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# Effects of African Panaxia Extracts on Staphylococcus Aureus and Klebsiella Pneumoniae from Bacteria Wound Infection in Tiko

Walter Ojong Ebot

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## Abstract

Background: Wound is the disruption of cellular and anatomic continuity of living tissue produced by physical, chemical, electrical or microbial insults to the tissue. Wound healing is the dynamic process of regeneration or repair of broken tissue, due to increasing health care costs, an ageing population, and a sharp rise in the incidence of diseases such as diabetes and obesity worldwide, the present study was aimed to achieve the following objectives. Objectives: This study aimed to determine the effect of African panaxia on bacteria wound infection. Methods: It was a Laboratory based experimental study made up of bacterial isolates from wounds, cultured on blood, EMB agar, followed by confirmatory biochemical tests and MI and MBC was done turbidity absorbance measured using spectrophotometry at 660nm. Data was analyzed using Microsoft excel 2010.

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*Index terms*— minimum inhibitory concentration; minimum bactericidal concentration.

## 1 Introduction

Wound is the disruption of cellular and anatomic continuity of living tissue produced by physical, chemical, electrical or microbial insults to the tissue. Wound healing is the dynamic process of regeneration or repair of broken tissue [1]. Chronic wounds are rapidly growing problem worldwide, due to increasing health care costs, an ageing population, and a sharp rise in the incidence of diseases such as diabetes and obesity [2]. The skin is under constant stress from the sun, smog, friction, tension, temperature, and other external factors. Therefore, under sufficient stress that causes injury, it results in wounds. Wounds may be classified as; open and closed, acute and chronic, avulsion and degloving, clean and contaminated, infected and colonized, laceration, incision and abrasion, puncture, penetration, and gunshot wounds. Nonetheless, they exist in various forms comprising crush injuries, ulcers, skin tears, bruises, and post-operative, which directly or indirectly affect human health conditions. If it is not treated correctly, it may ultimately lead to death. Wounds can be caused by various microorganisms such as bacteria, fungi, parasites, and viruses. Some of the commonly associated bacteria organisms include Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp., Acinetobacter spp [3]. Studies conducted in 2014 and 2021 reported a wound infection prevalence of 10% and 5.95% respectively [4,5]. Medicinal plants and synthetic drugs have been the most valuable sources of molecules with therapeutic potential throughout the history of mankind. Folk medicine of each civilization is based on natural products and, nowadays, medicinal plants still represent an important pool for the identification of novel drug leads [6]. African panaxia being a synthesized herbal medicine is made from various herbal plants such as ginseng root, Optimum gratisimum, Panax quiquifolium, Aloe-vera and water with active ingredients ginsenosides, methyl eugenol, saponins and salicylic acid respectively [7].

## 2 a) Rationale

The advent of the resistance that pathogenic microorganisms have developed against antibiotics has necessitated much attention to be paid on plant extracts and biologically active compounds isolated from natural plants used

43 in herbal medicine [8]. Despite the use of various synthesized herbal plants in the treatment of bacteria wound  
44 infection, there is limited information regarding the use of African panaxia in the treatment of wound infections.  
45 Furthermore, no study has been carried out in this study area. Hence there is need to investigate on the in-vitro  
46 activity of African panaxia on bacteria wound infections.

### 47 **3 b) Goal of study**

48 The goal of this study was to provide base line data on the effect of African panaxia on bacteria wound infection.

### 49 **4 c) Hypothesis**

50 There is no significant effect of African panaxia on bacterial wound infections.

### 51 **5 d) Objectives of study i. General objective**

52 The general objective of this study was to determine the effect of African panaxia on bacteria wound infection.

### 53 **6 ii. Specific objectives**

54 To determine the efficacy of distilled water extract of African panaxia on Staphylococcus aureus and Klebsiella  
55 species bacteria from wound infection.

56 To determine the efficacy of Luke warm water extract of African panaxia on Staphylococcus aureus and  
57 Klebsiella species bacteria from wound infection.

58 To determine the efficacy of ethanoic extract of African panaxia on Staphylococcus aureus and Klebsiella  
59 species bacteria from wound infection.

## 60 **7 II.**

## 61 **8 Materials and Methods**

### 62 **9 a) Study area and setting**

63 This study was carried out in Tiko, in Maflekumen Medical Teaching and Research Laboratory situated in Tiko  
64 is a subdivision of Fako Division in the South West Region of Cameroon with a [81]. The life style and occupation  
65 of inhabitants of Tiko including the dusty, windy and hot nature, farming, bike riding and much more favours  
66 the acquisition of wounds b humans thus making the area suitable for this study.

