Journal of Advances in Microbiology

10(3): 1-13, 2018; Article no.JAMB.40917
ISSN: 2456-7116

Studies of Bioactive Potentials of the Root Extracts of *Elaeis guineensis* Jacq. against Pathogens Implicated in Wound Infections

Paul Opeyemi Adeniyi[1], Oluwaseun Oyetunji Olasunkanmi[1*], David Ayinde Akinpelu[1,2], Oludare Femi Ajayi[1], Joseph Omololu-Aso[1] and Felix Oluwasola Olorunmola[3]

[1]*Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.*
[2]*Department of Plant Science, Free State University, Qwaqwa Campus, South Africa.*
[3]*Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Author POA carried out the chemistry aspect of the experiment, literature searches and statistical analysis. Author OOO performed the microbiological aspect of the experiments which include mode of action of the plant extract. Author DAA designed the project and proofread the manuscript. Author OFA collected the plant sample and prepared it for the extraction of the biological components. Author JOA carried out the preparation of media and other chemicals used for the experiments. Author FOO assisted in the microbiological analysis of the plant extract. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/40917
Editor(s):
(1) Grzegorz Cieslar, Professor, Department and Clinic of Internal Diseases, Angiology and Physical Medicine, Medical University of Silesia, Poland.
Reviewers:
(1) Fatunla, Oluwatoba Ayodele, Federal University of Technology, Nigeria.
(2) César Luiz da Silva Guimarães, Brazilian Institute of Environment and Natural Renewable Resources, Brazil.
(3) Muhammad Ali, Kano State University of Technology, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/24528

Original Research Article

Received 10th February 2018
Accepted 28th April 2018
Published 8th May 2018

ABSTRACT

This study was carried out at the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria, between April and October, 2014. It was designed to investigate the antimicrobial activities of the root extract of *Elaeis guineensis* Jacq. on some bacteria implicated in wound infection. This was with a view to obtaining the root extract *of E. guineensis* and testing its *in vitro* bactericidal

**Corresponding author: E-mail: olasunkanmioluwaseun@gmail.com;*

effects, the modes of action on various strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* implicated in wound infections as well as to determine the phytochemical components present in the plant extract that are responsible for the bioactivity.
The root of *E. guineensis* was harvested from Akinlalu area, Ile-Ife, Nigeria in the month of April, 2014. The plant sample was dried in hot air oven at 45ºC and then ground to fine powder. The powered root was cold extracted using methanol and distilled water (3:2 v/v). The methanolic extract obtained was concentrated *in vacuo* using rotary evaporator and then lyophilised. The methanolic crude extract was screened for phytochemicals and tested for antimicrobial activity against all the bacterial isolates associated with wound infection. The extract was later partitioned using different organic solvents in the increasing order of polarity starting from n-hexane, chloroform, ethyl acetate and butanol. The antimicrobial potentials of the crude extract and that of the partitioned fractions were determined by agar-well diffusion method. The minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) of the crude extract and the ethyl acetate fraction were also determined. Finally, the rate of kill, potassium ions and nucleotides leakages were determined using *Staphylococcus aureus* and *Pseudomonas aeruginosa* as representatives of Gram-positive and Gram-negative organisms.
The crude extract and the ethyl acetate fraction exhibited varying degrees of antimicrobial activity while other fractions did not show appreciable antimicrobial activities. The MIC of the crude extract ranged between 0.78 and 12.50 mg/mL and that of the ethyl acetate fraction ranged between 0.63 and 5.00 mg/mL. Phytochemical screening of the crude extract showed the presence of tannins, saponins, steroids and flavonoids. The time-kill assay revealed that the percentage of the cells killed increased with increase in the concentrations of the fractions as well as contact time intervals. A 100% kill was achieved by ethyl acetate fraction on *Staphylococcus aureus* at 3 x MIC within 120 minutes, whereas 100% kill was achieved by the same fraction on *Pseudomonas aeruginosa* at 3 x MIC within 30 minutes. Varying amounts of potassium ions as well as nucleotides were leaked by the ethyl acetate fraction from selected bacterial isolates leading to their death. The leakages of these materials were monophasic.
This study concluded that the crude extract along with the ethyl acetate fraction obtained from it exhibited appreciable bactericidal activities at minimal contact time and low concentrations. It also established the root extract of *E. guineensis* as a potential source of broad spectrum antibacterial drugs in the treatment of wound infections.

Keywords: Antimicrobial activity; Elaeis guineensis; pathogens; phytochemicals; wound infection.

1. INTRODUCTION

A wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity which provides a moist, warm and nutritive environment that is conducive for microbial colonization and proliferation [1]. Infection in a wound delays healing, causes wound breakdown, prolonged hospital stay, increased trauma care and treatment costs [2]. Wound infections are most common in developing countries such as sub-Saharan African and South Asian countries than in developed countries. The poor hygienic condition in these developing countries is the main cause of this problem in addition to inability to purchase drugs for the treatment of the wounds [3].

Many bacteria are known to cause wound infections [4] and those mostly implicated include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Proteus mirabilis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Enterococcus faecalis*. *Candida albicans* and *Candida tropicalis* have also been implicated as etiological agents [5-7]. The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics [8]. This has led us to our studies on the potency of *E. guineensis* root extract on some pathogens implicated in wound infections.

Elaeis guineensis is the common African oil palm and belongs to arecaceae family which comprises of 200 genera and 2500 species [9]. The stem is stout and stands erect and could attain a height of 30 m when fully grown. The natural habitat of this plant is in the rain forest of West Africa [10]. Juice obtained from squeezed leaf of *E. guineensis* is used to promote wound healing [11]. Soap prepared from fruit-husk ash of this plant is used for the treatment of headaches [12]. Oil obtained from fruit mesocarp

and palm kernel oil of *E. guineensis* are administered as poison antidote and can as well be used externally with several other herbs as lotion for skin diseases [13]. The pulverised roots of this plant are added to drinks to treat gonorrhoea, menorrhagia and as a cure for bronchitis [12]. The bioactivity of leaf extract of *E. guineensis* was reported against *Salmonella typhi, Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* [14]. All the test isolates were susceptible to the extract. The leaf extract of *E. guineensis* exhibited antioxidant property [15]. In addition, this plant also exhibited bactericidal effects against *Klebsiella pneumoniae* [16].

