dihydroxy-3(pentadecadienyl 8′,11′)benzene and 1,2, hydroxy-3(pentadecadienyl 8′)benzene (Indap et al. 1983). Other components isolated are, anacardoside (Majumdar et al. 2008) semecarpetin, nallaflavanone, jeediflavanone, semecarpuflavanone, galluflavanone (Murthy 1983, 1984, 1985a, b, 1987, 1988). Gedam et al. (1974) has shown the composition of bhilwanol from *S. anacardium*. Mona et al. (2010) have published a review article on *S. anacardium* in which they have mentioned the active principles present in it.

Reports have indicated that anacardic acid, a compound present in *S. anacardium*, possesses antibacterial activity against methicillin resistant *Staphylococcus aureus* (Muroi and Kubo 1996). Mohanta et al. (2007) has evaluated the antimicrobial activity of *S. anacardium* oils and nut extract.

In this context, the present study has been attempted for the first time to test the efficacy of *S. anacardium* as an alternative to conventional sodium chloride preservation. The main focus of this work was to exploit the antimicrobial activity of *S. anacardium* nut extract along with the dehydrating property of acetone to reduce the TDS in the tannery wastewater. As moisture content of the skins (about 70 %) is considered the main reason for bacterial attack and further putrefaction, we have standardized a method in which the moisture content was reduced to 20–25 % by applying nut extract dissolved in 10 % acetone for preservation.

Materials and methods

Skins

Freshly flayed goat skins of average weight 1 kg and average area of 5 sq ft collected from a local slaughter house located at Perambur, Chennai, India were used in the study.

Chemicals

Commercial grade sodium chloride and laboratory grade acetone were purchased from S D Fine-Chem Ltd, Mumbai, India.

Preparation of nut extract

150 g of *S. anacardium* dry nut was broken into pieces. 500 ml of acetone was added to it, plugged with cotton, and then kept on a rotary shaker at 200 rpm overnight. Then, it was filtered through a muslin cloth and centrifuged at 3,000 rpm for 15 min. The supernatant was collected and the solvent was evaporated. The powder thus collected was stored at 4 °C in airtight bottles, until use.

Standardization of optimum concentration of nut extract for preservation

Freshly flayed goatskins were cut into two halves. The left half was taken for experimental purpose and the right half was used as control. Different concentrations (0.25–2 %) of the extract were mixed with 10 % acetone, applied on the flesh side of the skin, and kept in a closed container for 1 h. The skins were then left open in the air for 30 min, folded flesh to flesh and kept aside for preservation at ambient temperature (ranging from 26 to 35 °C) in the tannery yard. Skins were shuffled every day and assessed periodically for physical changes like odor, hair slip, and development of worms which are indications of putrefaction (Sivaparvathi and Nandy 1974). Each experiment was repeated for at least thrice for reproducibility. After 1 month, the preserved skins were taken for soaking and processed into wet blue crusts. From the results obtained, the optimum concentration of the nut extract for preservation was found to be 1 % (w/w).

Antimicrobial activity of nut extract against isolated organisms

Sterile paper discs of 6 mm in diameter were taken and were made to absorb 0.1 ml of the extract having different concentrations (0.25–1 %). Known volume of acetone (0.1 ml) was added in blank paper discs and used as control. The organisms (*Bacillus subtilis*, *S. aureus*, and *Streptococcus pyogenes*) were inoculated individually on sterilized Mueller–Hinton agar plates. Air-dried discs were placed on the inoculated Mueller–Hinton agar surface. Plates were incubated at 37 °C for 18 h and the diameter of the inhibition zone was measured. The tests were performed in duplicates.

Viable count of microorganisms in salted and nut extract-treated skins

Skin samples (5 g) were cut during different stages of preservation and soaked separately by shaking the bottles in an orbital shaker at 200 rpm for 30 min. Then, 1 ml of the soak liquor was diluted to 10 ml with sterile water and kept for shaking to get a uniform suspension of bacteria. An aliquot of 0.1 ml of the resulting diluted solution was taken in sterile petri plates and molten nutrient agar was poured and shaken gently to get uniform distribution of the bacteria. Petri plates were incubated at 37 °C for 48 h (Cruickshank 1965). The bacterial population was determined and expressed as CFU/g of skin.

Table 2 Moisture content of the preserved skins

Days of preservation	Acetone + plant extract	Salt (40 %)
0	75	75
1	65	70
4	55	70
7	40	60
30	35	50

The values are mean values of three determinations

Determination of moisture content

Samples of cured skins were taken and the hair was removed and weighed and the moisture content was determined by drying in a drying chamber at 50–60 °C for 5–6 h (Bureau of Indian Standards, 1971) and the weight loss was calculated.

