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Preservation of African Sheepskin using *Momordica charantia* Biocide Recipe and Leather Production

Mikailu, A.S[1]., Igiri, B. E[1]*., Yakubu, M. K[3]., Akabuogu, E.P[2]., Suleiman, H[1]., Mohammed, S.Y[2]. Habila, B[2]. Joseph, C[4].

[1]Extension Centre, Nigerian Institute of Leather and Science Technology, Zaria, Nigeria
[2]Directorate of Research and Development, Nigerian Institute of Leather and Science Technology, Zaria, Nigeria
[3]Directorate of Polymer, Nigerian Institute of Leather and Science Technology, Zaria, Nigeria
[4]Directorate of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology, Zaria, Nigeria

**Correspondence: egwubernard2@gmail.com. Tel.: +234-706-859-7797*

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Abstract: Raw sheep skins are susceptible to bacterial degradation, necessitating preservation before leather processing. Traditional salt-based methods pose environmental and quality concerns. This study investigates the potential of *Momordica charantia* leaves extract as a salt-free alternative for sheepskin preservation. Parameters such as hydrothermal-stability, moisture content, and visual evaluation of skins were evaluated and processed into upper leather after preservation. Phytochemical test revealed that the extract contained alkaloids, saponins, tannins, flavonoids, steroids, and glycosides. The zones of inhibition of the extract against *Staphylococcus aureus*, *Bacillus* spp, *Staphylococcus* spp, *Coryne rbacterium* spp, *Micrococuss* spp, and yeast sp were 32 mm, 36 mm, 15 mm, 39 mm, 9 mm, and 33 mm. The curing assessment revealed that the recipe formulation is efficient in preserving raw goatskins. The shrinkage temperature of the wet-blue leather was 87% while the control was 89%. The shrinkage temperature of the chrome retanned leather was 94% while the control was 93%. The quality of the experimental wet-blue and chrome retanned leathers produced is comparable with the control leather,

Keywords: *Momordica charantia;* antimicrobial; raw goat skin*;* salt-free preservation; leather quality

1. Introduction

The leather industry generates high pollutant for the reason that leather is not a “friend” of the surroundings, as it plays a role in the environmental damage caused by the pollution of materials used in leather preservation and processing. In the light of this reason, raw hide/skin preservation has always been a challenge for leather manufacturers (Gudro *et al*., 2014). The raw hides and skins are flayed from the animals and deterioration of the skin starts within 5 to 6 h after flaying. Protein, which is the important component of raw skins and hides, is highly susceptible to bacterial action. It is necessary to preserve the protein matrix and temporarily arrest microbial physical attack (Kanagara & Chandra, 2002). The preservation has the aim to reverse flayed hide/skin into resistant to putrefaction raw

material suitable for transportation and storage during a particular time. Preservation is accomplished either by destroying active bacteria, preventing bacterial activity or preventing bacterial contamination (Covington, 2009). Wet salting, the conventional technique of curing, causes significantly higher pollution of tannery effluent, especially by total dissolved solids (TDS) and chlorides (Cl^-) (Sharma *et al*., 1996). The first stage is the preparation process which is mainly soaking, degreasing, dehairing, liming, deliming and bating, with the fundamental purpose of removing the unnecessary substances such as dirt, hair, grease and non-structural proteins, and to open up collagenous fibers. The second stage is tanning process in which hides and skins are subjected to acid treatment (pickling), and then tanned by using metal salts (chromium (III) salt) or vegetable tannins.

Leather artifacts have carried significant historical and cultural value throughout human history for all nations. The salt curing technique has a risk of being hazardous with significant negative environmental effects and researchers are currently experimenting with various plant formulations for skin preservation. *Azadirachta indica* (Neem) dried leaf powder (15%) was explored for use as a potential skin preservative with less salt because of its antimicrobial properties (Velappan *et al*., 2022). Goat skin was successfully preserved with 10% leaf paste, and 10% salt using the organic preservative *Calotropis gigantea* leaf paste (Hashem *et al*., 2018).