2. MATERIALS AND METHODS

This study was carried out at the Department of Microbiology, Obafemi Awolowo U niversity, Ile-Ife, Nigeria, between April and October, 2014.

2.1 Microorganisms Used for This Study and Preparation

Species of clinical isolates from wound infections which include *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were collected from stock culture of Microbiology Laboratory of University College Hospital, Ibadan, Oyo State, Nigeria. Typed cultures of National Collection of Industrial Bacteria (NCIB) and American Typed Culture Collection (ATCC) were obtained from culture collection of Prof. David Akinpelu, Department of Microbiology, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. Typed cultures used include *Staphylococcus aureus* (NCIB 8588), *Staph. aureus* (ATCC 6538), *Pseudomonas aeruginosa* (NCIB 950), *Pseud. aeruginosa* (ATCC 7700), *Klebsiella pneumoniae* (NCIB 418) and *Kleb. pneumoniae* (ATCC 4532). Nutrient broth (LAB M) and nutrient agar (LAB M) were used for sub-culturing the organisms while Mueller-Hinton agar (LAB M) was used for sensitivity testing. The organisms were first reactivated in nutrient broth for 18 h at 37ºC before use.

2.2 Collection of Plant Sample

Fresh roots of *E. guineensis* used were collected at Akinlalu area, Ile-Ife, Osun State, Nigeria in the month of April, 2014. The plant was identified in the Herbarium of Department of Botany, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. Voucher specimen of the plant was prepared (Voucher No. IFE-17479) and deposited for reference purposes.

2.3 Drying and Extraction of the Plant Sample

The root of *E. guineensis* was dried in hot-air oven at 45ºC until a constant weight of the sample was obtained. Three thousand gram of the powdered sample was cold extracted using a mixture of methanol and sterile distilled water (3:2) (v/v). This was allowed to stand for 4 days in a glass bottle with regular agitation. The supernatant collected from the mixture was filtered into a clean dried conical flask using Whattman's No. 1 filter paper. The filtrate collected was then concentrated *in vacuo* in a rotary evaporator to drive out the methanol. The aqueous part left was then lyophilised to collect dried extract of the plant sample.

2.4 Phytochemical Assay of the Crude Extract of the Plant Sample

The phytochemical assay of the extract was done to test for the presence of alkaloids, tannins, flavonoids, saponins, steroids and reducing sugars [17-18].

2.5 Solvent Partitioning of the Crude Extract

Different organic solvents were used for partitioning the crude extract according to their polarity. Hexane with the lowest polarity was first used but the solvent did not have affinity for the bioactive compounds present in the plant extract. Chloroform, ethyl acetate and butanol were used for further partitioning in that order. Various yields of the fractions were obtained and the weight of each fraction was noted. The fractions were kept in an air-tight container and kept in the freezer for further use.

2.6 Sensitivity Testing of the Fractions

The sensitivity testing of the crude extract and the fractions of the plant sample along with those of ampicillin and streptomycin used as positive controls was determined using agar-well diffusion methods [19-20] with some modifications. The test isolates were first re-activated in nutrient broth and later standardised to 10^6 cfu/mL of McFarland standard before use. One hundred

microliters of the standardised bacterial suspension were evenly spread out on Mueller-Hinton agar medium using a sterile glass spreader. A sterile 6 mm cork borer was then used to bore wells into the agar medium allowing a 5 mm distance to the edge of the plate. Solution of the crude extract and the fractions at concentrations of 25 mg/mL and 10 mg/mL respectively was filled up into each well. Spillage of the solution was prevented on the surface of the medium. The plates were allowed to stand on the laboratory bench for 1 h to allow proper in-flow of the extracts into the medium and incubated at 37ºC for 24 h without stock-pilling to allow even distribution of temperature. The plates were later examined for zones of inhibition which indicates susceptibility of the test isolates to the extracts. The effect of the extract on the isolates was compared with the standard antibiotics-ampicillin and streptomycin used as positive control.

2.7 Determination of Minimum Inhibitory Concentrations (MIC) of the Extracts

The MIC of the crude extract against the bacterial isolates was determined by preparing two-fold dilution of the extract [21]. Two millilitres of different concentrations of the extract solution was added to 18 mL pre-sterilised molten agar to give a final concentration regime of 0.391 mg/mL to 12.5 mg/mL. The same procedure was done for the fractions with concentrations ranging between 0.079 mg/mL and 5.00 mg/mL. The medium was then poured into sterile Petri dishes and allowed to set. The plates were left on the laboratory bench overnight to observe for sterility of the medium before streaking with the test isolates. The dry surface of the medium was then streaked with 18 h old standardised bacterial isolates and incubated at 37ºC for up to 72 h after which they were examined for the presence of growth. The MIC was taken as the lowest concentration that prevented the growth of the bacterial isolates.

2.8 Determination of Minimum Bactericidal Concentrations (MBC) of the Extracts

The MBC of the extract was determined by taking samples from plates with no visible growth in the MIC assay, sub-cultured on to freshly prepared nutrient agar medium and incubated at 37^0C for up to 48 h and later observed for growth [22]. The MBC was taken as the lowest concentration of the extract that did not allow any bacterial growth on plate surface.

