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Formulation and Antibacterial Studies of Ethanolic Leaf Extract of *Ziziphus mauritania* Herbal Cream

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ABSTRACT

Ziziphus mauritania leaves, fruits and seeds are used by Nigerian traditional herbalists for treatment of various ailments. This study investigated antibacterial property of ethanolic leaf extract which was formulated into *Ziziphus mauritania* herbal cream. The antibacterial activity of the ethanolic leaf extract was conducted against *Staphylococcus aureus* using cup plate method and was compared with Linezolid, Augmentin, Vancomycin and Cefpodoxime. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined. The extract-excipients compatibility tests were carried out using FTIR. The herbal cream was formulated at 1-5 % w/w after preparing the aqueous and oily phase and thereafter the cream was evaluated after 48 h. The extract had comparable antibacterial activity with Augmentin, Linezolide, Cefpodoxime and Vancomycin at concentrations of 1.56, 3.13, 6.25, 12.5, 25.0 and 50.0 mg/mL. The FTIR spectra of the extract-excipients mixtures revealed no additional peaks thereby implying that no possible interaction occurred. The formulated herbal cream passed the evaluation tests such as pH, appearance, viscosity, irritancy, type of smear. Herbal cream of *Ziziphus mauritania* leaves extract was formulated. The antibacterial action of the extract justified its traditional use in wound healing and because the herbal cream had no adverse effects on the skin, the formulation can be used externally in the treatment of various infections caused by *Staphylococcus aureus.*

Keywords: Herbal cream, FTIR, *Staphylococcus aureus, Ziziphus mauritania* leaves extract.

INTRODUCTION

Creams are semisolid dosage formulations containing one or more medicaments dissolved or dispersed in a suitable base. They are emulsions of either oil-in-water (O/W) or water-in-oil (W/O) type. They are basically meant for topical application (Okpanachi, 2021). According to the British pharmacopeia definition, creams are formulated to provide preparation that are essentially miscible with the skin secretion. They are intended to be applied to the skin or certain mucous membranes for protection, therapeutic or prophylactic purposes especially where an occlusive effect is not necessary. Herbal creams are products in which herbs are used in crude or extract form which serve as active ingredient that are dissolved or dispersed in a suitable base with varieties of properties like antioxidant, anti-inflammatory, antiseptic, emollient, antiseborrhatic, antikerolytic activity and antibacterial among others. An ideal cream should be readily washable; physically and chemically stable; have rapid

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onset of action; be non-irritant; non-allergic; non-toxic; biocompatible and bio miscible and have high rate of activity or affectivity.

Ziziphus mauritania also known as Indian jujube, Indian plum, Chinese date, Chinese apple; is a tropical fruit tree species belonging to the family *Rhamnaceae*. It is widely distributed in subtropical and Tropical countries.

Figure 1: Photograph of *Ziziphus mauritania* Plant (Gombe State University Campus)

Ziziphus mauritania is commonly called *Magarya* in Northern Nigeria. The leaves, fruits and seeds are commonly used by Nigerian traditional herbalists for treatment of various ailment including sexual deficiency, diabetes, obesity, fever, cough, convulsion, epilepsy, diarrhea, ulcers, digestive and urinary discomfort, sleep disorders, burning sensations skin rashes and ulcers. Various parts of the plant have also received scientific validation of its immunomodulatory, free radical antagonist, anticancer, anti-diarrhoeal hypoglycemic, antiulcer, antimicrobial, antimycobacterial and antiplasmodial activities (Alawode *et al*, 2020). This study investigated the antibacterial property of ethanolic extract of *Ziziphus mauritania* leaves and formulated *Ziziphus mauritania* herbal cream which can be used in the topical treatment of various infections caused by *Staphylococcus aureus.*

MATERIALS AND METHODS

Collection and Identification of *Ziziphus mauritania* Leaves

Ziziphus mauritania plant parts were collected from the shrub tree in the month of February, 2021 from Gombe State University Campus, Nigeria. The plant material was identified, authenticated and assigned a voucher number of "GSUH 245" in the Herbarium of the Department of Biological Science of Gombe State University.