Plants are a huge source of biocidal compounds, containing secondary metabolites involved in plant resistance mechanisms against microbial cells (Elbouzidi *et al*., 2024; Cherriet *et al*., 2023; Keita *et al*., 2022; Selles *et al*., 2013; Kumar *et al*., 2014;). These biocidal compounds may be directly exploited as plant extracts, phytochemical formulations or organic amendments, or used as model compounds for the development of chemically synthesized derivatives. Many plant-based preservative formulations (Iyappan *et al*., 2013; Kumar *et al*., 2014; Selvi *et al*., 2015; Zengin *et al*., 2014) have been reported for the preservation of animal skins and hides or as biocides used in the soaking process. Plant-derived biocides are advantageous because of their safety to environment, humans and animals, and selective mode of action. In addition, they can easily combine with other control practices, such as bio control agents and also synthetic biocides. In the light of this challenge, and in an attempt to salvage the situation, this study is undertaken to develop new, effective and more environmentally friendly biocide preservative, which is bacteriocidal.

2. Methodology

2.1 Collection of plants samples and preparation of extracts

Freshly *Momordica charantia* leaves were collected from Zaria, Kaduna State Nigeria. The leafs were carefully washed with tap water and dried under shade at room temperature and the dried material was ground to a fine powder and analytical grade solvent was used for the extraction. The resulting powder was weighed, and 500g of the plant materials was extracted using 2000 ml each of methanol, ethyl acetate, acetone and water as described by Eloff (1998) via maceration technique. After soaking, the extracts were shaken regularly at room temperature for 72 hours. The extract was then filtered using Whatman filter paper No 1. The filtrates were evaporated to dryness at 37°C using drying cabinet and kept until when needed.

2.2 Qualitative phytochemical screening of *M. charantia* extracts

Qualitative phytochemical screening for the identification of *M. charantia* secondary metabolites was performed according to standard techniques (Shah *et al*., 2014; Shaikh & Patil, 2008; Malar & Chinnacharmy, 2017). Detection was based on coloration and precipitation reactions with specific

reagents to assess the presence of saponins, alkaloids, tannins, anthraquinones, terpenoids, flavonoids, and cardiac glycosides.

Photo 1: Leaves and powder of *Momordica charantia*

2.3 Antimicrobial activity of *M. charantia* extracts

To assess the presence of antimicrobial compounds in the plants, antimicrobial susceptibility tests of the extracts was carried out using agar well diffusion method. This was done using the different extracts at different concentrations of 1.25mg/ml, 2.5mg/ml, 5mg/ml and 10mg/ml. The extracts at different concentrations were placed onto nutrient agar surface spread with 0.1 ml of bacterial culture (standardized to 0.5 McFarland standards (10^6 cfu. ml^{-1}) that were isolated from goat skins.
The plates were incubated at 37 ^{0}C for 24 h. The results were recorded by measuring the zone of growth inhibition around the wells. Control wells contained DMSO only. For comparison, standard synthetic biocide was used as positive control. The antibacterial spectra showing zone of inhibition in millimeters were calculated.

2.4 Collection of sheep skins

Freshly flayed sheep skins were procured from Tudun wada hides and skins canteen, Zaria, Kaduna State. Firstly, each goatskin was cut along backbone line into two halves: one half was further cut into two (quarter) (experimental analysis) and the corresponding halve were used for conventional salt curing as positive control for comparison purpose.