2.9 Determination of Rate of Kill on the Bacterial Isolates by Ethylacetate Fraction

The rate of kill of the bacterial isolates was determined using representative of each Gram positive and Gram negative organisms [23]. *Staphylococcus aureus* was chosen for Gram positive while Gram negative was represented by *Pseudomonas aeruginosa*. A 0.5 mL volume of standardised bacterial isolates suspension was added to 4.5 mL of different concentrations of the fraction. The suspension was thoroughly mixed and held at room temperature over a period of 2 h to determine the killing rate of the isolates. Exactly 0.5 mL of each of the suspension was withdrawn at time intervals and transferred to 4.5 mL of recovery medium containing 3% “Tween 80” to shake off the effect of the extract carry-over from the test isolates. The suspension was then serially diluted and plated for viable counts. The plates were incubated at 37ºC for 48 h before reading. Control plates containing the test cells without the extract were set up along with the experimental. The emergent bacterial colonies were counted and compared with the counts of the culture control.

2.10 Determination of Potassium Ion Leakage from the Test Isolates by the Fractions

Exactly 50 mL of harvested and washed cells (OD_{470nm} = 1.5) were placed in a clean 100 mL beaker which was magnetically stirred. A volume (5 mL) of ionic strength adjustment buffer (ISAB; 18.37 g of tetraethylammonium chloride in deionised water and made up to 100 mL) was added to the beaker. This ensured that the background ionic strength of all solutions was kept constant. The potassium ion sensing electrode (Qualiprobe QSE 314, EDT Instruments Waldershare Park, Dover, UK) and its reference electrode (Qualiprobe double junction reference electrode E8092, EDT Instruments) were placed into the cell suspension. The potential difference (mV) derived by the electrodes was measured using a Whatmann PHA 220 pH/mV meter (Whatmann Maidstone, UK). Bacterial cells were treated with various concentrations of the fraction of the plant extract relative to the MIC. The potassium efflux from the cells in the suspension was measured at time intervals over 2 h as a potential difference in mV.

These values were converted to concentrations of K^+ ions (M) by reference to a conversion graph, which had been constructed earlier using KCl standard solutions. The concentration of K^+ ions released was plotted against time.

2.11 Determination of Nucleotides Leakage from the Test Isolates by the Fractions

The leakage of nucleotides from the test cells was determined by washing cells of 18 h old test isolates which was then standardised (approximately 10^6 cfu/mL) and treated with different concentrations of the fractions relative to the MIC at various contact time intervals over 2 h [24]. Each suspension was centrifuged at 10000 rpm and decanted. Wavelength of the supernatant collected was determined at 260_{nm} to quantify the amount of nucleotide leaked by comparing with the standard curve already plotted. The blank constitute sterile distilled water inoculated with the test isolates.

3. RESULTS

The crude extract collected from powdered root of *E. guineensis* was dark brown in colour and the yield collected was 102 g. The crude extract exhibited antibacterial potentials at a final concentration of 25 mg/mL on all the test isolates associated with wound infections (Table 1).

Streptomycin and ampicillin exhibited antimicrobial activities against 18 out of 36 and 10 out of 36 bacterial isolates respectively. Zones of inhibition exhibited by the crude extract ranged between 13 mm and 22 mm, while streptomycin exhibited zones of inhibition ranging between 12 mm and 22 mm. On the other hand, zones of inhibition exhibited by ampicillin against the isolates were between 19 mm and 28 mm. The crude extract exhibited activities on both Gram positive and Gram negative organisms likewise streptomycin showed the same reaction but ampicillin only has activity on *Staph. aureus* which is Gram positive. Among the fractions collected from the crude extract only ethyl acetate fraction exhibited appreciable antimicrobial activities. Butanol fraction inhibited the growth of only two of the isolates while aqueous and chloroform fractions did not inhibit the growth of the test isolates (Table 2).

Thus, only ethyl acetate fraction was used for further tests while the rest were discarded. The zones of inhibition exhibited by ethyl acetate fraction against the test isolates ranged between 12 mm and 25 mm. All the isolates inhibited were both Gram positive and Gram negative. Thus both the crude extract and ethyl acetate fraction exhibited broad spectrum activities.

The MIC and the minimum bactericidal concentrations of the crude extract and ethyl acetate fraction were also determined. The MIC exhibited by the crude extract against the test isolates ranged between 1.56 mg/L and 12.5 mg/mL while the MBC were between 3.13 mg/mL and 12.5 mg/mL. On the other hand, the MIC exhibited by ethyl acetate fraction against the test isolates ranged between 0.313 mg/mL and 5.00 mg/mL. The MBC exhibited were between 0.625 mg/mL and 5.00 mg/mL (Table 3).

Investigation on phytochemical compounds of *E. guineensis* root extract revealed the presence of alkaloids, tannins, flavonoids, saponins, steroids and reducing sugars (Table 4).

The killing rate, potassium ions leakage and nucleotides were investigated to determine the bactericidal effects of root extract of *E. guineensis*. The extent and killing rate of ethyl acetate fraction of the extract was carried out on *Staph. aureus* and *Pseudomonas aeruginosa* (Figs. 1 and 2). At a concentration of 1 x MIC, the percentage of *Staph. aureus* cells killed after 15 mins contact time of the cells with the ethyl acetate fraction was 16.30%. The percentage of the cells killed rose to 51.34% after 60 mins of contact time. When the contact time increased to 120 mins, about 63.10% of *Staph. aureus* cells was killed (Fig.1). The concentration of the fraction was later increased to 2 x MIC and the test isolates were again subjected to the effect of the fraction. At 15 mins contact time of the cells with this concentration, 23.08% of the cells was killed and this rose to 95.50% after 120 mins. The same trend was observed when the concentration of the fraction was increased to 3 x MIC (Fig. 1). (Fig. 2) shows the extent and rate of kill of *Pseud. aeruginosa* at various concentrations and time. The same trend of reactions were observed in this test as shown in that of *Staph. aureus* (Fig.1). The percentage of *Pseud. aeruginosa* killed by ethyl acetate fraction increased with an increase in concentration and time (Fig. 2).

