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Original Research Article

In vitro Antibacterial Activity and Wound Healing Properties of Ethanol Extract of *Kigelia africana* Fruit in Rats

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ABSTRACT

The uneasy task of treating various wounds infested with multi-resistant bacteria is an increasing problem, prompting the need for alternatives therapies to conventional drugs. This study aimed at determining the *in vitro* antibacterial and wound healing activities of ethanol fruit extract of *Kigelia africana* on albino rats using the excision wound healing model. The crude extract of *K. africana* fruit was obtained using 95% ethanol in a Soxhlet extraction system. Using standard procedures, phytochemical analysis, *in vitro* antibacterial activity (against *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Salmonella typhi*.), and the subsequent minimum inhibitory concentrations (MIC) of the extract were determined. Simple ointments of varying concentrations (3, 5, and 10% w/w) of the extracts were formulated and used to screen for wound healing activity on experimental rats in groups of five (n=4). The antibacterial activities showed that the extract was effective against all the bacteria. The MIC values ranged from 0.98 to 125 mg/mL, *with* best value of 0.98 mg/mL against *P. aeruginosa*, followed by 15.63 mg/mL against *E. coli* and *S. aureus*, and then 62.5 mg/mL against *S. typhi* and *K. pneumoniae*. *K. africana* ointments significantly accelerated wound healing (P=0.000) with 5 and 10% w/w ointments having the highest percentage of wound contraction on the 18th and 20th days compared to the group treated with paraffin. The present study demonstrates that the ethanol extract of *K. africana* fruits contained bioactive compounds which promote an accelerated wound healing process and might harbour a novel therapeutic agent.

Keywords: *Kigelia africana*, Ethanol extract, Antibacterial, Wound healing.

Introduction

Plants and their metabolites are important sources of novel bioactive compounds, and they can be used as an alternative to synthetic drugs. Indigenous herbal medicine knowledge is a major source of modern medical knowledge.[1] Plants are used to treat various infectious diseases all over the world. They avail natural products that help fight infectious diseases.[2]

Kigelia africana (Figure 1) belongs to the Bignoniaceae family and is commonly known as the cucumber or sausage tree because of its large fruits, which hang from long fibrous stalks and have an average length and weight of 0.6 mm and 4 kg, respectively.[3] The flowers are bell-shaped, orange to reddish, 10 cm wide, and the leaves are opposite or in whorls of three, and 30 - 50 cm long. The individual flowers are oriented horizontally rather than hanging down[4], bisexual, and very large, measuring 2 to 4.5 cm in length, widening and curving upwards.[5] The sausage tree grows quickly and can reach maturity in 4 to 5 years. Traditional medicine has used various parts *K. africana* of to treat a variety of ailments.

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K. africana plant has many medicinal properties due to the presence of numerous secondary metabolites such as flavonoids, naphthoquinones, lignans, iridoids, and alkaloids among others.[6] The crude extracts from different parts of the plant have been used as antifungal, hepatoprotective, antiplasmodial, antioxidant, anticancer, antitumor, and antibacterial agents.[7] The ethanol extract of the stem/bark affects muscle coordination and stimulant effect on the central nervous system.[8] The aqueous leaf extract has also shown antidiarrheal activity as well the potential to increase fertility.[9] Due to the presence of active compounds in *K. africana*, the aqueous bark extract has shown wound healing activity particularly with burn and bacterial infections.[10,11] In West Africa, the leaves are used for stomach and kidney ailments, snakebites, and wounds, while the stem and twigs are effective against wounds, snakebite, and rheumatism as well as stomach and kidney ailments.

Figure 1: Picture of *K. africana* fruit

Fruits of *K. africana* are useful against constipation, gynaecological disorders, and haemorrhoids. It also acts as anti-psoriasis and anti-eczema.[12]

Many studies have described the wound healing activity of medicinal plants.[13,14,15] To reduce wound healing duration and minimize complications such as secondary microbial contamination, medical professionals often use synthetic/orthodox medications.[16,17] However, a major challenge facing the use of these medications is that most of the wound contaminating organisms are becoming highly resistant to their effects.[18] Also, the price of these medications are costly. Thus, there is a constant need to search for more effective and low-cost therapeutic approaches for wound healing. Most of the studies on *K. africana* used in traditional medicine over the years have been on the leaves, stems, twigs, and bark and not the fruits. Thus, this study aimed at evaluating the *in vitro* antibacterial activities and wound healing potentials of an ointment base containing ethanol extract of *K. africana* fruits for topical application on Wistar albino rats.

