EVALUATION OF SAPONINS CONTENT AND SURFACTANT PROPERTIES OF CRUDE EXTRACTS OF *Balanitesaegyptiaca* ROOTS FROM DEMSA AND GIREI LOCAL GOVERNMENT AREAS

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Abstract: To achieve green technology in the treatment of leather using bio-based surfactants, crude extracts of *Balanitesaegyptiaca* roots from Demsa and Girei Local Government Areas of Adamawa State, Nigeria, were evaluated for saponins content prior to its application in treatment of goat skin. High saponins content were recorded for both root extracts from Demsa (RexD) 89.4% and Girei (RexG) 88.1%, however, the slight variation in saponins contents and some other chemical functionalities are attributed to different geographical factors in the two locations, influencing the bioavailability of phytochemicals in the plant. The effect of temperature on the tensile strength and elongation of leather treated with the crude saponins extract and a synthetic surfactant were studied and the results showed that the surfactant properties of the crude extracts improved with increase in treatment temperature. The experiments further showed that at optimum operating temperature (35 – 40°C), the crude extracts will compete with synthetic surfactants in an efficient treatment of leather.

Keywords: *Balanitesaegyptiaca*, saponin, surfactants, leather processing

Introduction

Desert date with the botanical name *Balanitesaegyptiaca* belongs to the family Balanitsceae. About twenty-five known species of the plant are widely distributed through tropical Africa (Manji *et al.*, 2013). *Balanitesaegyptiaca* even though has the history of traditional use as a surfactant, has not been efficiently investigated to obtain data and information that will presentit as raw material with potentials in surfactant applications. *Balanitesaegyptiaca* plant is available in abundance inthe north eastern part of Nigeria (Manji *et al.*, 2013).

Saponins are structurally complex amphiphatic glycosides of steroids and triterpenoids that are widely produced in plants (Vincken *et al*., 2007). It is also found in some marine organisms like sea cucumbers and starfish (Van Dyck *et al*., 2010). The term saponin is derived from Latin; *sapo* means soap, because of it surfactant properties that form stable soap-like foam when mixed and agitated with water. The term saponin is chemically defined as a group of high molecular weight glycosides that consist of hydrphobicaglycone linked to a hydrophilic sugar moiety called sapogenin (Mayank *et al.*, 2011; Moses *et al*., 2014). It is classified as triterpenoid and steroidalsaponins because of their chemical structures (Sparg *et al*., 2004). It is further classified according to the carbon skeleton of the aglycon into 12 main classes, namely: tirucallanes, dammaranes, lupanes, cucurbitanes, hopanes, oleananes, 23-nor oleananes, taraxastereanes, ursanes, cycloartanes, lanostanes, and steroids (Vincken *et al*., 2007). Saponins are complex mixture; their composition may vary depending on the genetic background; the tissue type, the age and the physiological state of the plant, and environmental factors (Szakiel *et al*., 2011).

Saponins are abundant non-essential secondary metabolites of plants, which has found many applications both traditionally/domestically and at industrial scales (Dixon, 2001; Francis, 2002; DeGeyter *et al*., 2007). Saponins are also part of a diversity of secondary metabolites that have been demonstrated to play a role in the adaptation of plants to their environment. The production of secondary molecules plays part of the response to external factors and various biotic and abiotic stimulation, they contribute to the innate immunity as phytoprotectants and ptytoanticipins that are constitutively produced and inducible phytoalexind (Dixon, 2001). Because of their specific properties,the industrial application is growing fast, which sharply differentiate them from common surfactants as foaming and surface active agents (Kime *et al.*, 2015). Natural fat treatment from skins and leathers is compulsory to prevent some undesirable effects in finished products. Non degreased leatheris responsible for to feel hard, non-flexible, decreased in physical resistance and dye stains and also fear of white spots or vail on the skin surface because of the effect of free fatty acids and triglycerides of the skin (RamÓn, 2016).