The bactericidal effects of ethyl acetate fraction was also investigated on the test isolates by assaying for leakage of potassium ions from the cells (Fig. 3). The quantity of potassium ions

leaked from *Staph. aureus* cells at a concentration of 1 x MIC after 15 mins contact time with the fraction was 2.26 µg/mL. At 30 mins of contact time the leakage increased to 2.28 µg/mL. Finally, when the contact time reached 120 mins at the same concentration about 2.42 µg/mL of K^+ ion was leaked from the test cells. The leakage of K^+ ion continued to increase when the concentration of ethyl acetate increased to 2 x MIC and 3 x MIC with increase in contact time of the cells with the fraction (Fig. 3). The same trend of effects was observed when *Pseud. aeruginosa* was subjected to the effects of the fraction as shown in Fig. 4.

Table 1. The Sensitivity patterns exhibited by crude extract of *Elaeis guineensis* against the test isolates

Bacterial isolates	Zones of inhibition (mm)**			Statistics (F-value)	P-value
	Crude extract (25 mg/ml) (Mean ± SD)	Streptomycin (1 mg/ml) (Mean ± SD)	Ampicillin (1 mg/ml) (Mean ± SD)		
STA 1	16.00 ± 0.00	0	24.33 ± 1.15	1032.2	0.0001
STA 2	16.67 ± 1.15	15.67 ± 0.58	23.33 ± 0.58	78.3	0.0001
STA 3	19.00 ± 1.00	19.33 ± 0.58	19.00 ± 1.00	0.1429	0.8697
STA 4	20.00 ± 1.00	0	23.67 ± 0.58	4381	0.0001
STA 5	19.33 ± 1.15	21.00 ± 1.00	28.33 ± 1.15	56.273	0.0001
STA 6	17.00 ± 1.00	19.67 ± 1.15	21.50 ± 0.50	17.8	0.003
STA 7	16.00 ± 1.00	14.33 ± 0.58	24.67 ± 0.58	166.2	0.0001
STA 8	16.67 ± 1.15	0	24.33 ± 1.15	522.4	0.0001
STA 9	17.33 ± 0.58	0	25.00 ± 1.00	1107.2	0.0001
STA 10	18.00 ± 2.00	0	21.00 ± 1.00	232.2	0.0001
STA 11	16.33 ± 0.58	20.00 ± 1.00	0	765.3	0.0001
STA 12	17.00 ± 1.00	21.33 ± 0.58	0	858.3	0.0001
PA 1	16.00 ± 0.00	14.00 ± 1.00	0	684	0.0001
PA 2	18.67 ± 0.58	14.67 ± 0.58	0	1304	0.0001
PA 3	16.00 ± 1.00	0	0	768	0.0001
PA 4	19.33 ± 0.58	0	0	3364	0.0001
PA 5	16.17 ± 0.29	0	0	7921.9	0.0001
PA 6	14.00 ± 0.00	0	0	588	0.0001
PA 7	14.67 ± 0.58	14.33 ± 0.58	0	946.5	0.0001
PA 8	16.00 ± 0.00	15.00 ± 1.00	0	723	0.0001
PA 9	17.00 ± 1.00	0	0	867	0.0001
PA 10	15.00 ± 1.00	0	0	675	0.0001
PA 11	22.00 ± 1.00	0	0	1452	0.0001
PA 12	19.67 ± 0.58	21.33 ± 1.15	0	760.2	0.0001
KA 1	15.67 ± 0.58	0	0	2209	0.0001
KA 2	13.83 ± 0.76	0	0	984.1	0.0001
KA 3	16.33 ± 1.15	20.33 ± 0.58	0	626.6	0.0001
KA 4	17.00 ± 1.00	0	0	867	0.0001
KA 5	17.67 ± 0.58	20.00 ± 1.00	0	807.3	0.0001
KA 6	15.00 ± 1.00	0	0	675	0.0001
KA 7	16.67 ± 0.58	12.67 ± 0.58	0	1022	0.0001
KA 8	18.00 ± 0.00	13.33 ± 0.58	0	2356	0.0001
KA 9	18.00 ± 1.00	22.00 ± 1.00	0	618	0.0001
KA 10	17.00 ± 1.00	12.33 ± 0.58	0	520.8	0.0001
KA 11	20.33 ± 0.58	0	0	3721	0.0001
KA 12	21.33 ± 0.58	0	0	4096	0.0001

KEY: STA 1 - STA 5 = Staph. aureus (Pus Isolates), STA 6 - STA 10 = Staph. aureus (Surgical Wound Isolates), STA 11 = Staph. aureus, (NCIB 8588) S12 = Staph. aureus, (ATCC 6538);
PA 1 -PA 5 = Ps. aeruginosa (Pus Isolates), PA 6 - PA 10 = Ps. aeruginosa (Surgical Wound Isolates), PA 11 = Ps. aeruginosa (NCIB 950), PA 12 = Ps. aeruginosa (ATCC 7700),
*KA 1 - KA 5 = Kleb. pneumoniae (Pus Isolates), KA 6 - KA 10 = Kleb. pneumoniae (Surgical Wound Isolates), KA 11 = Kleb. pneumoniae (NCIB 418), KA 12 = Kleb. pneumoniae (ATCC 4532), P- value = Probability value, F- value = Fischer value, ** = Mean of three replicates*

Table 2. The Sensitivity patterns exhibited by *Elaeis guineensis* fractions on the test isolates