Materials and Methods

Ethical Clearance

The ethical clearance for the study was duly sort for and approval given by the Ethics Committee of the University of Nigeria (FPSRE/UNN/20/0009)

Experimental animals

A total of twenty (20) albino *Wistar rats* of either sex, approximately aged 9 – 10 weeks and weighing between 130 to 150g were obtained from the laboratory animal unit, Faculty of Veterinary medicine University of Nigeria, Nsukka. Following two weeks of acclimatization, they were fed on grower mash (commercial) and water *ad libitum* throughout the experimental period.

Plant collection and identification

Fresh fruits of *K. africana* were collected from Orba in Udenu Local Government Area of Enugu State, Nigeria, and was authenticated (Voucher numbers: PCG/UNN/0406 and INTERCEDD/069) by Mr. A. Ozioko of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka and Bioresources Development and Conservation Program (BDCP) Laboratory Nsukka in August 2020.

Extraction of plant material

The fruits were sliced into bits using a knife and allowed to air-dry at room temperature for 7 days, after which they were pulverized into a fine coarse powder using a mechanical milling machine. Two hundred (200) grams of the powdered sample was extracted using a soxhlet extractor with 95% v/v ethanol as solvent. The ethanol extract was concentrated using a rotary evaporator; the dried extract was transferred into a clean container and stored in the refrigerator at 4^{o}C for further use.

Phytochemical analysis

The primary phytochemical analyses were performed for qualitative estimation of various phytochemicals present (including tannin, phenol, alkaloid, flavonoid, saponin, steroid, hydrogen cyanide, terpenoid, glycoside, and polyphenol) in the fruit extracts of *K. africana* using standard methods.[19]

Preparation of ointments

Simple ointments containing 3, 5, and 10% w/w of the ethanol extract of the fruits of *K. africana* were prepared by fusion method[20] according to the formula as described in the table 1 below.

Table 1: Ointment preparation formula

Extract	Xg
Cetostearyl alcohol	5.0g
Wool fat	5.0g
Hard paraffin	5.0g
White paraffin	85.0g

The letter X represents 3, 5, and 10% w/w of the fruit extract that were incorporated in 100 g of the simple ointment base. After preparation, the ointments were aseptically transferred into a sterile cream plastic tube and labelled accordingly.

Bacterial strains

A total of six multidrug-resistant bacteria isolates including; *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Salmonella typhi* were collected from the Department of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nsukka. The microorganisms were sub-cultured onto a sterile nutrient agar plate, incubated at 37^{o}C for 24 h.

Antibacterial screening

The antimicrobial screening was determined using the agar well diffusion method on Muller-Hinton agar (MHA) (Oxoid, UK) with an inoculum density equivalent to 0.5 McFarland turbidity standards. The inoculum density was equivalent to 1.5 x 10^8 colony forming unit (CFU) per mL. A quantity of 1 g of the extract was dissolved in 4 mL of dimethylsulphoxide (DMSO) which yield an initial concentration of 250 mg/mL. Subsequently, two-fold serial dilution was made from the stock solution to obtain 125 mg/mL to 7.8 mg/mL concentrations. One hundred microlitres (100 µL) of the standardized (using McFarland standard) overnight culture of each isolate were collected and inoculated onto a sterile Muller-Hinton Agar plate. The inoculum was spread over the entire surface of the plate using a sterile glass rod and allowed to dry with the lid in place. A sterilized 6 mm cork borer was used to make wells in the center of the divided area. A 45 µL of different concentrations of the extract were pipetted into the wells. The plates were left for 15 minutes at room temperature for pre-diffusion after which the plates were incubated at 37^{o}C for 24 h. The inhibition zone diameter was measured and recorded. The experiment was done in triplicate to minimize error. The inhibition zones of the extract were compared with that of ciprofloxacin (1 mg/mL) as positive control. Few drops of DMSO were pipetted into one well as the negative control.

Minimum inhibitory concentration (MIC)

The Agar dilution method was used to determine the minimum inhibitory concentration of the fruit extract using the different dilutions (125 mg/mL to 7.8 mg/mL) obtained from the stock solution (250 mg/mL). Each of the dilutions was poured into a sterile petri dish and sterile molten Muller-Hinton agar was added into the dishes bearing different dilutions, mixed properly, and allowed to solidify. A loopful of the bacterial suspension equivalent to 1.5 x 10^8 CFU per mL was streaked on the surface of the agar. The plates were allowed to stand for 30 minutes until the inocula have been completely absorbed by the media. The plates were incubated at 37^{o}C for 24 h and the MIC was determined as the least concentration showing no visible growth as compared with the growth in the control (ciprofloxacin (1 mg/mL)) plate.