The potential of the root from *Balanitesaegyptiaca* plant as bio-based surfactant for the treatments of leather in one way will increase the understanding and to help to expand the use of mostly neglected plants in our area. The local sourcing of *Balanitesaegyptiaca* inclined this study towards sustainability, while its natural sourcing inclined the study towards green production. In furtherance to our previous study (Kime *et al*., 2015; Barminas *et al*., 2016), this study therefore seeks to investigate some other surfactant properties of the extracts from the root of *Balanitesaegyptiaca* to more comprehensively report its potentials as a surfactant.

Materials and Methods

Collection of plant materials

Balanitesaegyptiaca plant grown in Demsa and Girei, Local Government Areas of Adamawa state, Nigeria were used in this study. Root of matured plant were collected (Plate 1). The random sampling method used by Ponarulselvam *et al.* (2012), Kime *et al.* (2015) and the sampling technique by Ninfaa *et al.* (2014) with little modifications was adopted. The fresh plant samples were collect and the voucher specimen numbered, and kept in Chemistry Research Laboratory of Modibbo Adama University of Technology, Yola for analysis. The root sample were then being pulverized and ground with pestle and mortar and sieved to obtain a dried powder samples as crude source of surfactant. The general available surface-active agent, synthetic detergent is purchased from the market in Girei, Adamawa state.

Plate 1: *Balanitesaegyptiaca* plant (A) and the root (B)

Method of extraction

Extractions, determination of the presence and the amount of saponins from root of Balanitesaegyptiaca plant

The procedure outlined by Barminas (2016) which described the determination of saponins by the gravimetric method of AOAC (1990) was used for the extraction. With this, 5 g of each of the dried grounded powdered samples was weighed into a thiamble and transferred into a Soxhlet extractor chamber fitted with a condenser and a round bottom flask containing 200 cm^3 of acetone. This was heated was on mantle at 60ºC for 3 h, to exhaustively extract lipid and interfering pigments, after which the solvent was distilled off. The defatted sample was further being transferred into another Soxhlet extractor fitted to both a condenser and a dry weigh round bottom flask containing 200 cm^3 of methanol, and heated on mantle for another 3 h. The methanol was recovered by distillation at the end of the extraction, and the extract was transferredinto the oven, dried and cooled in desiccator. The percentage of crude saponins extract was calculated as shown below:

$$\% \text{ of } Saponin = \frac{Weight\ of\ Saponin}{Weight\ of\ Sample} \times \frac{100}{1}$$

Selection of animal and sampling of skin for treatment

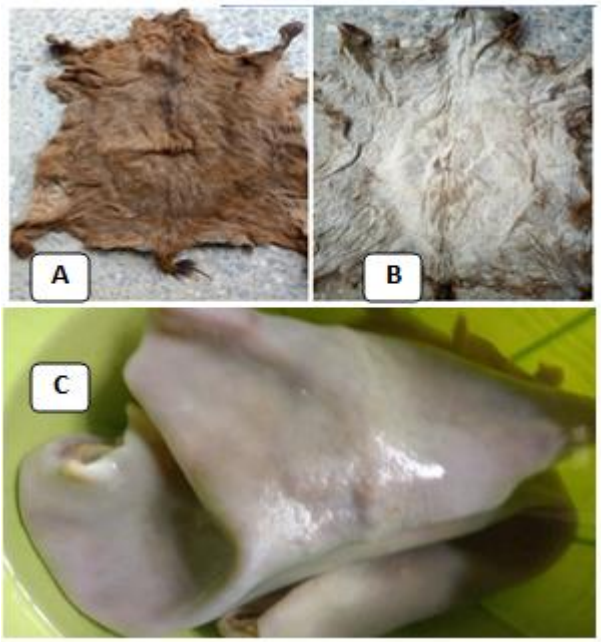
The skin was purchased fromhousehold, a farmer reared in Girei Local Government province comprised of 11 kids and 13 adult goats, with a ratio of 6 males to 18 females in which the male were randomly selected. Girei area is located at longitude 12º.1'23" to 12º.33'38" E and latitude 12º.11'51" to 12º.41'49" Nbordering Camaroon and Mandara mountains. The climate of Girei is generally hot and the land-use type is basically arable farming and animal production. The goat is grazed in a free range throughout the spring, summer and autumn without any amounts of supplementary forage and grain (Salehi & Bitaraf, 2013). The male goat was slaughtered and the skin cured salted. The salted skin was place in the shade 20^0C-30% humidity for at least two weeks to get dried.