2.5 Preservation and quality evaluation of goatskins

Freshly flayed sheepskins (taken not later than 1 h after flaying) were used for preservation, quality evaluation and cured with different concentrations of preserving materials. The percentages used for the study were based on the green weight of the skin as depicted in Table 1. Each of the half skins were cured using 30% (on fresh skin side) common salt (positive control) and left for 30 days of storage. Each of the quarter skin was preserved at different concentrations of 30% of *Momordica charantia* biocides and left for 30 days of storage under laboratory condition. Quality of preservation was assessed organoleptically observing any physical changes such as hair slip, smell and appearance of mucous surface of skin at an ambient temperature and monitored daily, which are indicators of putrefaction (Daniyan & Muhammad, 2008).

Table 1: Experimental Design of the percent and concentration of the preserving materials used

Experiment	Codes	Percent (w/w) of preservation materials used	Green weight (g)
Preservation with *Momordical charantia*	Sample 1	30% of 5mg/ml of *Momordical charantia*	243.79
	Sample 2	30% of 1mg/ml of *Momordical charantia*	259.29
	Sample 3	Control (30% of Nacl)	700

2.6 Leather making and physical strength properties of wet blue and chrome retanned leather

The goatskin that was preserved for 30 days were processed into leather using chrome tanning process and retanned by syntan (4%) and mimosa (10%) to make upper leathers. The wet blue leathers were tested for their shrinkage temperature. After 48 hours, the wet blue leathers were retanned and the crust leather physical strength properties such as shrinkage temperature, tensile strength, elongation at break, moisture content, and resistance to compression were determined and assessed in comparison with conventional salt-cured skin.

2.7 Statistical analysis

All data were expressed as the average value of duplicate measurements. One way ANOVA with Turkey′s post-hoc test was used to compare between positive controls and the experimental. Confidence limits were set at $p<0.05$ and data was analyzed using Graph Pad Prism 8.0.2 version.

3. Results and Discussion

3.1 Phytochemical screening

The qualitative phytochemical screening of crude extracts of *M. charantia* (bitter melon) leaf for possible presence of different secondary metabolites is shown in Table 2. Previous research has identified *M. charantia* as rich source of various phytochemicals (Farombi & Owoeye 2011; Joseph & Jini 2013). The qualitative phytochemical analysis in this study revealed the presence of biologically active secondary metabolites such as flavonoids, saponins, terpenoids, and alkaloid. The least phytochemical was steroids as it was only detected in the aqueous extracts of the plant (Table 2). This finding corroborates that of (Seyi-Samson *et al.*, 2024), with the exception of alkaloids and terpenoids which was absence in their finding.

Table 2: Preliminary phytochemical screening profile of *M. charantia* extracts

Secondary metabolites	Extracts of *M. charantia*			
	ME	EA	AC	AQ
Flavonoids	+	+	+	+
Terpenoids	+	+	−	+
Alkaloid	+	+	−	+
Tannins	+	+	−	+
Saponins	+	+	+	+
Steroids	−	−	+	−
Cardiac glycosides	+	+	−	+
Anthraquinones	−	+	−	+
Phenols	+	+	−	+

+: present, **−:** absent, ME: Methanol, EA: Ethyl acetate, AC: Acetone, AQ: Aqueous

3.2 Antimicrobial activity

The phytochemicals are known for their medicinal importance, demonstrating various pharmacological, biochemical, and physiological effects in modern medicine (Rajani *et al.*, 2024).

The antimicrobial test against the microorganisms isolated from sheepskin revealed that the organisms were susceptible to the *M. charantia* extract at 10mg/ml concentration (Table 3). The results indicate that the leaf extracts of *M. charantia* demonstrated antimicrobial activity against sheepskin putrefying bacterial isolates. The most significant inhibitory activity was observed against *Bacillus spp* with an inhibition zone of 36 mm. Furthermore, our findings are in agreement with Khalid *et al.* (2021) research, which reported the antimicrobial activity of *M. charantia* leaf extracts against bacterial isolates including *P. multocida*, *E. coli*, and *S. aureus* (Khalid *et al.*, 2021). The result also agreed with Ihsan *et al.* (2023) finding that *M. charantia* exhibited more significant growth inhibition compared to antibiotics when tested against *E. coli*, *P. aeruginosa*, and *S. aureus* (Ihsan *et al.*, 2023).