Wound healing activity

Creation and contamination of excision wound

Twenty rats were anesthetized with 10 mg/kg body weight of xylazine hydrochloride and 50 mg/kg Ketamine hydrochloride. Their dorsum was shaved with a sterilized razor blade and disinfected with methylated spirit. A circle of diameter 2 cm (20 mm) was marked on the skin of the lumbar region. The wounds were created along the marked areas of the skin surface using forceps, surgical blades, and pointed scissors and cleaned with 70% ethanol. The rats were randomLy assigned into five groups of four animals per group. The wound on each animal was contaminated by flooding 1 mL of a standardized broth culture of multidrug-resistant *P. aeruginosa* using a sterile micropipette. The wounds were not treated for 48 h post contamination to ensure colonization and establishment of infection. After 48 h post contamination, the wounds were cleaned with methylated spirit, after which the respective treatments were applied topically daily until complete healing occurred. Group one was considered as negative control (untreated, but administered with soft

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paraffin with no known antimicrobial effects); Group two, three, and four were treated with 3, 5, and 10% ointments respectively, while group five were treated with 1% Gentamicin ointment (reference standard).

Percentage wound Contraction
The wound diameter of each rat was measured every two days from the 2nd to 26th days post wounding (DPW) using a transparent meter ruler. The percentage of wound contraction was calculated using the following formula.[21]

Percentage wound contraction

$$\text{Percentage wound contraction} = \frac{\text{Initial wound size} - \text{wound size on the day of measurement}}{\text{Initial wound size}} \text{X } 100\%$$

Statistical analysis
Using SPSS Version 20, data were analyzed using one-way analysis of variance (ANOVA; DUNNET; POST HOC TEST). All the values were expressed as mean ± standard error mean (SEM), the difference between the mean values of treated and the control groups and among the treated groups were considered significant at $P < 0.05$

Results and Discussion

Phytochemical analysis
The result of the phytochemical analysis of the fruit of *Kigelia africana* revealed the presence of tannin, phenol, alkaloid, flavonoid, saponin, steroid, hydrogen cyanide, terpenoid, glycoside, and polyphenol, which have numerous pharmacological effects and have been extensively used as drugs in the medical field (Table 2). Phenol was recorded to be the most abundant followed by tannin, then alkaloids and flavonoids. These constitute the active principles of the plants and are responsible for the antimicrobial and wound healing activities of the plant. Tannins are known to have antimicrobial, astringent and protein coagulatory properties that aid in wound healing and together with the contribution of the other secondary metabolites which gives a positive indication that *K. africana* fruit has wound healing properties.[22] Tannins are also known for their sticky nature; hence, they aid wound contraction and epithelisation. Phenols and flavonoids present in the fruit extract can denature and coagulate proteins in the microorganisms hence contributes effectively to the antimicrobial activities of the plant.[23] Several studies have reported that flavonoids have an antioxidant and anti-inflammatory property which is known to promote tissue healing.[24] The alkaloids present in the fruit extract can bind microbial DNA and bring about cell death.

Antibacterial activity of ethanol extract of K. africana
The extract exhibited very high antibacterial activity to the growth of all the tested organisms which justified its traditional use in microbial infections. Some of the concentrations of the extracts had comparable inhibition activity with the positive control (ciprofloxacin, 1 mg/mL) as shown in Table 3, especially against *P. aeruginosa, K. pneumoniae, E. coli,* and *S. aureus*. The lowest concentration (7.8 mg/mL) used had the best inhibition zone recorded across most of the organisms tested. The negative control DMSO produced no zones of inhibition across the tested organisms. The observations is in line with the findings of some researchers who reported that the extracts of *K. africana* may be used as the alternative source for treating several infectious diseases caused by various pathogens.[25,26]

Table 2: Phytochemical components of ethanol extracts of *K. africana* fruits

Bioactive Compound	Ethanol extract
Tannin	+
Phenol	+
Alkaloid	+
Flavonoid	+
Saponin	+
Steroid	+
Hydrogen cyanide	+
Terpenoid	+
Glycoside	+
Polyphenol	+