Skin Treatment (Leather)

The procedure described by RamÓn (2016) was used. The weight skin was recorded, before and after salted. The leather was measured by using the methods introduced by the International Organization for Standardization (2002a, b, c; 2006). Where the crude saponins extract obtained from the root of *Balanitesaegyptiaca* plant were used as synthetic surfactants/enzymes. The applied sequence of processes steps and techniques on goatskins storage and beam house operations were followed by these steps (Table 1 and Plate 2):

Table 1: Goatskin storage and leather operations (Hauber & Knodler, 2008)

Process unit	Inputs
Curing and storing	Salt, cooling and drying
Soaking	Water, surfactant, alkali
Flashing	Cold water
Liming and unhairing	Water, surfactant (root extracts), lime and alkali sulphides
Washing after unhairing	Water
Deliming and bating	Ammomium salts, water gabaruuwaa plant
Rinsing	Water and gabaruuwaa plant (*Acacia nilotica*)
Treatment/degreasing	Surfactant (root extracts) and water
Pickling before tanning	Water, salt and fungicides



Plates 2: Flayed goatskin, salted (A), dry (B) and parchment (C)

Statistical analysis by two-ways ANOVA of Root of Balanitesaegyptiaca plant

The quantitative data obtained was statistically analyse by calculating the mean of three replicates follow by calculation of the Sum of Square, Variance, Standard Deviation and Standard error. The results were presented as mean ± standard error and correlation coefficient was determined.

Surface tension measurement

The surface tension was measured using the drop weight method by Agu (2010), with some modifications as follows; a burette was clean with detergent and was thoroughly rinsed with distilled water. This was allowed to dry before being clamped with its tap turned off. The burette was filled with distilled water. A pre-weighed 10 cm^3 measuring cylinder was clamped below to receive the droppings from the burette. The burette was then opened and adjusted at a regular drop wise interval of 2 seconds. Drops were collected up to a total volume of 2 cm^3. The time taken to obtain this was recorded using a stop watch and the weight of the liquid was obtained. During this experiment, the pressure was kept constant by blocking the burette top with a tissue paper to limit the influx of air, thereby stabilizing the rate of flow of the liquid. This was repeated for the extracts at different concentrations (0.01, 0.03, 0.06, 0.1, 0.3, 0.6, and 0.8 mg/dm^3). Averages of triplicate determinations were recorded. All analysis was carried out at room temperature, and the surface tension was calculated using the formula below:

$$(r_2) = r_1 n_1 p_2 / n_2 p_1$$

Where r_2 = surface tension of sample r_1 = surface tension of distilled water (standard solution) n_1 = Number of drops of water n_2 = Number of drops of samples p_1 = Density of water p_2 = density of sample A surface tension of 72.13 Nm^{-1} was adopted for distilled water, taken as the standard solution at 25°C.

Emulsion formation/capacity

The procedure by Agu and Barminas (2013) was adopted. That is 50 cm^3 paraffin oil and 50 cm^3 sample solutions were mixed in a beaker. The mixture was homogenized using an improvised method with a 100 cm^3 glass syringe. This improved homogenization process involved repeated cycles of

sucking and rapid expulsion of emulsion from the syringe. This was done to ensure proper droplet break-up until a creamy homogenous emulsion is obtained. Emulsion Capacity is expressed as the amount of oil emulsified and held per gram of sample as given by Padmashree *et al.* (1987);
Emulsion Capacity = X/Y x 100/1
Where X = Height of emulsified layer; Y = Height of whole solution in the syringe