Bacterial isolates	Zones of inhibition (mm)**			
	Aqueous fraction (10 mg/ml)	Chloroform fraction (10 mg/ml)	Ethyl acetate fraction (10 mg/ml)	N-butanol fraction (10 mg/ml)
STA 1	0	0	15.67 ± 0.58	0
STA 2	0	0	14.50 ± 0.87	0
STA 3	0	0	19.33 ± 1.15	14.33 ± 0.58
STA 4	0	0	18.00 ± 0.00	0
STA 5	0	0	16.00 ± 1.00	0
STA 6	0	0	16.33 ± 0.58	0
STA 7	0	0	18.00 ± 1.00	0
STA 8	0	0	14.33 ± 0.58	0
STA 9	0	0	17.33 ± 0.58	0
STA 10	0	0	20.00 ± 1.00	13.83 ± 0.29
STA 11	0	0	18.00 ± 0.00	0
STA 12	0	0	19.33 ± 0.58	0
PA 1	0	0	15.00 ± 1.00	0
PA 2	0	0	15.67 ± 1.15	0
PA 3	0	0	14.00 ± 1.00	0
PA 4	0	0	15.00 ± 0.00	0
PA 5	0	0	13.67 ± 0.58	0
PA 6	0	0	15.67 ± 0.58	0
PA 7	0	0	13.00 ± 1.00	0
PA 8	0	0	20.33 ± 0.58	0
PA 9	0	0	17.33 ± 1.15	0
PA 10	0	0	18.00 ± 1.00	0
PA 11	0	0	25.67 ± 1.53	0
PA 12	0	0	24.33 ± 0.58	0
KA 1	0	0	17.67 ± 0.58	0
KA 2	0	0	14.50 ± 0.50	0
KA 3	0	0	14.00 ± 0.00	0
KA 4	0	0	16.67 ± 1.15	0
KA 5	0	0	14.00 ± 1.00	0
KA 6	0	0	14.00 ± 0.00	0
KA 7	0	0	14.00 ± 1.00	0
KA 8	0	0	12.33 ± 0.58	0
KA 9	0	0	14.17 ± 0.29	0
KA 10	0	0	14.00 ± 0.00	0
KA 11	0	0	20.00 ± 1.00	0
KA 12	0	0	20.33 ± 1.53	0

*KEY: STA 1 - STA 5 = Staph. aureus (Pus Isolates), STA 6 - STA 10 = Staph. aureus (Surgical Wound Isolates), STA 11 = Staph. aureus, (NCIB 8588) S12 = Staph. aureus, (ATCC 6538); PA 1 - PA 5 = Ps. aeruginosa (Pus Isolates), PA 6 - PA 10 = Ps. aeruginosa (Surgical Wound Isolates), PA 11 = Ps. aeruginosa (NCIB 950), PA 12 = Ps. aeruginosa (ATCC 7700), KA 1 - KA 5 = Kleb. pneumoniae (Pus Isolates), KA 6-KA 10 = Kleb. pneumoniae (Surgical Wound Isolates), KA 11 = Kleb. pneumoniae (NCIB 418), KA 12 = Kleb. pneumoniae (ATCC 4532), ** = Mean of three replicates*

Table 3. The minimum inhibitory concentrations and minimum bactericidal concentrations of the crude extract and ethyl acetate fraction exhibited against the test isolates

Bacterial isolates	Crude extract		Ethyl acetate fraction	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
STA 1	3.13	6.25	5	ND
STA 2	3.13	6.25	1.25	2.5
STA 3	6.25	12.5	5	ND
STA 4	1.56	3.13	5	ND
STA 5	3.13	6.25	1.25	2.5
STA 6	3.13	6.25	1.25	2.5
STA 7	3.13	6.25	1.25	2.5
STA 8	3.13	6.25	5	ND
STA 9	1.56	3.13	5	ND
STA 10	3.13	6.25	1.25	2.5
STA 11	3.13	6.25	2.5	2.5
STA 12	3.13	6.25	1.25	2.5
PA 1	6.25	12.5	2.5	5
PA 2	6.25	12.5	ND	ND
PA 3	6.25	12.5	5	ND
PA 4	12.5	ND	5	ND
PA 5	6.25	12.5	5	ND
PA 6	12.5	ND	5	ND
PA 7	12.5	ND	5	ND
PA 8	6.25	12.5	2.5	5
PA 9	12.5	ND	5	ND
PA 10	12.5	ND	5	ND
PA 11	12.5	ND	5	ND
PA 12	12.5	ND	5	ND
KA 1	12.5	ND	5	ND
KA 2	12.5	ND	5	ND
KA 3	6.25	12.5	5	ND
KA 4	6.25	12.5	5	ND
KA 5	12.5	ND	5	ND
KA 6	3.13	6.25	5	ND
KA 7	6.25	12.5	5	ND
KA 8	12.5	ND	5	ND
KA 9	6.25	12.5	5	ND
KA 10	12.5	ND	5	ND
KA 11	0.78	1.56	0.63	1.25
KA 12	6.25	12.5	5	ND

KEY: STA 1 - STA 5 = Staph. aureus (Pus Isolates), STA 6 - STA 10 = Staph. aureus (Surgical Wound Isolates), STA 11 = Staph. aureus, (NCIB 8588) S12 = Staph. aureus, (ATCC 6538);
PA 1 - PA 5 = Ps. aeruginosa (Pus Isolates), PA 6 - PA 10 = Ps. aeruginosa (Surgical Wound Isolates), PA 11 = Ps. aeruginosa (NCIB 950), PA 12 = Ps. aeruginosa (ATCC 7700),
*KA 1 - KA 5 = Kleb. pneumoniae (Pus Isolates), KA 6 - KA 10 = Kleb. pneumoniae (Surgical Wound Isolates), KA 11 = Kleb. pneumoniae (NCIB 418), KA 12 = Kleb. pneumoniae (ATCC 4532), ** = Mean of three replicates. ND = Not done, MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration.*

Leakage of nucleotides from the test cells by ethyl acetate fraction was also studied. At 15 mins contact time of *Staph. aureus* cells with the ethyl acetate fraction and at a concentration of 1 x MIC, about 27.3 µg/mL of nucleotide was leaked out of the test cells (Fig. 5). When the time interval got increased to 60 mins, about 36.5 µg/mL of nucleotides was leaked. Finally, when the contact time reached 120 mins, the quantity of nucleotides that leaked out of *Staph. aureus* cells at the same concentration of 1 X MIC was 3.87 µg/mL. As the concentrations and contact

time increased in the experiment, the quantity of nucleotides continued to increase as well (Fig. 5). The same trend of events was observed when *Pseud. aeruginosa* was treated with the ethyl acetate fraction (Fig. 6).