Key: '+' = present

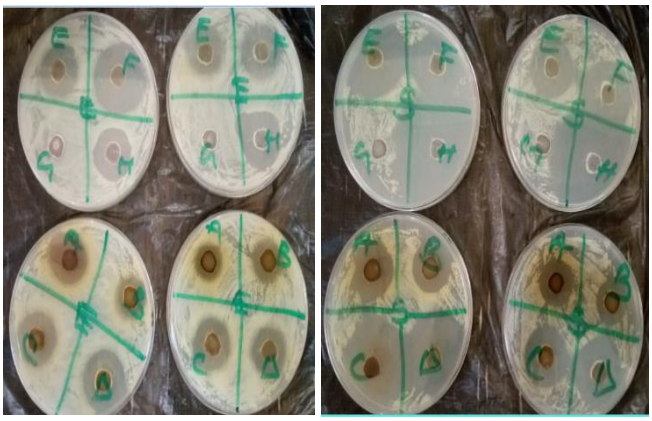

Figure 2: Antibacterial activity of fruits extract of *K. africana*

Table 3: The inhibition zone diameter (IZD) of the ethanol extracts of *K. africana* fruits against the test organisms

Conc. mg/mL	Inhibition Zone Diameter (IZD) mm					
	P. aeruginosa	***K. pneumoniae***	***E. coli***	***S. aureus***	***S. typhi***	***B. subtilis***
A (250)	20 ± 0.0	21.5 ± 3.5	19 ± 1.0	21 ± 1.0	14.5 ± 1.5	19 ± 0.0
B (125)	19.5 ± 0.5	24 ± 2.0	19 ± 0.0	21 ± 1.0	12 ± 1.0	17.5 ± 1.5
C (62.5)	25 ± 0.0	27 ± 2.0	22 ± 0.0	30 ± 5.0	14.5 ± 0.5	14.5 ± 0.5
D (31.25)	27 ± 3.0	30.5 ± 0.5	24.5 ± 0.5	32.5 ± 2.5	12 ± 0.5	13.5 ± 0.5
E (15.63)	28.5 ± 0.5	30.5 ± 0.5	25 ± 0.0	29 ± 2.0	10 ± 0.0	12.5 ± 0.5
F (7.8)	32 ± 2.0	32 ± 1.0	28.5 ± 1.5	33.5 ± 2.5	9 ± 0.0	10 ± 0.0
G (DMSO 2 drops)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
H (Cipro 1mg/mL)	41.5 ± 0.5	42 ± 0.0	28.5 ± 3.5	41 ± 0.5	33 ± 1.0	40 ± 0.0

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Table 4: The effect of the *K. africana* fruit extract in wound contraction

Group (Treatment)	Mean ± standard error of mean wound contraction at days post wounding (cm)												
	2	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**
Paraffin^{ab}	2 ± 0.00^{ac}	2 ± 0.00^{ae}	1.9 ± 0.04^{ae}	1.83 ± 0.03^{ae}	1.75 ± 0.029^{ad}	1.68 ± 0.03^{ad}	1.58 ± 0.03^{ad}	1.55 ± 0.03^{ae}	1.43 ± 0.03^{ad}	1.43 ± 0.03^{ad}	1.3 ± 0.04^{ad}	1.18 ± 0.03^{ad}	1.13 ± 0.03^{ab}
3% Extract^{aa}	2 ± 0.00^{ab}	1.88 ± 0.03^{bd}	1.68 ± 0.05^{bd}	1.53 ± 0.06^{bd}	1.45 ± 0.029^{bc}	1.35 ± 0.03^{bc}	1.28 ± 0.05^{bc}	1.05 ± 0.03^{bd}	0.88 ± 0.05^{bc}	0.6 ± 0.04^{bc}	0.32 ± 0.063^{bc}	0.10 ± 0.04^{bc}	0.0 ± 0.00^{ba}
5% Extract^{ba}	1.93 ± 0.05^{aa}	1.75 ± 0.03^{cc}	1.6 ± 0.04^{cc}	1.4 ± 0.04^{cc}	1.00 ± 0.058^{ca}	0.70 ± 0.06^{ca}	0.45 ± 0.03^{ca}	0.35 ± 0.03^{cc}	0.13 ± 0.03^{ca}	0.05 ± 0.03^{ca}	0.025 ± 0.03^{ca}	0.0 ± 0.00^{ca}	0.0 ± 0.00^{ca}
10% Extract^{ca}	1.9 ± 0.04^{aa}	1.78 ± 0.03^{db}	1.65 ± 0.03^{db}	1.5 ± 0.04^{db}	1.15 ± 0.065^{db}	0.75 ± 0.03^{db}	0.48 ± 0.03^{db}	0.3 ± 0.04^{db}	0.1 ± 0.04^{da}	0.0 ± 0.00^{da}	0.0 ± 0.00^{da}	0.0 ± 0.00^{da}	0.0 ± 0.00^{da}
Gentamicin ointment^{da}	1.83 ± 0.03^{ba}	1.55 ± 0.03^{ea}	1.3 ± 0.04^{ea}	1.08 ± 0.025^{ea}	0.80 ± 0.041^{ea}	0.55 ± 0.03^{ea}	0.033 ± 0.03^{ea}	0.10 ± 0.04^{ea}	0.0 ± 0.00^{ea}	0.0 ± 0.00^{ea}	0.0 ± 0.00^{ea}	0.0 ± 0.00^{ea}	0.0 ± 0.00^{ea}