Fourier transform infra-red (FT-IR) measurement
The FT-IR analysis of the root extracts were carried out at American University of Nigeria using PerkinElmer FTIR spectrometer Vision 10.03.02. Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. When interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined (Ashokkumar & Ramaswamy, 2014)

The tensile strength measurement of the leather
Standard Test Method for Tensile Strength of Leather, ASTM D2209-00 and D4704 (2015), was adopted. The machine used was Monsanto Tensometer type 'w', serial number 9875, made in UK. The tensile strength is the force (Kg) per unit area of cross section (sq. Cm) required to cause a raptured of the test specimen. The tensile strength of the specimen was calculated using the following formula;

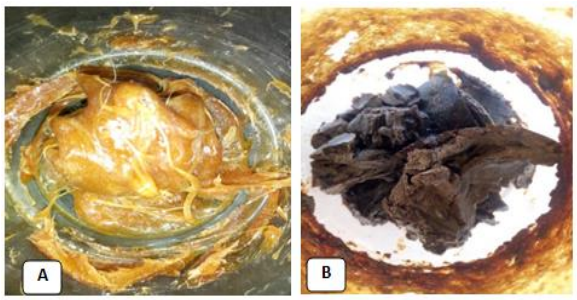
$$\text{Tensile strength} = \frac{breaking\,load\ (kg)}{thickness\,(cm)\ x\ width}$$

b) Percentage elongation at break for specimen was calculated from the distance of the jaws after breaking had occurred;

$$\%\ \text{elongation at break} = \frac{distance\ increased\ by\ breaking}{distance\ of\ the\ two\ \ jaws\ in\ normal} x\,100$$

Results and Discussion

Extraction and determination of the presences of bio-surfactants (extracts) from root
Saponins has been identified as the phytochemicals playing the surfactant role in the plant samples (Kime *et al*., 2015; Barminas *et al*., 2916). The dried crystalline Saponins extracts obtained from the *Balanitesaegyptiaca* plant roots collected from Demsa and Girei appeared in different colours (Plate 3A and B). This was attributed to the different types of soils in the locations from which the samples were collected.



Plates 3: Crude saponins extract of root from Demsa (A) and Girei (B) local government areas, Adamawa State, Nigeria

Saponins' yields were calculated to be 89.4 and 88.1 % for root samples collected from Demsa and Girei LGAs, respectively. The high content of saponin in these root samples of *Balanitesaegyptiaca* may be as a result of the plants protective or defence mechanism against soil pathogen attacks. Saponins are active antimicrobial phytochemicals which may be produced more in plant parts that are more prone to microbial attack to naturally build resistance and defence (Cragg & David, 2001; Haralampidis *et al.*, 2002). Saponins content and composition in plants depends on factors that can influence biosynthesis and active transport of phytochemicals in the plant, such as; cultivar (provenance), physiological state, geographical location, chronological age of the plant, percentage humidity of the harvested material, situation and time of harvest (Chapagain, 2006; Henna *et al.*, 2010; Faizal, 2013). These justify the variation in saponins content obtained in the samples from the two different locations. Saponins yield from both samples showed the potentials of the extract obtained as bio-based surfactants for the treatment of skins.

Statistical analysis by two-ways ANOVA Root and fruit of Balanitesaegyptiaca plants from Demsa and Girei LGA
The Univariate Analysis of Variance between subject factors using two-ways ANOVA in SPSS conducted on the crude saponins extract revealed difference in the amount of saponin extracts between the two selected LGA at $p<0.05$ with F-value of 7.424. The result clearly implies that different LGA have different effect on the amount of saponins that was obtained in *Balanitesaegyptiaca* plant. This is not surprising because the two LGA are located in two different areas and have different environmental characteristics which include elemental composition, climate, soil and relief, as such, the vegetation characteristics will differ from one another since these environmental factors affect vegetation characteristics. This difference in amount of crude saponins extract Demsa has the highest impact than Girei LGA.
Local Government Ares has it significant at 5 % = 0.013
P = 0.05
P < 0.05
LGA and plant parts has it sig. at 5% = 0.30
P = 0.05
P < 0.05