Table 4. Phytochemical screenings of the root extract of *Elaeis guineensis*

Chemical Test	Result
Alkaloids	Negative
Reducing sugar	Negative
Tannins	Positive
Flavonoid	Positive
Steroids	Positive
Saponins	Positive

4. DISCUSSION

The biocidal effects of root extracts of *E. guineensis* were investigated against a panel of organisms associated with wound infections. These organisms include various clinical isolates of *Staph. aureus*, *Kleb. pneumoniae* and *Pseud. aeruginosa*. Both crude extract and ethyl acetate fraction obtained from the plant sample exhibited inhibitory effects on the growth of all the test isolates. Aqueous and chloroform fractions did not show any activities against the organisms while butanol inhibited the growth of two isolates. This is an indication that ethyl acetate will serve as a good solvent for the extraction of bioactive components from *E. guineensis* root.

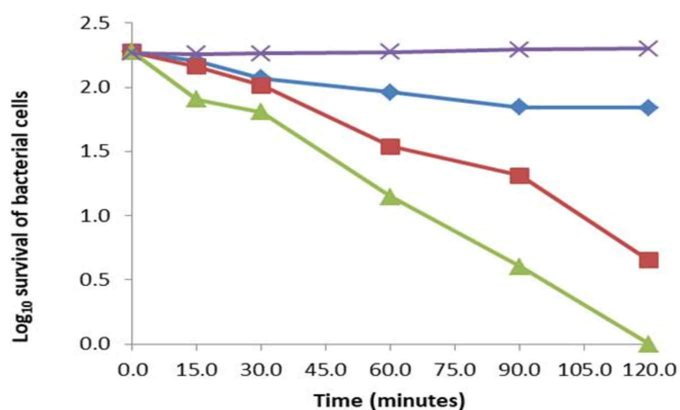

Fig. 1. The extent and the rate of killing of *Staph. aureus* cells by the ethyl acetate fraction at 1 x MIC (), 2 x MIC (), 3 x MIC () and control (). Each point represents the mean log_{10} survival of bacterial cells at a particular time interval in the presence of the fraction

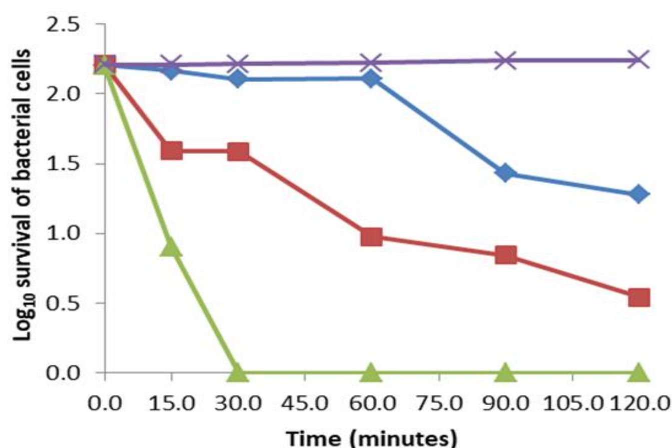

Fig. 2. The extent and the rate of killing of *Ps. aeruginosa* cells by the ethyl acetate fraction at 1 x MIC (), 2 x MIC (), 3 x MIC () and control (). Each point represents the mean log_{10} survival of bacterial cells at a particular time interval in the presence of the fraction

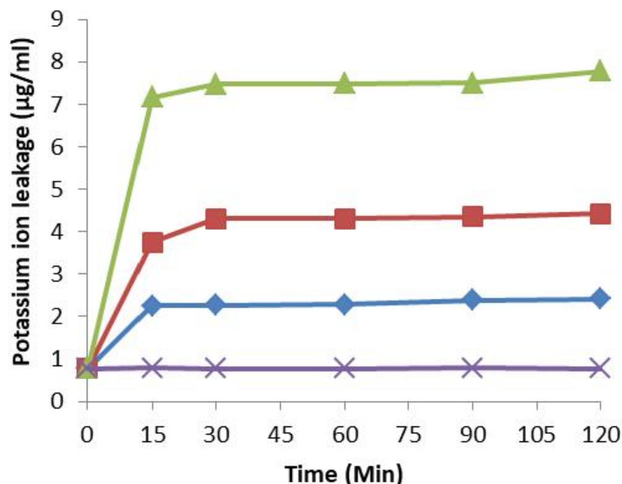

Fig. 3. The Effect of the ethyl acetate fraction on potassium ion leakage from *Staph. aureus* cells at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the amount of potassium ions leaked (µg/ml) from the cells at a particular time interval in the presence of the fraction

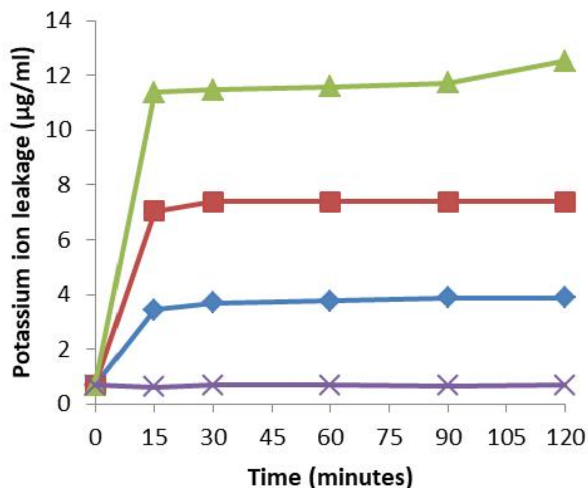

Fig. 4. The effect of the ethyl acetate fraction on potassium ion leakage from *Ps. aeruginosa* cells at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the amount of potassium ions leaked (µg/ml) from the cells at a particular time interval in the presence of the fraction

Comparison of the activities of ethyl acetate fraction with those of the standard antibiotics – ampicillin and streptomycin, one could conclude that ethyl acetate fraction compared favourably with these standard antibiotics. The fraction also exhibited broad spectrum activities against the test bacterial isolates. Thus, the root extract of *E. guineensis* appears to be a potential source of antibacterial compounds that could be relevant in the treatment of wound infections caused by these pathogens. Hence, results obtained from this study corroborate the usefulness of the plant in folklore remedies especially among many tribes in West Africa.