NB: The first letter 'a' of the superscript is for comparison of the significance at $p < 0.05$ of negative control (Paraffin) against the other treatments, while the second superscript 'a' of the superscript is for comparison of the positive control (Gentamicin) against the other treatment.

Table 5: The effect of ethanol extract of *K. africana* fruit on Percentage increase in wound contraction

Group (Treatment)	Mean ± standard error of mean wound contraction at days post wounding												
	2	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**
Paraffin^{ab}	0.0 ± 0.0^{ac}	0.0 ± 0.0^{ab}	5.00 ± 2.0^{ae}	8.75 ± 1.3^{ae}	12.5 ± 1.4^{ad}	16.25 ± 1.3^{ad}	21.25 ± 1.3^{ad}	22.50 ± 1.4^{ae}	28.75 ± 1.3^{ac}	28.75 ± 1.3^{ac}	35.00 ± 2.0^{ac}	41.25 ± 1.3^{ac}	43.75 ± 1.3^{ab}
3% Extract^{aa}	0.0 ± 0.0^{ab}	6.25 ± 1.3^{ba}	16.25 ± 2.4^{bd}	23.75 ± 3.2^{bd}	27.50 ± 1.4^{bc}	32.50 ± 1.4^{bc}	36.25 ± 2.4^{bc}	47.50 ± 1.4^{bd}	56.25 ± 2.4^{bb}	70.00 ± 2.0^{bb}	83.75 ± 3.2^{bb}	95.00 ± 2.0^{bb}	100 ± 0.0^{ba}
5% Extract^{ba}	3.75 ± 2.4^{aa}	12.50 ± 1.4^{ca}	20.00 ± 2.0^{cc}	30.00 ± 2.0^{cc}	50.00 ± 2.9^{ca}	65.00 ± 2.9^{ca}	77.50 ± 1.4^{ca}	82.50 ± 1.4^{cc}	93.75 ± 1.3^{ca}	97.50 ± 1.4^{ca}	98.75 ± 1.3^{ca}	100 ± 0.0^{ca}	100 ± 0.0^{ca}
10% Extract^{ca}	5.00 ± 2.0^{aa}	11.25 ± 1.3^{da}	17.50 ± 1.4^{db}	25.00 ± 2.0^{db}	42.5 ± 3.2^{db}	62.50 ± 1.4^{db}	76.25 ± 1.3^{db}	85.00 ± 2.0^{db}	92.50 ± 4.3^{da}	100 ± 0.0^{da}	100 ± 0.0^{da}	100 ± 0.0^{da}	100 ± 0.0^{da}
Gentamicin ointment^{da}	8.75 ± 1.3^{ba}	22.50 ± 1.4^{ea}	35.00 ± 2.0^{ea}	46.25 ± 1.3^{ea}	60.00 ± 2.0^{ea}	72.50 ± 1.4^{ea}	83.75 ± 1.3^{ea}	95.00 ± 2.0^{ea}	100 ± 0.0^{ea}	100 ± 0.0^{ea}	100 ± 0.0^{ea}	100 ± 0.0^{ea}	100 ± 0.0^{ea}

NB: The first letter 'a' of the superscript is for comparison of the significance at $p < 0.05$ of negative control (Paraffin) against the other treatments, while the second superscript 'a' of the superscript is for comparison of the positive control (Gentamicin) against the other treatment.