Table 3: Antimicrobial activity of *M. charantia* extract against indigenous microbes from goatskins

Plant	Extracts	Microbes	Zone of Inhibition (mm) at Different Concentrations (mg/ml)				Positive control (mm)	*p* value
			10	5.0	2.5	1.25		
M. charantia	ME	*Staphylococcus aureus*	32	14	13	11	39	0.0009
	EA		21	10	0	0		
	AC		27	13	11	5		
	AQ		10	0	0	0		
	ME	*Bacillus spp*	36	0	0	0	45	0.0004
	EA		27	0	0	0		
	AC		35	0	0	0		
	AQ		0	0	7	0		
	ME	Yeast	33	26	17	15	55	<0.0001
	EA		32	0	0	0		
	AC		30	0	0	0		
	AQ		12	10	0	0		
	ME	*Staphylococcus* spp	0	0	0	0	52	<0.0001
	EA		0	0	0	0		
	AC		0	0	0	0		
	AQ		15	10	0	0		
	ME	*Corynerbacterium* spp	25	14	9	7	37	<0.0001
	EA		18	12	10	9		
	AC		10	9	10	7		
	AQ		0	0	0	0		
	ME	*Micrococuss* spp	0	6	5	0	38	<0.0001
	EA		0	9	5	0		
	AC		0	8	6	0		
	AQ		0	0	0	0		

3.3 Skin degradation evaluation

Hair slip is the first indication of putrefaction when the protein is degraded by the bacteria. During putrefaction process, there is a breakdown of the protein to amino acid and further break down to ammonia (Covington 2009; Didem & Meral 2010). Consequently, in this study, physical evaluations such as hair slip, odor, and putrefaction were taken as measurements for assessing the efficacy of the preservation technique. The comparative visual observations on the degree of putrefaction are displayed in Table 4. Putrefaction indications, hair slip and odor were not observed on the experimental preservation of the skins treated with two different concentrations of *M. charantia* up to 30 days curing storage.

Table 4: Preserved skin degradation evaluation for 30 days at difference concentrations of curing agents

Percentage of curing agent	Skin degradation evaluation		
	Hair loosening	Odor	Putrefaction
30% of 5mg/ml of *M. charantia*	No hair slip	No odor	No
30% of 1mg/ml of *M. charantia*	No hair slip	No odor	No
Control (30% NaCl)	No hair slip	No odor	No

3.4 Suitability of preserved skin

The suitability of the preservation technique was tested by producing leather from the preserved skins and the behavior of the skins during processes was observed, as well as assessing properties of the leather produced from goatskins (Figure 1 and Table 5). In the first soaking process, skins were soaked in water to remove the preservative from curing and increase the moisture so that the skin can be further treated (Covington 2009).