Preparation of *Ziziphus mauritania* Leaves for Extraction

The leaves were plucked and thoroughly washed using water to remove dirt and other impurities and then shade dried in an open space with regular movement for aeration to ensure proper drying. The leaves were size reduced to obtain a coarse powder. One

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hundred gram (100 g) was weighed on an electronic balance and transferred into a stainless steel bowl. One liter of 90 % v/v ethanol was poured into the bowl containing the powder and stirred using a stirring rod thereafter placed on a hot water bath and stirred continuously for about 30 min. It was then kept for 24 h before filtering using filter paper to obtain the filtrate thereafter, the filtrated solution was evaporated by rotary evaporator at 70°C to get the extract which was dried at room temperature. The percentage yield of the extract was determined. The extract was transferred in a previously cleaned air tight container and stored in a desiccator until required for use.

Antibacterial Assay

The antibacterial activity of the *Ziziphus mauritania* leaves extract was determined using the cup plate method as described by Abdallah, 2017. The tested bacterial strain (*Staphylococcus aureus*) was sub-cultured for 18 h and then a working bacterial sample was prepared and adjusted to be equivalent to 0.5McFarlands (1.5×10^8 cfu/mL). Thirty (30) mL of hot autoclaved Muller-Hinton agar (Watin-bio life, KSA) was prepared and poured into a sterile disposable-petri-dish (size 100* 15 mm) and left for 15 min to solidify. The bacteria cultured was swabbed onto the surface of the plate using a sterile cotton swab. A cup/hole was made in the middle of each plate using a sterile cork borer (number 8) aseptically. One hundred (100) µL of various dilutions of the extract was placed in each cup made with each plate having different concentrations of the plant extract. A standard antibiotic disc was placed on each plate [Vancomycin (30 µg), Augmentin (30 µg), Linezolid (30 µg), Cefpodoxime (10 µg)]. The plates were incubated at 35 ^{o} C for 24 h. The diameter of zones of inhibition for each antibiotic standard and the extract was measured to the nearest millimeter and recorded. The standard deviation of three determinations was recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extract was determined using broth dilution method. 1 g of the extracts was dissolved in 5 mL of sterile distilled water. Serial (two fold) dilutions of the extract were carried out in properly-labeled test tubes using Mueller Hinton Broth (MHB) as diluents. Each test tube containing the broth and the extract was inoculated with the standardized organism (0.5 McFarlands *Staphylococcus aureus*). A tube containing sterile Mueller Hinton Broth without any organism was used as a broth sterility control (tube 10). A tube containing the sterile broth with the organism without the extract was used as the organism viability control and a tube containing the sterile broth and 1mL extract was used as the extract control. The tubes were then incubated at 37 ^{o}C for 24 h. After the incubation period, the tubes were observed for the presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

The results from the MIC were used to determine the MBC. A sterile Mueller Hinton agar plate was divided into 6 quadrants on which a loopful from two tubes with lower concentration than the MIC and 3 tubes with higher concentrations than the MIC, were streaked. The plate was then incubated at 37 ^{o}C for 24 h. After the incubation period, the plate was then examined for the presence or absence of growth. This was done so as to determine whether the antimicrobial effect of the extract was bactericidal or bacteriostatic.

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Figure 2 contain some selected photographs of the antibacterial assay.