Determination of nitrogen content

The cured samples of known weight (5 g) were kept in distilled water ten times its weight, shaken well in a bottle at 30 rpm for 3 h to extract the soluble nitrogenous compounds. The extract was then filtered through a filter paper, digested, and the amount of nitrogen was determined by Kjeldahl method (Bureau of Indian Standards 1971).

Determination of hydrothermal stability of the skin

The thermal stability of the skins is normally assessed by measuring shrinkage temperature (Nutting and Borasky 1949). Shrinkage meter was used to determine the shrinkage temperature of the preserved skin. Test samples (20×3 mm) were taken and hooked in the holder which was then immersed in a bath containing a glycerine/water solution in the ratio of 70:30. The temperature was gradually increased by heating. The temperature at which the sample starts shrinking

Table 1 Standardization of the concentration of *S. anacardium* nut extract

Concentration of acetone + plant extract (%)	Volatile nitrogen g/kg (48 h)	Skin degradation evaluation (48 h)	
		Hair loosening	Odor
10+0.25	3.60±0.05	Hair slip	Presence of odor
10+0.50	3.00±0.03	Hair slip	Light odor
10+1.0	2.75±0.02	No hair slip	No odor
10+2.0	2.20±0.02	No hair slip	No odor

The values are mean ± standard deviation of three determinations and assessment made on four samples of each experiment

Table 3 Bacterial count in the skin preserved skins

Days	Acetone + plant extract	Salt (40 %)
0	2×10^{3}	2×10^{3}
1	4×10^{10}	6×10^{10}
4	3×10^{9}	5×10^{10}
7	3×10^{8}	4×10^{10}
15	2×10^{8}	4×10^{10}
30	2×10^{6}	3×10^{10}

The values are mean values of three determinations

Table 4 Antibacterial activity of *S. anacardium* nut extract

S. no.	Name of the organism	Zone of inhibition (diameter in mm) Concentration	
		0.5 %	1 %
1.	*Bacillus subtilis*	13	21
2.	*Staphylococcus aureus*	8	15
3.	*Streptococcus pyogenes*	11	20

was noted as the shrinkage temperature of that particular skin.

Measurement of pollution load generated in leather processing

The skin samples, after different time point of preservation, were subjected to soaking. The liquor from the soaking operation was collected and analyzed for BOD, COD, TDS, SS and chlorides, using standard analytical procedures (Eaton et al. 1995).

SDS-PAGE

The stability and fiber structure of the collagen extracted from preserved skin samples were analyzed by running sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (Laemmli 1970).

Results and discussion

Curing is the protective treatment administered soon after the hides and skins are flayed. Curing agents are supposed to create an environment for the skins in which the contaminant organisms cannot function. Freshly flayed hides/skins contain a variety of microorganisms, which are derived from air, water, soil, and extraneous filth, apart from the local inhabitance of the skin. The skin, with 65–70 % moisture, serves as a biological matrix and perfect medium for the microbes to thrive and hence undergoes disintegration by the hydrolytic enzymes secreted by these organisms, resulting in defective raw material with voids and ultimately producing poor quality leather.

In this present study, we explored the possibility of using *S. anacardium* nut extract as an alternative to salt for the curing process. The standardization of optimum concentration of *S. anacardium* extract for ambient preservation of skins, keeping the solvent concentration fixed at 10 % is depicted in Table 1. Of the extract, 1 % was found to be effective in preserving the skins for more than 1 month. There was no hair slip or foul odor observed in 10 % acetone and 1 % extract group indicating that there is no putrefaction of the skins though the volatile nitrogen content is higher (2.75 g/kg) for 2 % nut extract when compared to 1 % (2.20 g/kg).

Moisture content is one of the most important factors that could be used to assess the ability of curing agents for preservation. The moisture content of the preserved skins at different time point intervals is shown in Table 2. The moisture content of acetone plus plant extract and salt treaded skins were reduced to 13 and 6.6 % respectively after 24 h. It was reduced to 26 % in *S. anacardium*-treated skins on day 4 whereas it remains the same (6.6 %) in salt-preserved skins. There was about 46 and 20 % decrease in the moisture content in the extract-treated and salt-treated skins respectively, after 7 days. The moisture content at the end of 30 days was reduced to 53 and 33 % respectively for extract-treated skins and salt-treated skins. The greater reduction in moisture content in extract-treated skins might be due to the hygroscopic/dehydrating properties of acetone.