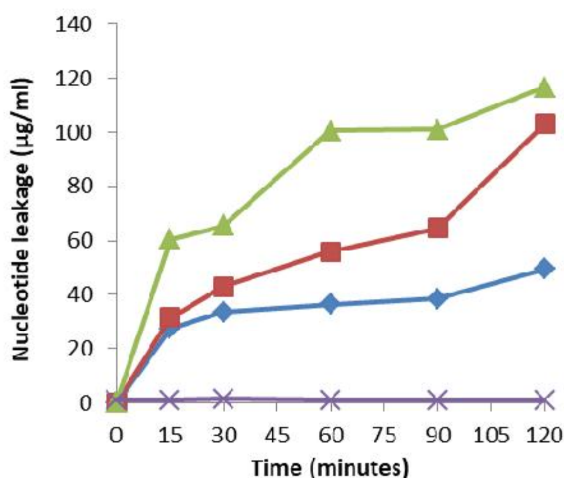

Fig. 5. The effect of the ethyl acetate fraction on nucleotide leakage from *Staph. aureus* cells at 1 x MIC (), 2 x MIC (), 3 x MIC () and control (). Each point represents the µg/ml of nucleotides leaked from the cells at a particular time interval in the presence of the fraction

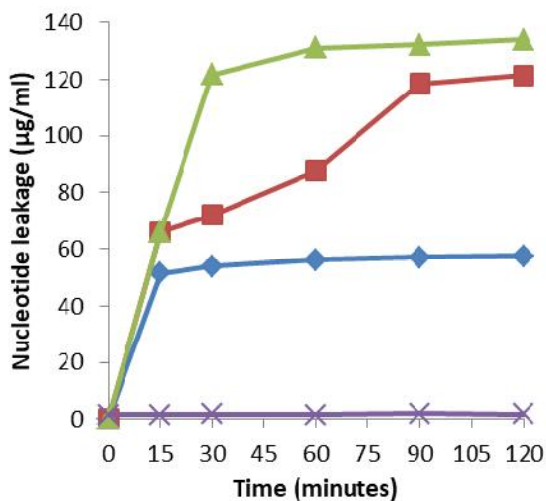

Fig. 6. The effect of the ethyl acetate fraction on nucleotide leakage from *Ps. aeruginosa* cells at 1 x MIC (), 2 x MIC (), 3 x MIC () and control (). Each point represents the µg/ml of nucleotides leaked from the cells at a particular time interval in the presence of the fraction

Elaeis guineensis root extracts revealed the presence of some phytochemicals which include alkaloids, tannins, flavonoids, saponins, steroids and reducing sugars. These phytochemicals are known to play important roles in biological activities of medicinal plants [18] and thus, produce a definite physiological action on human body. The crude extract and ethyl acetate fractions obtained from the root extract of *E. guineensis* exhibited low MIC and MBC. For example, the lowest MIC exhibited by the crude extract was 0.78 mg/mL while the lowest MBC was 3.13 mg/mL. On the other hand, the lowest MIC exhibited by ethyl acetate traction was 0.313 mg /mL while the lowest MBC was 0.625 mg/mL. The antibacterial activity of medicinal plant extract is considered significant if the MIC is less than or equal to 200 mg/mL [25]. The MIC and MBC exhibited by both crude extract and ethyl acetate fraction

were far below 200 mg/mL. This is an indication that root extract obtained from *E. guineensis* exhibited significant antimicrobial activities against the test isolates. Such plant could be a good source of antimicrobial drug that can be used to combat the infections caused by microorganisms.

From our observations, *E. guineensis* extract exerted a biocidal effect on the test cells through leakage of protoplasmic inclusions from the cells. Damage to bacterial membrane cause the release of cytoplasmic constituents of the cell such as DNA, RNA, potassium and other materials [26]. Our investigation on biocidal effects on the test isolates were based on rate of killing of the test isolates along with the leakage of potassium ions and nucleotides from their cells. The ethyl acetate fraction exhibited appreciable kill rate against *Staph. aureus*, *Kleb. pneumoniae* and *Pseud. aeruginosa*. As the concentrations of the fraction got increased with increase in contact time, there appears to be an increase in population of the test cells killed (Figs. 1 and 2). This is an indication of monophasic effects been exhibited by the extract. The ethyl acetate fraction also exhibited appreciable leakage of potassium ions and nucleotides from the test cells which is an indication of damaged to the cytoplasmic membrane of the cells and thus led to the cells death. Leakage of potassium ions from bacterial cells could lead to deactivation of important enzymes required for cell metabolism and this effects could be lethal on the cells and thus lead to their death [27]. Hence, permeability of the cytoplasmic membrane will lead to the loss of cellular matters and, consequently results in cell death [28]. Our findings have shown that *E. guineensis* root extracts exhibited biocidal effects on the test isolates through cytoplasmic membrane disruption leading to the exit of protoplasmic inclusion. The antibacterial potentials shown by *E. guineensis* root extract on the organisms implicated in wound infections showed a significant therapeutic effectiveness and thus supported its usefulness in folklore remedies.

5. CONCLUSION

The antimicrobial potency exhibited by *E. guineensis* root extract to kill or inhibit test isolates used for this study at low concentration and minimal contact time has shown that drugs formulated from this plant for clinical trials will go a long way in health care delivery.