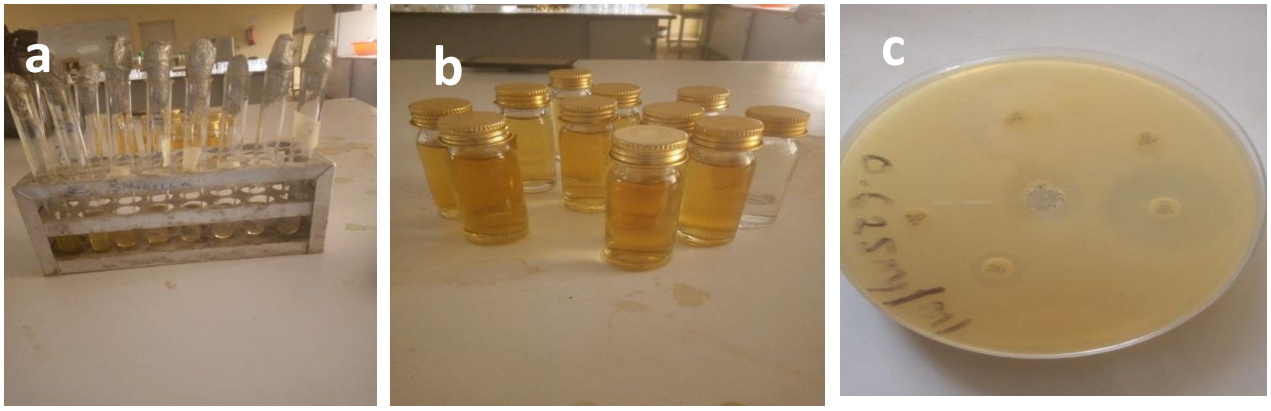

Figure 2: Photographs of Antibacterial Assay (a) Serial Dilution of Plant Extract (b) Muller Hinton Nutrient Agar (c) Selected Cultured Plate Showing Zones of Inhibition

Formulation Studies

Drug-Excipient Interactions (FTIR)

The method of Okpanachi, *et al*, (2020) was adapted to check for Drug-Excipient interactions using FTIR (Perkin Elmer L1600401 Spectrum Two DT GS, Waltham, USA). Five milligrams of the sample was mixed with 150 mg KBr in a mortar and pestle. The mixture was compressed into a tablet using a Sigma KBr press. The tablet was placed in the sample compartment and scanned at a range of 4000 to 400 cm^{-1}. Equal portion of the extract with equal portion of each excipient (White soft paraffin, Cetyl alcohol, Propylene glycol and Sodium lauryl sulphate) used in the formulation was used to check if there was new formation of peak.

Preparations of Aqueous Cream

An aqueous cream of *Ziziphus mauritania* herbal cream was formulated using the formula in Table 1.

Table 1: Formula for *Ziziphus mauritania* herbal cream

Ingredients	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)	F4 (% w/w)	F5 (% w/w)
Extract	1.0	2.0	3.0	4.0	5.0
White soft paraffin	25.0	25.0	25.0	25.0	25.0
Cetyl alcohol	25.0	25.0	25.0	25.0	25.0
Propylene glycol	12.0	12.0	12.0	12.0	12.0
Sodium lauryl sulphate	1.0	1.0	1.0	1.0	1.0
Water to	100	100	100	100	100

Preparation of Oily Phase

The method of preparation of the oily phase described in BPC, 2004 was adopted. White soft paraffin (25 % w/w) was weighed and placed in a previously cleaned beaker, 25 % of cetyl alcohol was also weighed and added to the beaker, 12 % of sodium lauryl sulphate and 12 % of propylene glycol was measured and added to the beaker and they were all melted using a preheated water bath at 70 °C. This served as the base of the cream.

Preparation of Aqueous Phase

One gram (1 g) of the ethanolic extract of *Ziziphus mauritania* leaves was weighed and

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transferred into a clean beaker and 20 % of distilled water was used to dissolve the extract using a preheated water bath at 70 ° C. The already dissolved extract was added to the melted base under the same temperature with a constant stirring in the same direction for 10 min to obtain a homogenous mixture of the cream (F1) then allowed to cool at room temperature. The same procedure was repeated with 2 g, 3 g, 4 g and 5 g of the extract to get F2, F3, F4 and F5 formulations respectively. The cream was then evaluated for various physical parameters.

Evaluation of the Cream

The following methods of evaluation of cream carried out by Shivathaya *et al*., 2022 were adapted.

Physical Properties: The colour, texture and odour of the cream was observed.

pH Determination: A suspension of 2 % w/v the formulated cream was prepared using distilled water and shaken for 5 min. It was then allowed to stand for 10 min thereafter the pH of supernatant was determined using a pH meter.

Viscosity test: Ten gram (10 g) of F1 was weighed and dissolved in 40 mL of distilled by continuous stirring for 5 min to make a suspension. The viscosity of the suspension was determined with a viscometer at 20 revolution per minute at room temperature. The same procedure was carried out for F2 to F5.

Stability studies: The product was evaluated at both room and accelerated temperature for 60 days to determine its stability or degradation.