### 67 **10 b) Study design and duration**

68 This was a Laboratory based experimental study designed that was conducted from November 2022, to June  
69 2023.

### 70 **11 c) Specimens and sampling**

71 This study made use of bacteria isolates from people with bacteria wound infection in Tiko community.

### 72 **12 d) Ethical consideration**

73 An introductory letter was obtained from MAFLEKUMEN Higher Institute of Health Sciences TIKO (AP-  
74 PENDIX A) and was taken to regional delegation in Buea for the approval of the project. An administrative  
75 authorization was obtained from the regional delegation (APPENDIX B) and was presented to the administration  
76 of MAFLEKUMEN. An authorisation was gotten from the MAFLEKUMEN administration to carry out the  
77 research.

### 78 **13 e) Data collection and techniques i. Sample collection**

79 Bacterial isolates were obtained from Maflekumen diagnostic laboratory. Preparation of MacConkey agar, blood  
80 agar and EMB was done byweighing the powder using an electronic balance and dissolved in distilled water  
81 following the manufacturer's instructions and was cooked to obtain the gel using a Bunsen burner and allowed to  
82 cool to 40°C. The agar was poured into petri dishes and allowed to solidify. The samples were inoculated in the  
83 plate and read after 24hrs. Presumptive identification of bacteria was done based on colony characteristics, gram  
84 reactions were recorded. Confirmatory biochemical tests were done to confirm the bacteria. For staphylococcus  
85 aureus, and Klebsiella species respectively.

### 86 **14 ii. Catalase test**

87 A drop of Hydrogen peroxide was placed on a slide and a colony of isolated bacteria picked and emulsified on the  
88 slide containing the hydrogen peroxide. The appearance of air bubbles indicate catalase positive.

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## 15 iii. Coagulase test

A drop of normal saline was placed at both ends of the same slide, one labeled test and the other control. A colony of the isolated bacteria was emulsified on each drop of the normal saline. Serum was placed on the test path and emulsified and nothing was added on the control. The presence of coagulation indicates coagulase.

## 16 iv. Indole test

Test organism was inoculated in a bijoux bottle containing 3 ml of sterile tryptone water. Incubate at 35-37 °C for up to 48 h. 0.5 ml of Kovac's reagent was added and shake gently, examination for a red color in the surface layer within 10 minutes macroscopically.

## 17 v. Extraction of African panaxia (Alcohol, distilled water and luke warm water)

African panaxia was bought from the Moghamo express in Mutengene and transported to the laboratory for sensitivity testing on the bacteria isolates. One gram of African panaxia was weighted on an electric scale balance and put in a 250 ml flask, followed by adding 100 ml of solvent (95% ethanol). The flask was then left at room temperature for two days preceding filtration funnel and Wattman No. 1 filter paper. The filtrate was concentrated under decreased pressure with an evaporator at 40°C. This crude extract was saved at 4°C until use, this extract of African panaxia was considered as the 100% concentration for ethanol extract, different stock solution for distilled water were made equally and also for luke warm water respectively. Then the concentrations (100%, 75%, 50%, and 25%) were made by diluting the concentrated extract of African panaxia with appropriate volumes of sterile distilled respectively for luke warm water. Serial dilutions were made to determine the minimum inhibitory, and bactericidal concentration respectively.

i. Different stock solutions of African panaxia were made (Absolute alcohol, luke warm water and distilled water, in which different volumes were used 100, 75, 50 and 25 in which the isolated species of bacteria were used to test for the minimum inhibitory concentration and minimum bactericidal concentration using dilution technique and absorbance was measured using a spectrophotometry machine at a wavelength of 660nm.

ii. A solution of the isolated bacteria was prepared and standardized by matching to the 0.5 McFarland turbidity standards using sterile saline to produce approximately  $1.5 \times 10^8$  colony forming units per ml. iii. Serial dilutions were made on the different stock solutions of African panaxia using four sterile dry tubes per isolate and per stock solution respectively. iv. Two (2ml) of nutrient broth was placed in each sterile test tube, followed by adding 2ml of each stock solution in the first tube, mix well and transfer 2ml to the next tube continuously and to the fourth to remove 2ml and discard respectively 1drop of the bacterial suspension was placed in each test tube respectively.

v. They labeled test tubes were sealed and incubated at 37 °C for 18 to 24hours in which the and minimum inhibitory concentration and minimum bactericidal concentration recorded by checking the turbidity of each tube and absorbance was measured using a spectrophotometer at 660nm following the control of the absorbance of 0.5 McFarland standard and Azithromycin.

f) Data analysis Data was analyzed using Microsoft excel and the results was presented in tables and figures III.