Performance test of crude saponin extracts in root of Balanitesaegyptiaca plant
Figure 1 present and compare the emulsion capacity of 1 g of *B. aegyptiaca* root extract from Demsa (RexD) and Girei (RexG) as function of time. High emulsion capacities (>90%) were recorded for both extracts in the first two hours of the evaluation, followed by a gradual decrease with time. This showed that the saponins in the crude extracts obtained from the roots of *Balanitesaegyptiaca* plant from Demsa and Girei can be a good and rapid emulsifying agent which can server as a bio-based surfactant. The emulsion capacity of the root extract from Girei (RexG) dropped lower than that of Demsa (RexD) at 5 h of evaluation. This showed that RexD contain more surfactant saponins than RexG, this is supported by the differential distribution of specific saponins in the plants and saponin production has been found to vary in individual organs and tissues (Faizal, 2013).

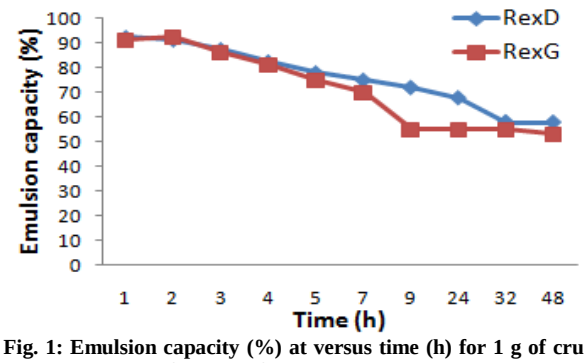


Fig. 1: Emulsion capacity (%) at versus time (h) for 1 g of crude saponins extract of the root from Demsa and Girei LGAs

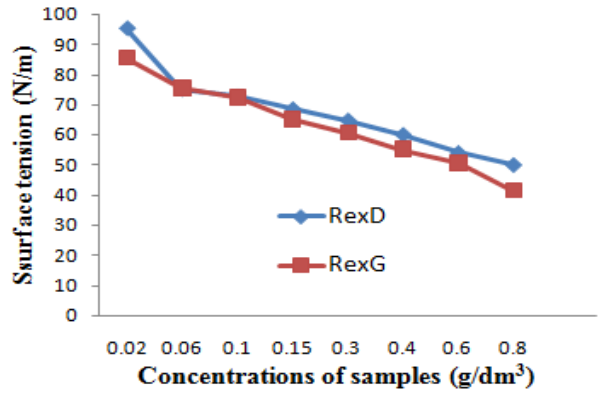


Fig. 2: Surface tension of crude saponin extract at various concentrations (g/dm^3) at 30^{o}C from Demsa and Girei LGAs

Figure 2 shows and compares the surface tension of different concentrations of RexD and RexG at 30-^{0}C (room temperature). Reduction of surface tension by surfactants allows efficient contact with materials hence a better cleansing power. Surface tension decreases with increase in concentrations of both RexD and RexG as expected of a surfactant. The results obtained in this experiment are comparable with those reported by Kime *et al*. (2016). Reduction in surface tension is more rapid with RexG. This showed that RexG disperse faster in solution than RexD, and this may be due to presence of impurities or other phytochemicals interfering and hindering the dispersion of surfactant saponins in the RexD (Feizal, 2013).

Fourier transform infrared spectrometer (FT-IR) measurement

FTIR spectra of RexG and RexD are comparatively presented in Fig. 3. There are many striking similarities in the spectra of the two extracts. The spectra of both extracts showed a broad band with a peak at 3411.80 cm^{-1} and a narrow band at 2911.33 cm^{-1}, and these attributes to O-H and C-H vibrations respectively. Another broad band appeared between 1044.97 - 1236.97 cm^{-1} and this can be associated with C-O, C-OH and C-C stretching vibrations. These bands are characteristic of saponins presence in the crude extracts. However, some differentiating peaks e.g. 2374.38 and 911.52 on RexG, showed that there are some variations in the chemical functionality of RexG compared to RexD. These variations in chemical functionalities are deemed to be responsible for the variations observed in the emulsion properties of the extracts.