Minimum inhibitory concentration of K. africana fruit extract

The relative level of antimicrobial activity was further evaluated by determining the MIC values of the extract against the bacterial species which were shown to be susceptible by agar well diffusion assay. The ethanol extract of *K. africana* fruit was effective and inhibited microbial growth at low concentrations. The best value obtained was 0.98 mg/mL against *P. aeruginosa*, followed by 15.63 mg/mL against *E. coli* and *S. aureus* and then 62.5 mg/mL against *S. typhi* and *K. pneumoniae* (Figure 3). Though out of the six bacteria used in the study, only two are Gram-positive bacteria, the result shows that the extract has better antimicrobial activity against Gram-negative bacteria than the Gram-positives. No MIC was recorded for *Bacillus subtilis.*

Wound contraction

Management of wounds is very vital because improper wound healing may contribute to undesirable consequences such as chronic wounds, non-healing wounds, and delayed wound healing.[27] Several factors such as age, presence of pathogens/contaminants, antimicrobial activity of some medicaments may contribute to wound healing. Many medicinal plants and products have been shown to possess satisfactory wound healing efficacy with no or little toxicity and are less expensive than synthetic drugs.

The present study revealed that ethanol extract of *K. africana* fruit at varying concentrations can exert significant cutaneous wound healing activity. The effects of the extract, gentamicin ointment (standard drug), and soft paraffin (control) in the excision wound model were evaluated by measuring the wound size and wound contraction. The comparison of the effect of the topical application of the three ointment concentrations (3,5 and 10)% w/w of the plant fruit extract against the controls on the time required for wound healing was statistically significant ($P < 0.000$) (Table 3). Following treatment on the second day, wound healing was recorded only for 5% extract (1.93 ± 0.05), 10% extract (1.9 ± 0.04), and gentamicin (positive) (1.83 ± 0.03) groups, although the values among these three were not statistically significant (between 5 and 10%, $P = 1.0$; between 5% and gentamicin, $P = 0.834$; between 10% and gentamicin, $P = 0.768$) as shown in Table 4. Thus, comparable with that of the reference drug (gentamicin), confirming the effectiveness of this medicinal plant in wound healing. No contraction was recorded for the negative control (paraffin) until the sixth day. The wound size gradually decreased at varying rates to the treatment till the eighteenth-day post wounding (DPW) where total healing was observed for the positive control alone (Table 5).

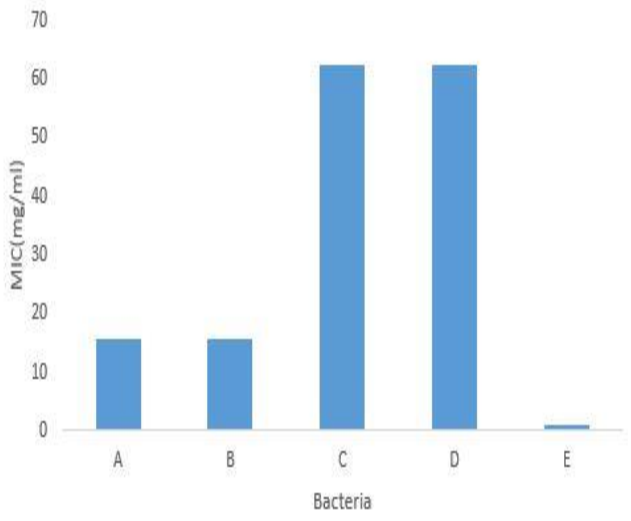

Figure 3: Minimum Inhibitory Concentration (MIC) of the Ethanol extracts of *K. africana* fruits. Key: A= *E. coli*, B = *S. aureus*, C = *S. typhi*, D = *K. pneumonia*, E = *P. aeruginosa.*

Generally, there was increasing potency of the treatments with increasing concentration used (3%, 5%, and 10%) of the extracts. Total healing was observed for the 10% extract group on the twentieth day, 5% extract group on the twenty-fourth, and 3% extract group on the final day of observation. Total healing was not observed with the negative control group throughout the treatment period. From the study, the best effect was obtained with the gentamicin group, followed by 10% extract and 5% extract groups. These results suggest that the ethanol extract of *K. africana* fruits had some influence on the wound healing in rats, by inhibiting wound contaminants that delay healing compared to the untreated wound at various phases of the wound healing process. The antimicrobial effects is most likely as a result of the phytochemicals present in the plant fruit. However, there is need to isolate and characterize the bioactive agents or compounds responsible for the above biological activities from this plant extract.

Conclusion

There is considerable scope for improvement on this plant since it has been used in treating some microbial diseases. The antibacterial activity of *K. africana* fruit shows that the crude ethanol extract has inhibitory activity on all the microorganisms tested. The study shows that the ethanol extract of *K. africana* fruits promotes wound healing and could be a source for new healing agent. Our findings could have clinical implications and could help with drug development in both human and veterinary medicine.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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