In vitro Antibacterial Efficacy of Velvet Tamarind (*Dialium guineense* Wild) Root Extract

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ABSTRACT

This study was carried out to investigate the *in vitro* antibacterial efficacy of velvet tamarind (*Dialium guineense* Wild) ethanol root extract against three common clinical bacterial isolates (*Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae*) using standard agar well diffusion assay and ciprofloxacin as reference drug. The efficacy (bactericidal and or bacteriostatic effects) of the crude extract was determined by time-kill assay at single and twice the minimum inhibitory concentrations (MIC). The phytochemical contents of the root extract were also determined using standard techniques. The three bacteria were sensitive to 10 mg/mL of the extract with zone of inhibition diameter ranging from 20.4 ± 1.02 mm (*S. aureus*) and 15.2 ± 0.25 mm (*K. pneumoniae*). The inhibitory effect was significant ($p<0.05$) when compared with 0.002 mg/mL ciprofloxacin (18.8 ± 0.01 to 24.5 ± 0.05 mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts ranged from 0.625 – 1.25 mg/mL and 1.25 – 5 mg/mL, while ciprofloxacin gave ≤ 0.064 mg/mL. Time-kill assay revealed that 2 x MIC of extracts achieved bactericidal activities (≥ 3 $\log_{10}$) on all the isolates post 6 h interaction, with almost complete elimination of the bacterial density within 24 h. The observed antibacterial efficacy could be attributed to the relatively high quantities of alkaloids (6.2 ± 0.09 mg/100g), phenols (4.6 ± 0.29 mg/100g), tannins (4.3 ± 0.50 mg/100g) and saponins (4.1 ± 0.41 mg/100g), among other phytochemicals, in the root extract. These findings suggest that *D. guineense* root extract possess *in vitro* bactericidal activities against some important public health bacteria and thus, could be considered as a potential candidate in developing new antimicrobial agent against these pathogens.

Keyword: Antibacterial efficacy, time-kill assay, *Dialium guineense,* clinical isolates

INTRODUCTION

The growing public interest and awareness of natural medicines have led the pharmaceutical industries and academic researchers to pay more attention to medicinal plants. Consequently, several plants and their products have been scientifically reported to possess phytochemicals such as glycosides, alkaloids, saponins, tannins, terpenoids, and flavonoids, which are frequently, implicated in *in vitro* and *in vivo* antimicrobial activities of plant products. One of such plants with great medicinal value is *Dialium guineense* Wild (Fabaceae).

D. guineense Wild (Fabaceae) is a tree characterised by possessing a hairy leafy crown, smooth greyish bark and whitish flowers which bears densely velvet black fruits that are more or less circular, flattened and enclosing dry, brownish, sweet relatively acidic edible pulp (Hutchinson and Daniel, 1958). It grows to an average height of 30m and is found mostly in the rain forest region of West Africa. The common names of the plant are black velvet or velvet tamarind (English), 'Icheku' (Ibo, Eastern Nigeria), 'Awin' (Yoruba, Western Nigeria), Tamarinier noir (French) (Ezeja et al., 2011; Ogu and Amiebenomo, 2012). All the parts of this plant have been reported to possess medicinal value. Among some women in south-eastern Nigeria, the ripe fruits are chewed to improve lactation and check genital infection (Nwosu, 2000; Ogu et al., 2012; Ajoboye et al., 2015). The twig or bark is chewed as remedy for dental and stomach ache among the Esan tribe of Edo State in Nigeria (Idu et al., 2009; Ogu et al., 2012). The leaves, stem and root bark are used as remedies for diarrhoea, severe cough, bronchitis, wound, stomach aches, toothache malaria fever, jaundice, ulcer and haemorrhoids (Amponsah, 2002; Bero et al., 2009; Ogu et al., 2012). Scientific study revealed that the fruits, leaves and stem extract posses anticancer (Balogun et al., 2013), molluscicidal activity (Odukoya et al., 1996) antiplasmodial properties (Adumanya et al., 2013), antimicrobial activities (Orji et al., (2012; Ogu et al., 2013; Ajoboye et al., 2015), analgesic and anti-vibrio properties (Akinpelu et al., 2011). The phytochemical components identified in the leaf and stem bark extracts were tannins, phlobitaninns, alkaloids, flavonoids, saponins, steroids, terpenoids, resins, steroids/triterpenes and cardiac glycosides (Ogu et al., 2012). In addition, flavonoids, alkaloids, tannin, saponins, oxalates, glycosides and essential oils were reported in the fruit pulp (Ajoboye et al., 2015; Moronkola et al., 2017).