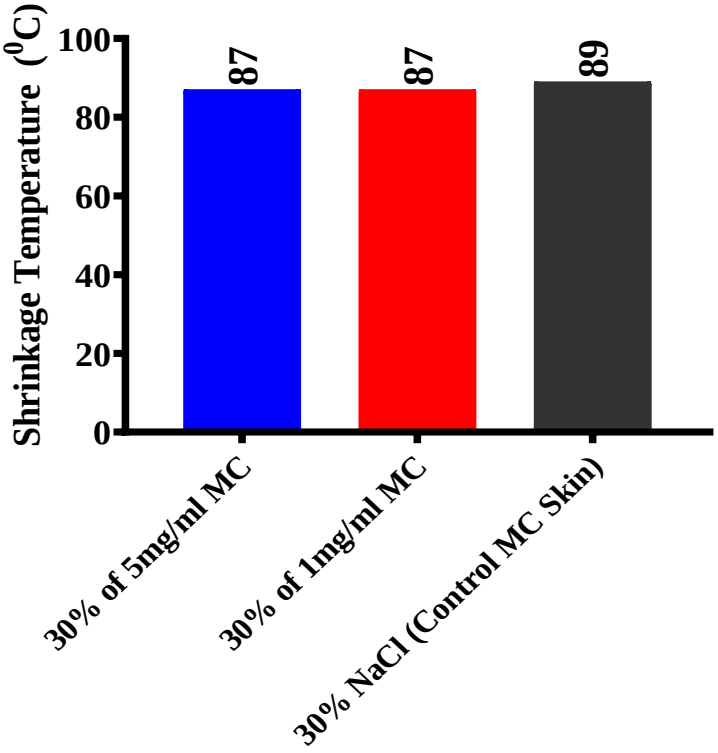

Figure 1: Shrinkage temperature of wet blue leather of experimentally preserved 30% of *M. charantia* and control (30% common salt) sheepskin. MC = *M. charantia*

Table 5: Effect of technological processes on retanned leather properties of *M. charantia* biocide recipe and salt preserved sheepskins

Index	Conc.of *M. charantia* & salt	Chrome retanned leather produced from preserved skin
		MC
Shrinkage temperature of leather [^{0}C]	30% of 5mg/ml of recipe	94
	30% of 1mg/ml of recipe	94
	30% NaCl, control	93
Tensile strength [N/mm^2]	30% of 5mg/ml of recipe	19
	30% of 1mg/ml of recipe	15.5
	30% NaCl, control	24.4
Elongation at break (%)	30% of 5mg/ml of recipe	81
	30% of 1mg/ml of recipe	56
	30% NaCl, control	67.7
Hardness	30% of 5mg/ml of recipe	71.7
	30% of 1mg/ml of recipe	75.7
	30% NaCl, control	68
Moisture content (%)	30% of 5mg/ml of recipe	51.73
	30% of 1mg/ml of recipe	49.19
	30% NaCl, control	51.38
Resistance to compression	30% of 5mg/ml of recipe	1.90
	30% of 1mg/ml of recipe	1.95
	30% NaCl, control	1.75
Lastometer	30% of 5mg/ml of recipe	41.68
	30% of 1mg/ml of recipe	32.79
	30% NaCl, control	57.22
Water vapour permeability	30% of 5mg/ml of recipe	0.041
	30% of 1mg/ml of recipe	0.003
	30% NaCl, control	0.104
Thickness (mm)	30% of 5mg/ml of recipe	1.79
	30% of 1mg/ml of recipe	1.30
	30% NaCl, control	2.13
Apparent density	30% of 5mg/ml of recipe	0.39
	30% of 1mg/ml of recipe	0.32
	30% NaCl, control	0.45

MC; *M. charantia*

After soaking the preserved skins, the physical observation reveals that the salted skin absorbs water and rehydrate completely. Also, the 30% of 1mg/ml *M. charantia* absorb, rehydrated and was softer than the 30% of 5mg/ml *M. charantia* and the salt preserved skin. Physical observation during bating revealed that 30% of 1mg/ml *M. charantia* had a good porosity than the salt preserved skin.

The leather samples produced were tested and qualitative indexes were determined (Figure 1 and Table 5). In determining the properties of the leather, the index, which is very sensitive to collagen structural

changes, is the shrinkage temperature of skin. The results of the skin shrinkage temperature determination (Figure 1 and Table 5) did not show any serious changes in derma structure. The shrinkage temperature of the skin increases when the leather was retanned (Hassan *et al.*, 2023; Kasmudjiastuti *et al.*, 2019).

As the main protein in skin is collagen, its condition determines the quality of the leather produced and leather quality depends upon the condition of the raw material. The derma of the leather produced was more accessible for chromium compounds. Consequently, the higher content of Cr_2O_3 leads to higher shrinkage temperature, which is the main reason for the higher shrinkage temperature of the leather produced from the skin preserved with the biocide recipe for 30 days (Kherbache *et al.*, 2016). The tensile strength of the experimental skin was 19 and 15.5 while that of the salt skin was 24.4 N/mm^2 (Table 5).