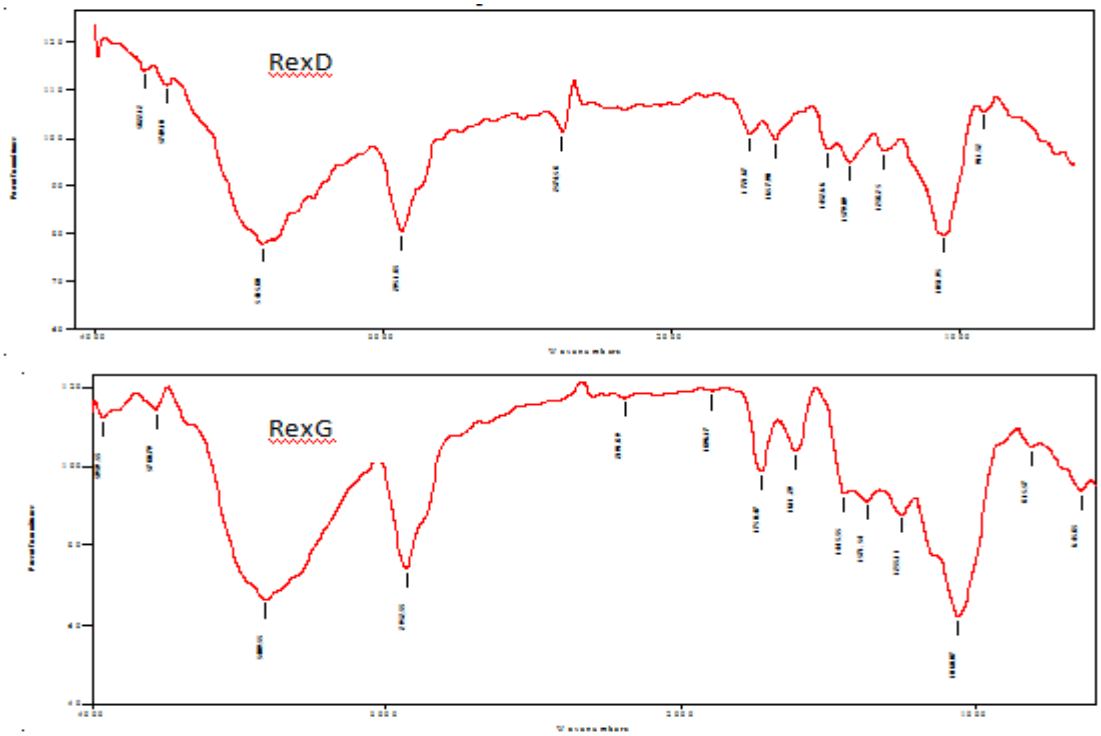
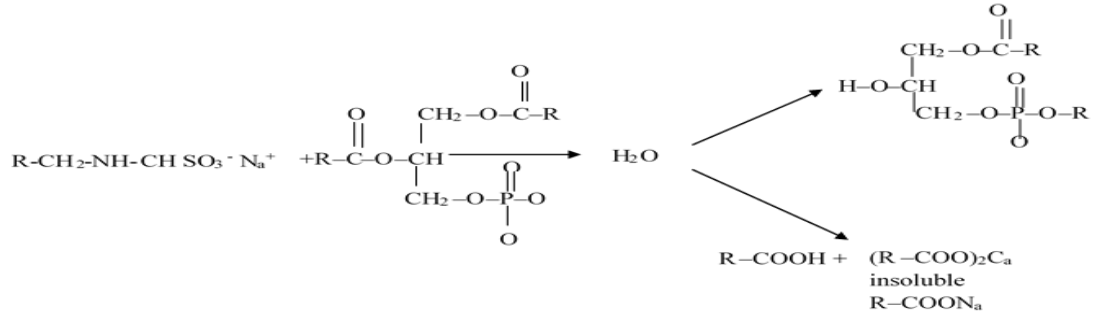