## 18 Results and Discussion

### 19 a) Results

This chapter presents the results obtained from the effect of African panaxia on Staphylococcus and Klebsiella isolated from wounds. Based on extract with distilled water the concentration with 75% and 50% stocks were effective in inhibiting the growth of Staphylococcus and Klebsiella respectively. Also using a stock of 50% and 25%, it exhibited bactericidal properties on Staphylococcus and Klebsiella respectively as presented on figure 1 below. [83]. Also, Ohammed et al., in 2019, also revealed that the most common bacteria isolated from wounds were Staphylococcus aureus, and Klebsiella pneumonia [84]. Also, these findings agree with other findings by Obi et al., in 2015 who reported that common bacteria isolates from the different types of wounds were Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis and Acinetobacter baumannii [85].

## 20 To determine the minimum inhibitory concentration of African panaxia on Staphylococcus aureus and Klebsiella pneumoniae from wound infections

The minimum inhibitory concentrations were determined using extract from different concentrations such as distilled water, luke warm water and ethanoic extracts of the African panaxia. From the findings it was revealed that African panaxia extracts of distilled water and luke warm water were more inhibitory at 75%, 50% stock respectively than Bactericidal. These findings are similar to results of Korukluoglu et al. in 2010 who reported

144 that extraction of aqueous solvent resulted in a product with greater overall antimicrobial activity than extraction  
145 with water, as aqueous extracts of all the olive oil displayed little or no antimicrobial activity against any of the  
146 bacteria tested [786]. Similarly Weerakkody et al., (2010) [5] observed that water extracts of oregano and  
147 rosemary had little or no antimicrobial activity compared to ethanol or hexane extracts. Again, Sofia et al.,  
148 (2007) [6] reported that water extracts of mustard, cinnamon, garlic and clove had good inhibitory activities  
149 against *E. coli* and *S. aureus*,

### 150 **21 To determine the minimum bactericidal concentration of** 151 **African panaxia on Staphylococcus aureus and Klebsiella** 152 **pneumoniae from wound infections**

153 Comparing results found in this study with those of the literature, we notice in a previous work on antimicrobial  
154 activity of some medicinal plants from Tunisia, that methanolic extracts of *C. monspeliensis* leaves have shown  
155 an interesting activity against *P. aeruginosa*, *S. aureus*, *E. faecalis* with inhibition zones diameters of 18.0, 20.0  
156 and 15.0 mm, respectively. 26 Whereas, water-methanol extracts of fruit peels of pomegranate (*P. granatum*)  
157 have demonstrated a moderate activity when they were tested on *S. aureus*, *P. aeruginosa* and *K. pneumoniae*  
158 (13.0, 18.0 and 16.0 mm, respectively) [27]. This activity of pomegranate peels could be attributed to tannins,  
159 for which antimicrobial activity has been demonstrated. [24] On the other hand, the results found in the study  
160 concerning the activity of *R. tripartita* aerial parts extracts are in agreement with other previous works which  
161 found significant antibacterial activity of leaves alcoholic extracts against methicillin-resistant *S. aureus*, 16 and  
162 no activity against *E. coli* and *P. aeruginosa*. 29 For *W. frutescens*, El Bouzidi et al. have reported different  
163 antibacterial activities of leaves methanolic extracts against *S. aureus* (11.5 mm), *K. pneumoniae* (18.0 mm), *P.*  
164 *fluorescens* (14.5 mm) and no activity against *E. coli*. 30 IV.

### 165 **22 Conclusion**

166 Based on the results obtained from the study, it could be concluded that the most common bacteria isolates  
167 obtained from this study were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.  
168 African panaxia extract from distilled water was more inhibitory than bactericidal on *Staphylococcus aureus*  
169 and *Klebsiella pneumoniae* and the lastly the African panaxia extract from ethanol was totally bactericidal on  
170 both *Staphylococcus aureus* and *Klebsiella pneumoniae*.

171 V.

### 172 **23 Recommendations**

173 From the results obtained from this study, the following recommendations can be made Ethanoic extract of  
174 African panaxia should be used on wounds infected with *Klebsiella pneumoniae* and *Staphylococcus aureus* to  
175 obtain maximum success. Also other natural herbs should be used to determine their inhibitory and bactericidal  
176 properties on *Klebsiella pneumoniae* and *Staphylococcus aureus*.<sup>1</sup>

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Based on the inhibitory and bactericidal property of Africa panaxia on staphylococcus and Klebsiella, of all the different concentrations made with distilled water, at 25% stock concentration, the extract of African panaxia was both inhibitory and bactericidal on Staphylococcus and Klebsiella. African panaxia extract with look warm water revealed that the extract was bactericidal at 50% stock and 25% stock concentration on Staphylococcus and bacteriostatic at 25% stock concentration on Klebsiella. Finally with alcoholic extract of the African panaxia, the plant extract was bactericidal at 95% stock concentration on Staphylococcus and Klebsiella and bactericidal and bacteriostatic at 75% stock concentration on Staphylococcus and bacteriostatic on Klebsiella as presented on table 1 below.

Figure 1: Table 1 :



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