After feel: This test was carried out after applying the cream on the skin. Properties such as emollient nature, slipperiness and amount that remains after applying the cream on the skin was evaluated.

Smear type: After applying the cream on the skin, the smear type was observed this was done to know if the cream is oily or aqueous.

Removal: This was carried out after washing the cream under running tap water with minimal force to determine its wash ability

Irritancy test: This test was conducted using 11 healthy volunteers for 10 days. The cream was applied on the forearm for 1 h daily to check for any visible reactions such as rashes, edema.

RESULTS AND DISCUSSION

The physical properties of ethanolic extract of *Ziziphus Mauritania* leaves is shown in Table 2. The yield of the ethanolic extract was 12.7 % w/w.

Table 2: Physical Properties of ethanolic extract of *Ziziphus mauritania* leaves

Property	Description
State	Crystalline powder
Smell	Odourless
Color	Dark green
Taste	Bland

Antibacterial Assay of Ethanolic extract of *Ziziphus mauritania* Leaves

The outcome of the antibacterial activity of the extract screened for biological activity against *Staphylococcus aureus* (most common causatives agents of both primary and secondary infectious skin disorders) using Linezolide, Augmentin, Vancomycin and Cepodoxim as standard antibiotics are shown in Table 3 while the MIC and MBC results are presented in Tables 4 and 5 respectively. The antibacterial activity of the plant extract against *Staphylococcus aureus* is in conformity with a study carried out by Muharrami, *et al.*, 2019 which showed that the ethanolic extract of *Ziziphus mauritiana* leaves had activity at concentrations of 1 %, 10 %, 20 %, 30 % and 40 %. The results obtained in this study revealed that the

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average zone of inhibition for the extract was comparable to that of the standard antibiotic discs (Augmentin, Cepodoxime, Vancomycin, Linezolid) used as control. The MIC obtained was 0.78 mg/mL while the MBC was 1.56 mg/mL. In formulating a cream containing this extract, it is important to ensure that these concentrations are considered for the cream to become pharmacologically active against *Staphylococcus aureus*. The antibacterial properties of the aqueous extract of *Ziziphus mauritania* leaves is said to be due to the presence of phenolic compound (Nazif, 2002).

Table 3: Antibacterial Zone of Inhibition of Ethanolic Extract of *Ziziphus mauritania* Leaves compared to Linezolid, Augmentin, Vancomycin and Cefpodoxime

	Zone of Inhibition (cm)				
Conc. of extract (mg/mL)	**Extract**	**Linezolid** (30µg)	**Augmentin** (30µg)	**Vancomycin** (30µg)	**Cefpodoxime** (10µg)
10.00	1.4	2.0	-	1.3	-
5.00	1.0	1.3	1.2	1.7	-
2.50	1.7	1.0	0.2	0.9	0.2
1.25	1.2	4.0	1.0	2.2	1.3
0.62	-	2.2	-	-	-
0.31	1.9	-	-	2.9	-
0.16	1.5	2.1	-	-	1.9
0.07	1.1	-	-	-	-
0.04	1.6	2.1	0.9	1.3	1.3
0.02	-	-	-	-	-
Average	**1.9**	**2.1**	**1.0**	**1.4**	**1.3**

Table 4: Minimum Inhibitory Concentration of ethanolic extract of *Ziziphus mauritania* leaves.

Test tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Conc. mg/ml	100	50	25	12.5	6.25	3.31	1.56	0.78	0.39			
Result	-	-	-	-	-	-	-	-	+	-	+	-

Key: - (Negative); + (Positive) MIC is 0.78 mg/mL

Table 5: Minimum Bactericidal Concentration of ethanolic extract *Ziziphus mauritania* leaves

Test tube	**2**	**3**	**4**	**5**	**6**	**7**
Conc. mg/mL	50	25	12.5	6.25	3.31	1.56
Result	-	-	-	-	-	-

Key: - (Negative). The MBC = 1.56 mg/mL. We can also conclude that the extract has bactericidal activity on *Staph. aureus*