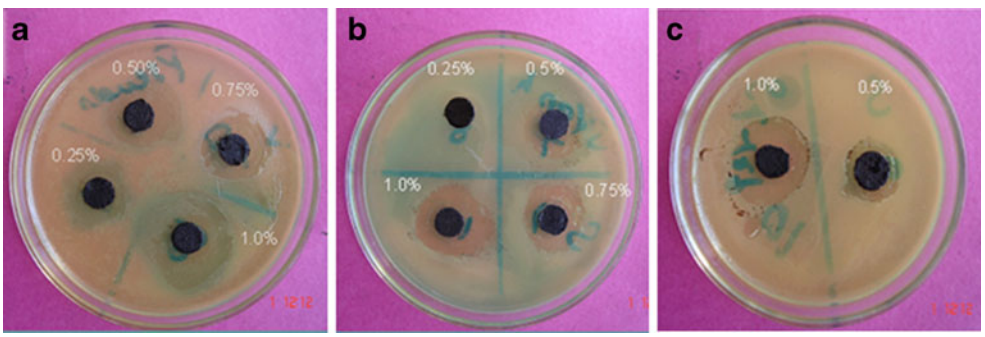

Fig. 1 Antibacterial property of *Semecarpus anacardium* against **a** *Bacillus subtilis* **b** *Staphylococcus aureus* **c** *Streptococcus pyogenes*

Table 5 Total extractable nitrogen (g/kg) in the preserved skins

Days	Acetone + plant extract	Salt (40 %)
0	2.30±0.01	2.30±0.01
1	2.66±0.02	3.60±0.03
4	2.52±0.03	4.00±0.04
7	2.48±0.04	4.40±0.05
15	2.45±0.05	5.30±0.07
30	2.41±0.04	5.10±0.02

The values are mean ± standard deviation of three determinations

Kanagaraj et al. (2005b) has shown a 34 % reduction in salt-cured skins after 48 h. The results obtained in these experiments are quite contradictory. There were only 13 and 6.6 % reduction in the moisture content even after 4 days in our experiments. This might be due to the changes in the climatic conditions during which the experiments have been carried out.

The bacterial count in the preserved skins is shown in Table 3. The main purpose of performing bacterial count was to determine the number of bacteria present in the new method of preservation without salt. The bacterial count was 6×10^{10} in the case of salt curing method on day 1 whereas it was 4×10^{10} in acetone treated skin. At the end of 30 days, the bacterial count has been reduced to 2×10^{6} in *S. anacardium*-treated skins whereas it was 3×10^{10} in salt-preserved skins. The drastic positive reduction in the microbial content might be due to the reduction in moisture content which is known to be directly proportional to the bacterial count and the antimicrobial activity of the plant extract.

Antimicrobial activity of the extract at different concentration against *B. subtilis*, *S. aureus*, and *S. pyogenes* is shown in Fig. 1 and Table 4. It was observed that at 1 % concentration, the zone of inhibition is maximum.

It has been reported that petroleum ether nut extract of *S. anacardium* contains phytochemicals like terpenes, flavonoids, phenolics, saponins, alkaloids, and glycosides—these phytochemicals posses antimicrobial activity (Zabin et al. 2012). The presence of phenolic compounds in the extract may contribute antibacterial activity. Phenolic compounds are thought to be toxic to micro organisms, inhibiting the enzymes which are essential for the growth of microorganism.

Table 6 Shrinkage temperature (°C) of preserved skins

Days	Plant extract	Salt (40 %)
0	70±2	70±2
1	68±2	69±2
4	70±2	69±2
7	71±2	70±2
30	71±2	71±2

The values are mean ± standard deviation of three determinations

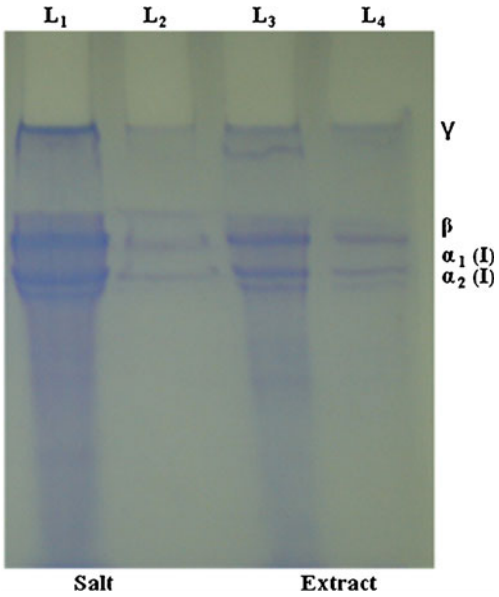

Fig. 2 SDS-PAGE of collagen extracted from *Semecarpus anacardium* and salt-preserved skin samples after 30 days of preservation. *Lane 1* 2–25 and 10 μg of salt-treated collagen respectively, *lane 3*, 4–25 and 10 μg of extract-treated collagen respectively

A major setback to the constant source of export earning from leather industry is brought about by some indigenous microorganisms of the skin causing degenerative and putrefactive changes damaging the flayed skin (Rashid et al. 2008). Curing is known to preserve the skin from the time of flaying to processing in the tannery. Thus, our study proves that bactericidal activity of *S. anacardium* can be highly useful in cutting down the decomposition of skin during the time elapsing between pre-tanning processes.