Fig. 3: FTIR spectrum of crude saponin extracts from root of *Balanitesaegyptaica* plant from Girei (RexG) and DemsaRexD



Scheme 1: Equation of the reaction between surfactants and phospholipids

Treatment of goatskin leather

Scheme 1 presents the possible reactions of surfactants (crude saponin extracts) and the Phospholipids in skins. Skin, especially domestic goat skins contain fats and oils which are removed in the tannery by liming, but liming is inadequate in achieving an efficient removal of these substances. Palop *et al.* (2000) studied the effectiveness of degreasing with lipase enzymes and standard degreasing with ethoxylated fatty alcohol as synthetic surfactant. Treatment of tanned leather with natural surfactants (bio-based surfactants)is however necessary to sustain green technology. When the saponin extracts used as surfactants are applied in aqueous medium as pickling phase, there isa combined action involving rupture of the membranes surrounding the fat cells and triglyceride splitting, which all help in the degreasing process. The fat is composed of fatty acids (10 %), triglycerides (56 %), waxes (23%), phospholipids (6%), and cholesterol (5%). The breakdown of the triglyceride with surfactants follow a mechanism in which fatty acid composition is increased from 10 to about 40 %, hence enhancing the emulsification of monoglycerides, diglycerides and fatty acid glycerol which were formed in the rupture of the triglycerides (Palop *et al.*, 2000). The reaction products are composed of glycerides, monoglycerides, diglycerides, fatty acid and surfactants. Increase in concentrations of the surfactant will increase the dispersion of fats on the leather. The test was done with the same amounts of surfactant, using 30 g amount of crude extracts and synthetic surfactant per 11.5 cm heights and 1.5 cm width of leather under different temperature of 30, 35, 40 45, 50^{o}C. Parchment from goat skin and dry leather showed different physical characteristics and most notably the colour of wet leather which changed upon drying (Plates 4).

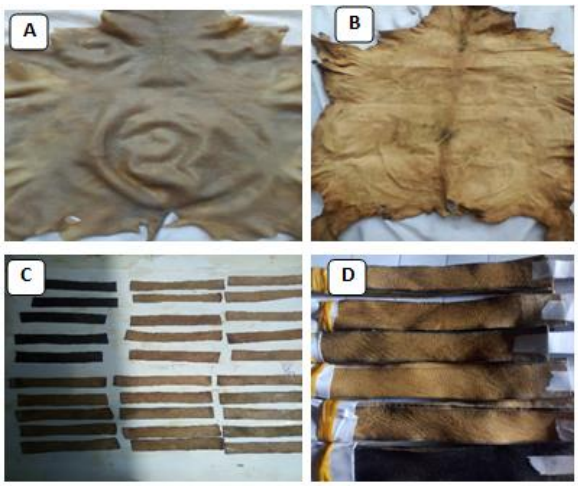


Plate 4: Fleshing operation to remove excess fat (A), finished leather (B), piece samples (C and D) of goatskin treat with 30 g/ml of crude saponins extract solution from *Balanitesaegyptiaca* plant (Demsa and Girei) and synthetic surfactant at various temperature (^{0}C)

Plate 5: Image representing the uses of male goat skin treated with crude saponin extracts (root) of *Balanitesaegyptiaca* plant

Determination of tensile strength of goatskin leather treated with crude extracts and synthetic surfactants at different temperature

Figure 4 present the effect of process temperature on the tensile strength of goatskin leather treated with 30 g of the crude extracts and a synthetic surfactant. Increase in degreasing efficacy (emulsification of fats) by surfactants and the operating temperature employed in treating leather samples will result in a reduction in tensile strength of the final product obtained (Dennis, 2016). The results showed that tensile strength of the leather decreased as the process temperature was increased. The tensile strength of the leathers treated with the synthetic surfactant is lower compared to those of the crude extracts at all operating temperatures. RexD treated leathers are exhibits higher tensile strengths than RexG at all operating temperatures except at 45°C.