ACKNOWLEDGEMENTS

We wish to acknowledge Prof H. C. Illoh of Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria for identifying the plant sample.

Our gratitude also goes to Free State University Qwaqwa Campus, South Africa.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev. 2001; 14(2):244-69.
2. Alexander MF. Wound infection. In: Alexander MF, Fawcett JN, Runciman PJ, editors. Nursing practice hospital and home, the adult. New York: Churchill Livingstone. 1994;703.
3. Sasidharan S, Rajoo N, Rathinam X, Lachimanan YL, Rajoo A. Wound healing potential of *Elaeis guineensis* Jacq leaves in an infected Albino rat model. Molecules. 2010;15:3186-99.
4. Yah SC, Enabulele IO, Eghafona NO, Udemezue OO. Prevalence of Pseudomonas in burn wounds at the University of Benin Teaching Hospital, Benin City, Nigeria. J Clin Exp Anatomy. 2004;3(1):12-5.
5. Enweani IB, Esumeh FI, Akpe RA, Tatfeng MY, Isibor JO. Bacteria associated with post-operative wounds. J Contem Issues. 2003;1:183-8.
6. Kaplan NM, Smadi AA, Taani MI, El-Qudah MA. Microbiology of wound infection after caesaria section. Est Meditter Health Journal. 2003;9(5-6):1068-74.
7. Isibor JO, Oseni A, Eyaufe A, Osagie R, Turay A. Incidence of aerobic bacteria and *Candida albicans* in post-operative wound infections. Afr J Microbiol Res. 2008;2:288-91.
8. Akinjogunla OJ, Adegoke AA, Mboto CI, Chukwudebelu IC, Udokang IP. Bacteriology of automobile accident wounds infection. Int J Med Sci. 2009; 1(2):23-7.

9. Keay RWJ. Trees of Nigeria. Oxford, UK: Oxford University Press; 1989.
10. Obahiagbon FI. A review: Aspects of the african oil palm (*Elaeis guineesis* jacq.) and the implications of its bioactives in human health Am J Biochem Mol Biol. 2012;2(3):106-119.
11. Irvin TT. Wound healing. Arch Emerg Med. 1985;2:3-10.
12. Sasidharan S, Logeswaran S, Latha LY. Wound healing of *Elaeis guineensis* leaf extract ointment. Int J Mol Sci. 2012; 13(1):336-47.
13. Chong KH, Zuraini Z, Sasidharan S, Devi PVK, Latha LY, Ramanathan S. Antimicrobial of *Elaeis guineensis* leaf. Pharmacology online. 2008;3:379–86.
14. Chang JL, Lin HV, Blauwkamp TA, Cadigan KM. Spenito and split ends act redundantly to promote Wingless signalling. Dev. Biol. 2008;314(1):100-11.
15. Sasidharan S, Sharmini R, Vijayarathna S, Yoga Latha L, Vijenthi R, Amala R, Amutha S. Antioxidant and hepato protective activity of methanolic extracts of *Elaeis guineensis* Jacq leaf. Pharmacology Online. 2009;3:84-90.
16. Soundararajan V, Zuraini Z, Yeng, C, Lachimanan YL, Jagat RK, Sreenivasan S. The antimicrobial efficacy of *Elaeis guineensis*: characterization, *in vitro* and *in vivo* studies. Molecules. 2012;17:4860-77.
17. Harborne JB. Phytochemical methods – a guide to modern techniques of plant analysis. London: Chapman and Hall. 1998;60-6.
18. Trease GE, Evans WC. Textbook of Pharmacognosy, London, UK: Balliere, Tindall. 2002;57(9):343–83.
19. Russell AD, Furr JR. The antibacterial activity of a new chloroxylenol preparation containing ethylene diamine tetraacetic acid. J Appl Bacteriol. 1977;43:253.
20. Irobi ON, Moo-Young M, Anderson WA. Antimicrobial activity of Annato (*Bixa orellana*) extract. Int J Pharmacognosy. 1994;34:87-90.
21. Akinpelu DA, Kolawole DO. Phytochemical and antimicrobial activity of leaf extract of *Piliostigma thonningii* (Schum.). Sci Focus. 2004;7:64-70.
22. Olorundare EE, Emudianughe TS, Khasar GS, Koteyi SA, Irobi DN. Antibacterial properties of leave extract of *Cassia alata*. Bio Res Com. 1992;4:113-7.
23. Odenholt I, Lowdinand E, Cars O. Pharmacodynamics of telithromycin *in vitro* against respiratory tract pathogens. Chemotherapy. 2001;45:23-9.
24. Heipieper HJ, Diefenbach R, Kewelol H. Conversion of cis unsaturated fatty acids to Trans, a possible mechanism for the protection of phenol degrading *Pseudomonas putida* P 8 from substrate toxicity. Appl Environ Microbiol. 1992;58: 1847-51.
25. Suffredini IB, Paciencia MLB, Varella AD, Younes RN. Antibacterial activity of Brazilian Amazon plant extracts. Braz J Inf Dis. 2006;10(6):400-2.
26. Stojkovic DS, Jelena Z, Marina S, Jasmina G, Isabel CFR, Ferreir TJ, et al. Antibacterial activity of *Veronica montana* L. extract and of protocatechuic acid incorporated in a food system. Food Chem Toxicol. 2013;55:209-13.
27. Cox SD, Mann, CM, Marhan JL, Gustafson JE, Warmington JR, Wyllie SG. Determining the antimicrobial action of tea tree oil. Molecules. 2001;6:87-91.
28. Cao MT, Wang R, Helmann JD. Antibiotic that inhibit cell wall biosynthesis induce expression of the *Bacillus subtilis* σ W and σ M regulons. Mol. Microbiol. 2002;45: 1267-76.

© 2018 Adeniyi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24528