The amount of extractable nitrogen in the processed skin is an important parameter to be considered to assess the effectiveness of the preservative. Total extractable nitrogen for the skin preserved with acetone plus plant extract and salt-treaded skin is shown in Table 5. The total extractable nitrogen progressively increases with increased duration of preservation. The saltless preservation with the plant extract showed 2.41 g/kg when compared to the corresponding salt-cured skin (5.10 g/kg) at the end of 30 days. The

Table 7 Reduction in pollution load in soaking liquor

Pollution load in soaking liquor	Acetone + plant extract	Salt (40 %)
BOD	9.0	11
COD	12.5	26
TDS	16.0	281
TSS	13.0	37.0
Cl	0.6	200

The values are expressed as g/kg of raw material; mean values of three determinations

decrease in volatile nitrogen content might be due to the fact that the extract acts as a bactericide, thus inhibiting the putrefaction.

Putrefaction leads to destabilization of collagen, in turn, the skin structure. Hydrothermal stability measure in terms of shrinkage temperature is one of the direct indicators of structural stability. Shrinkage temperature is an index of any structural changes in the skin matrix. The shrinkage temperature of the preserved skins at different time point intervals is depicted in Table 6. There is no significant change in the shrinkage temperature of the salt-cured and plant extract cured skins showing that there is no deleterious effect developed on the skin matrix by the newly developed method.

Collagen is the structural glycoprotein present in the skins. SDS-PAGE of collagen obtained from the extract-treated and salt-treated skins are depicted in Fig. 2. Degradation of collagen by proteolytic and collagenolytic bacteria, results in poor quality leather. Collagen was extracted and isolated from both the methods of preservation after 30 days. SDS-PAGE was run for the collagen samples to observe whether the collagen fibers have been affected by the curing process. The banding pattern observed was similar in both the treatment confirming that collagen was not degraded or affected by the treatment with plant extract (Preethi et al. 2006).

The reduction in the pollution load in the control and experimental groups is shown in Table 7. Even though there is no significant change in the BOD levels, there is a great reduction in COD (52 %) in the experimental soak water when compared to the controls. The main pollution problem of the leather industries, TDS, is reduced from 281 g (salt treated) to 16 g in experimental groups.

Despite the strong smell and its fast evaporation rate, acetone is surprisingly not toxic. The US EPA (EPA US 2003) has specifically listed acetone as one of the few solvents that is not listed as hazardous air pollution and does not cause cancer or other serious illness unless it is taken orally. Conversely, care has to be taken during handling process as it is highly inflammable. It is well known that acetone has a tendency to absorb moisture from the inner layer to the surface layer and reduce the moisture level of the skins. This property of acetone, along with the bactericidal property of *S. anacardium*, provides an effective and useful method of preservation of goatskins. Moreover, this reported method of preservation is cost-effective and does not require any special skill.

It is well known that environmental risk minimization and management is one of the main objectives of cleaner production (Gupta et al. 2002). The most popular and traditional system of using common salt in the curing process causes adverse effects on the environment. A search for pollution free, environment friendly, and cleaner curing system is on and is also listed as one of the top priorities in the leather technology area. The attempted study has been performed with this intention using an eco-friendly medicinal plant namely *S. anacardium* as a substitute to common salt for the curing process. Its use had given some added advantages like reduction in the usage of water and removal of whole fat at the first step due to the degreasing property of acetone. Normally, for salt-cured skins, 300 % (v/w) of water is used for soaking, and the water is changed at least three times to ensure complete removal of the salt. One time wash with 300 % (v/w) water was found to be more than sufficient in our method.

On the whole, we experienced nil difficulty during leather processing with the skins preserved with acetone plus plant extract system and the final leather quality compares very well with the leathers from salt-cured skins.

Conclusions

S. anacardium is a plant with a number of active principles present in it. The nut extract of this plant is capable of preserving the freshly flayed skins for more than 30 days because of the antimicrobial components present in it as reported by Zabin et al. (2012).

The curing method using *S. anacardium* provides an eco-friendly option to overcome the environmental problems produced by using salt. The present study substantiates that this method can be used effectively as an alternative curing agent to sodium chloride. In summary, the potential use of newly developed eco-labeled products like this may substitute pollution-causing chemicals for preservation of skins and hides in leather industry.

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