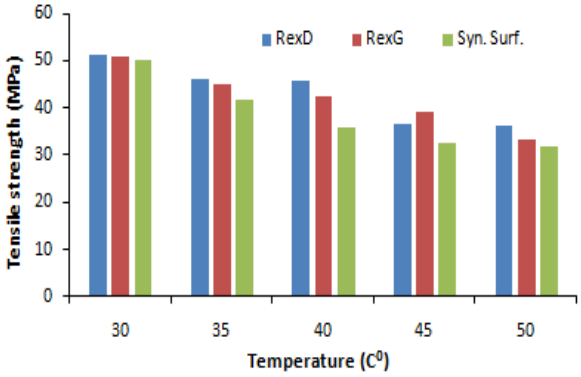


Fig. 4: Tensile strength of leather treated with 30 g of RexD, RexG and synthetic surfactants at different temperature

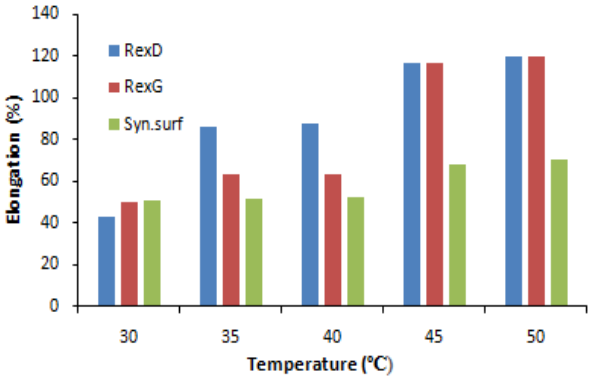


Fig. 5: Elongation (%) of leather treated with 30 g of RexD, RexG and synthetic surfactants at different temperature

Determination of elongation of goatskin leather treated with crude extracts and synthetic surfactants at different temperature

Figure 5 show the elongation (%) of leather treated with 30 g of crude extracts and a synthetic surfactant. Degreasing (emulsification of fat) increase flexibility and this is expected to increase the elongation of the treated leather (Yu-Fenchen *et al.*, 2010; Ork *et al.*, 2014; Diafari, 2017). In these results, elongation of the treated leather generally increased as the treatment temperature was increased while using all the surfactants. However, there was a boost in the elongation of leather treated with both RexD and RexG at 35°C and higher temperatures, compared to the synthetic surfactant. Comparing the extracts, RexD treated leather exhibits

significantly higher elongation at 35 and 40°C, and stay slightly high at 45 and 50°C than RexG treated leather. The results obtained for both extract at 30°C, and for RexG at 35 and 40°C fall with the range (30 – 80%) specified for leather used in manufacture of linings (KEBS, 2012). These results show that the surfactant properties of the crude extracts are competitive when compared with those of the synthetic surfactant. Hence it provides a potential replacement for the synthetic materials.

Conclusion

Crude extracts from root of *Balanitesaegyptiaca* grown in Demsa and Girei LGAs were evaluated as natural surfactants in the treatment of leather, and the results obtained show the potentials of the natural resources as a replacement for the synthetic surfactants. Saponins contents in the crude extracts were high, and saponins in plants have been extensively used as detergents, foaming and surface-active agents. There were variations in the saponins content and some chemical functionalities of the crude extracts obtained from different locations and these were attributed the geographical parameter that factored in the bioavailability of phytochemicals in the plants. Comparison of temperature effects on the tensile strength and elongation of leather treated with the crude extracts and a synthetic surfactant showed that the surfactant or emulsifying properties of the crude extracts improves with increase in treatment temperature. The experiments also showed that the crude extracts may perform at optimum within 35 – 40°C, and compete well with synthetic surfactants.

Conflict of Interest

Authors declare that there is no conflict of interest related to this paper.

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