Drug excipient compatibility study

The knowledge of FTIR spectrum of the *Ziziphus mauritania* leaves extract was required to investigate and predict any physicochemical interactions between components of a formulation containing the extract and therefore be applied to the selection of suitable chemically compatible excipients (Wells, 2002). Also, FTIR spectroscopic technique can be used for qualitative analysis of practically all compounds (Bunaciu *et al*., 2011). The FTIR fingerprints features are important in the identification of the main components of the herbal plant extract and it can also distinguish the geographical origins of samples easily (Bunaciu *et al*., 2011). The observed individual peaks of white soft paraffin, cetyl alcohol, sodium lauryl sulphate, propylene glycol and the extract remained unchanged when compared with the FTIR spectral data of

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the extract-excipient mixtures as shown in Figures 3 to 6.

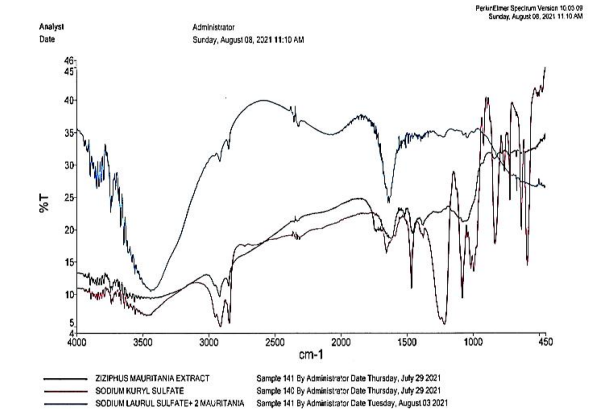

Fig. 3: FTIR overlay of *Z. mauritania*, Sodium Lauryl Sulphate and mixture.

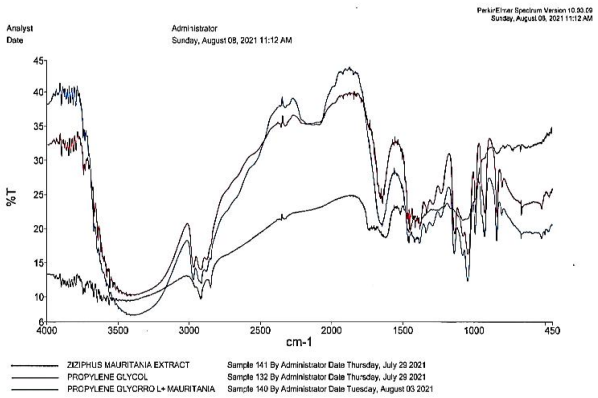

Fig. 4: FTIR overlay of *Z. mauritania*, Propylene glycol and mixture.