Conclusion

This study successfully demonstrated the potential of *Momordica charantia* leaves extract as a salt-free alternative for short-term raw sheepskin preservation. The extract effectively preserved the skins, resulting in comparable leather quality with improved softness and porosity. While tensile strength was slightly lower, the extract allowed better access to chromium compounds, leading to a higher shrinkage temperature. Further research is needed to assess long-term effectiveness, cost-efficiency, and scalability for wider adoption in the leather industry.

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References

Cherriet S., Merzouki M., El-Fechtali M., Loukili E., *et al.* (2023) In silico investigation of aristolochia longa anticancer potential against the epidermal growth factor receptor (EGFR) in the tyrosine kinase domain, *Moroccan Journal of Chemistry*, 11 (04), 1074-1085

Covington T. (2009). Tanning chemistry; The science of leather. Cambridge: The Royal Society of Chemistry. 2009

Daniyan S.Y., and Muhammad H.B. (2008). Evaluation of the antimicrobial activities and phytochemical properties of extracts of *Tamaridus indica* against some diseases causing bacteria. *Afri. J. Biotech*, 79(14), 2451-2453

Didem B. and Meral B. (2010). Examination of bacterial population in salt, salted hides, soaked hides and soaked liquors. *J. Ameri and Leather Chem. Asso.* 105; 321.

Elbouzidi A., Taibi M., Laaraj S., Loukili E.H., Haddou M., *et al.* (2024). Chemical profiling of volatile compounds of the essential oil of grey-leaved rockrose (Cistus albidus L.) and its antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer, *Frontiers in Chemistry*, 12, 1334028

Eloff JN. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants. *Journal of Ethnopharmacology*, 60(1), 1-8

Farombi E.O. and Owoeye O. (2011). Antioxidative and Chemo-preventive Properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. *IJERPH.* 8(6), 2533–2555. Available at: http://doi.org/10.3390/ijerph8062533

Gudro I., Valeika V. and Sirvaitytė J. (2014). Short term preservation of hide using vacuum: influence on properties of hide and of processed leather. *PLoS ONE*, 9 (11), e112783.

Hashem M.A., Momen M.A., Hasan M. (2018). Leaf paste aided goat skin preservation: Significant chloride reduction in tannery. *J. Environmental Chemical Engineering*. 6(4):4423–8. https://doi.org/10.1016/j.jece.2018.06.050

Ihsan M., Din I.U., Alam K., Munir I., Mohamed H.I. and Khan F. (2023). Green Fabrication, Characterization of Zinc Oxide Nanoparticles Using Plant Extract of *Momordica charantia* and *Curcuma zedoaria* and Their Antibacterial and Antioxidant Activities. *Appl Biochem Biotech,* 195(6), 3546–3565. Available at: http://doi.org/10.1007/s12010-022-04309-5

Iyappan K., Ponrasu T., Sangeethapriya V., Gayathri V.S. and Suguna L. (2013). An eco-friendly method for short term preservation of skins/hides using *Semecarpus anacardium* nut extract. *Environ. Sc. Pollution Research,* 20(9), 6324-6330.

Joseph B. and Jini D. (2013). Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency. *Asian Pacific Tropi Dis.* 3(2), 93–102. Available at: http://doi.org/10.1016/S2222-1808(13)60052-3

Kanagaraj J. and Chandra N.K. (2002). Alternatives to salt curing techniques - A review. *Journal of Scientific and Industrial Research* 61, 339–348.