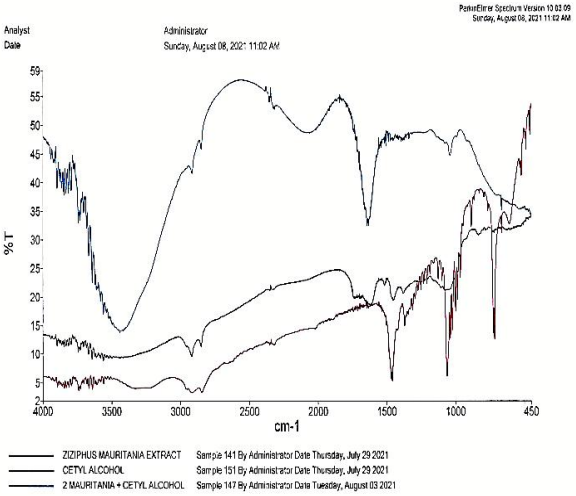

Fig. 5: FTIR overlay of *Z. mauritania*, Cetyl alcohol and mixture

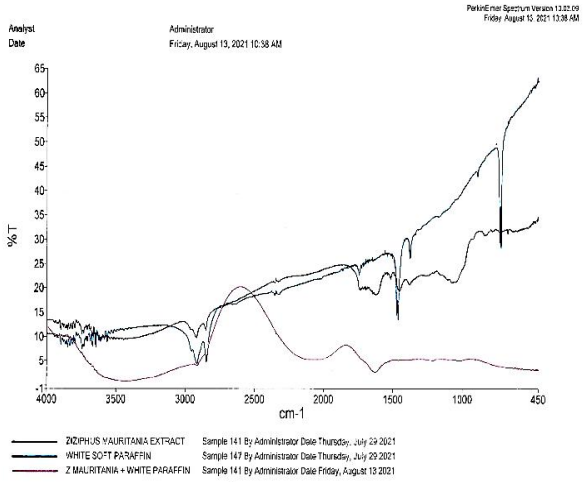

Fig. 6: FTIR overlay of *Z. mauritania*, White soft Paraffin and mixture

Some Physicochemical and *In vivo* Evaluation of the Cream

The results of some physicochemical and irritancy tests of the formulated *Ziziphus mauritania* cream on the skin are presented in Tables 6 and 7 respectively.

The determination of physicochemical properties is important in the standardization and quality control of herbal medicines (Regupathi and Chitra, 2015). The pH of human skin ranges from 4.5 to 6.0 (Lambers *et al*, 2006). Therefore, any formulation intended for application to skin should have pH closer to this range. The result obtained showed that the pH of the formulations is within the range of the skin pH. Any noticeable fluctuations in the pH recorded may be due to the absorption of CO_2 from air by the formulation or changes in the extract of *Ziziphus mauritiana* (Yosipovitch *et al.,* (2007). It was found that the viscosity of the formulated creams F1-F5 increased when decreasing the rate of shear, so the viscosity

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of cream is inversely proportional to rate of shear. For thixotropic fluid, viscosity is a function of shear rate and time and may increase or decrease with respect to shear rate and time (Morrison, 2003). All the formulated cream were tested for homogeneity by visual inspection. They were tested for their appearance with no lumps found. In accelerated stability testing, a product is stored at elevated stress condition (Robert, 2003). The formulations were stable with no degradation observed, this may be attributable to the constituting components of the preparations as well as the documented antimicrobial activity of *Ziziphus mauritania* (Akhtar *et al*., 2016; Abalaka *et al*., 2010). Degradation at recommended storage conditions could be predicted based on the degradation at each stress condition and known relationship between the accelerated factor and the degradation rate. Stability studies helps in determining the shelf life of the product. When the formulated creams were kept at room temperature for two months the results showed that the products retained their physical characteristics such as color and homogeneity. One of the major characteristics of cream is that it should be non-irritant in nature. The result obtained for the formulation used on eleven (11) healthy volunteers for 10 days revealed no allergic reactions, erythema and edema. The absence of erythema and edema in tested healthy volunteers may be attributed to the antioxidant and anti-inflammatory effects of the *Ziziphus mauritania* plant (Akhtar *et al*., 2016; Mesaik *et al.,* 2018).

Table 6: Physical parameters of *Ziziphus mauritania* Herbal Cream (F1-F5) at room temperature (25^{o}C) and at Accelerated Temperature (35^{o}C)

Cream	Viscosity (20 rpm)	pH	Parameters				
			Homogeneity	Appearance	After feel	Type of smear	Removal
F1	46.1	5.25	No color change	No color change	Emollient	Non greasy	Easy
F2	61.4	5.44	No color change	No color change	Emollient	Non greasy	Easy
F3	65.8	5.34	No color change	No color change	Emollient	Non greasy	Easy
F4	42.7	5.11	No color change	No color change	Emollient	Non greasy	Easy
F5	38.2	4.98	No color change	No color change	Emollient	Non greasy	Easy

Table 7: Irritability Studies of *Ziziphus mauritania* Herbal Cream (F1-F5)

Formulation	Irritancy	Erythema	Edema
F1	NIL	NIL	NIL
F2	NIL	NIL	NIL
F3	NIL	NIL	NIL
F4	NIL	NIL	NIL
F5	NIL	NIL	NIL

CONCLUSION

The ethanolic extract of *Ziziphus mauritania* leaves has antibacterial activity against *Staphylococcus aureus* and cream of various strengths were formulated. The antibacterial action of the extract justified its traditional use in wound healing especially in the treatment of infections caused by *Staphylococcus aureus.* The plant extract can be used as a substitute for the standard antibiotics because it is cheaper and readily available and because the herbal cream had no adverse effects on the skin, the formulation can be used externally in

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the treatment of various infections caused by *Staphylococcus aureus.*

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