Kasmudjiastuti E., Pidhatika B., Griyanitasari G., and Pahlawan I. F. (2019) The effect of alum addition on shrinkage temperature, chemical properties, and morphology in the manufacture of vegetable-tanned leather, *IOP Conf. Series: Materials Science and Engineering*, 602, 012044, doi:10.1088/1757-899X/602/1/012044

Keita K, Darkoh C, Okafor F. (2022) Secondary plant metabolites as potent drug candidates against antimicrobial-resistant pathogens. *SN Appl Sci.,* 4(8), 209. https://doi.org/10.1007/s42452-022-05084-y

Khalid Z, Hassan MS., Mughal SS., Hassan SK. and Hassan H. (2021). Phenolic Profile and Biological Properties of *Momordica charantia*. *Chem. Biomole Eng.* 6(1), 17–29. Available at: https://doi.org/10.11648/j.cbe.20210601.13

Kherbache S., Bouzidi N., Bouzidi M. A., Moussaceb K., Tahakourt A. K. (2016) The behavior of the concretes and mortars reinforced by metallic fibers wastes as substitution of cement, *J. Mater. Environ. Sci.* 7 (1), 18-29

Kumar M.P., Velmurugan P., Sreeram K.J., Rao J.R. and Nair B.U. (2014). Reuse of solid waste from juice industry (*citrus sinensis* peel) in the extraction of antioxidants with enhanced activity through polymer encapsulates for the preservation of skin. *J. Ameri Leather Chemists Assoc*, 109(7), 231-238.

Malar G. and Chinnachamy C. (2017). Phytochemical screening, total flavonoid, total terpenoid and anti-inflammatory activity of aqueous stem extract of *Salacia oblonga*. *J. Chemical and Pharmaceutical* 10, 550

Rajani V., Umadevi S. and Naga Raju C. (2024). A Review on Exploring the Phytochemical and Pharmacological Significance of *Indigofera astragalina*. *Pharmacogn Mag.* 3. Available at: https://doi.org/10.1177/09731296231215911

Selvi A.T., Kanagaraj J. and Saravanan P. (2015). Preservation of goatskin using *tamarindus indica* leaf extract -green process approach. *J. Society of Leather Technologists and Chemists*, 99(3), 107-114.

Selles C., Dib M.E.A., Djabou N., F Beddou, *et al.*, (2013) Antimicrobial activity and evolution of the composition of essential oil from Algerian Anacyclus pyrethrum L. through the vegetative cycle, *Natural Product Research* 27 (23), 2231-2234

Seyi-Samson E., Zaynab-Aderonke O., Temiloluwa-Femi O., Nwachi-Idume O., Adekunle-Totoola A., Jamiu-Monsur O. and Ayomide-Oluwatobiloba O (2024). Phytochemical screening and antibacterial activities of *Momordica charantia* and *Vernonia amygdalina* extracts on some selected enteric isolates. *TMR Modern Herbal Medicine* 7(1), 2

Shah S., Hussain M., Aslam M. and Rivera G. (2014). Natural Products; Pharmacological Importance of Family Cucurbitaceae: a Brief Review. *Mrmc* 14, 694–705.

Shaikh J. and Patil M. (2020). Qualitative tests for preliminary phytochemical screening: an overview. 8,603–8

Sharma P.K., Varma S.K., Datta K.S., Kumar B. and Angrish R. (1996). Differential response of wheat to chloride and sulphate salinities at germination and early seedling growth. *Haryana Agricultural University Journal of Research* 26, 1–7.

Velappan B., Gnanasekaran S., Victor J.S., Alagumuthu T., Nagarajan V., Chinnaraj V.K. (2022). Characterization and application of dried neem leaf powder as a bio-additive for salt less animal skin preservation for tanneries. *Environmental Science and Pollution Research*. 29(3):3763–72. https://doi.org/10.1007/s11356-021-15653-0

Zengin A.C.A., Colak S.M., Zengin G. and Kilic E. (2014). Eco-friendly soaking process using tannic acid as an alternative bactericide. *Archives of Environmental Protection*, 40(1), 3-12.