Despite the fact that the diverse traditionally acclaimed medicinal potentials of *D. guineense* leaf and stem bark extracts had been scientifically validated by previous researchers, there is however, paucity of scientific information on the phytochemical contents and antibacterial efficacy of its root bark extract. The current study was therefore undertaken to investigate the phytochemical and antibacterial efficacy of the ethanol root extract so as to augment the available substantiated reports on the acclaimed ethno-medicinal potential of this plant in southern Nigeria.

MATERIALS AND METHODS

Source of test micro-organisms

The pure clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, were collected from Lahor Research Diagnostic and Environmental Consortium (LRDEC) in Benin City, Nigeria. They were maintained on Luria Bertani (LB) medium (1% Tryptone, 1% sodium chloride, 0.5% yeast extract) until used for the study.

Collection, preparation and extraction of plant material

Fresh roots of *D. guineense* (Figure 1) were collected manually from the forest along Amai-Obiaruku in September, 2015. The roots were thoroughly washed, chopped into bits, air-dried

to crispiness on the laboratory workbench for two weeks. The dried materials were reduced to coarse form using pestle and mortar and further pulverized to very fine particles with an electric blender (Super Search Model 2815). 100 g of the powdered root was subjected to soxhlet extraction and exhaustively extracted with 500 mL of ethanol for about 48 h. The extracts were filtered and concentrated under reduced pressure using rotary evaporator to a slurry sticky mass with yield of 3.4 % (w/w). The required dose of the extract was reconstituted appropriately before the assay.

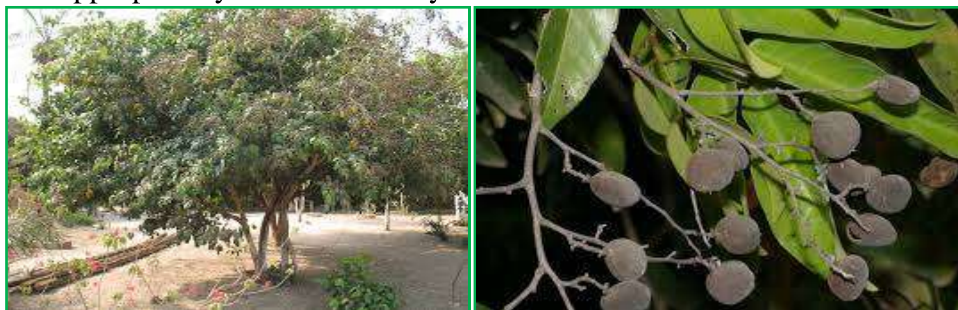

Figure 1: *D. guineense* (Wild) plant

Sterility test of the root extract

The extract was tested for growth or contaminants. This was carried out by inoculating 1mL on sterile Mueller Hinton Agar (Hi-Media, India) and incubated at 37 ^{o}C for 24h. The plates were observed for growth. Absence of growth in the extracts after incubation for 24 h indicated sterility. The sterile extract was then assessed for antimicrobial activity.

Antimicrobial susceptibility testing

The agar well diffusion method of Ogu et al. (2013) was adopted for this assay. Mueller Hinton Broth (Hi-Media, India) was prepared as specified by the manufacturer, autoclaved and poured aseptically into sterile Petri dishes and allowed to gel. Then, a loopful of the previously standardized bacterial cell suspension (10^6 cfu/mL) was streaked evenly on each gelled agar plate. The crude root extract was reconstituted in 20 % Dimethyl Sulfoxide (DMSO) to obtain the working concentration of 10 mg/mL. 100µL of each extract was inoculated into three wells (6 mm diameters) earlier bored with a sterile cork borer in each plate. The negative control was 100µL of 20 % DMSO, while the positive control contained 100 µL of ciprofloxacin (0.002 mg/mL (Ranbaxy Pharmaceuticals India). The plates were allowed to stand for 30 min on the work bench for pre-diffusion of the extracts to proceed before the growth of the organism commenced. The plates were incubated at 37 °C for 24 h. The whole experiment was carried out in triplicate and the antibacterial activity of the extract was determined after incubation period by measurement of mean zones of inhibition diameters produced by the extract against the test bacteria and results were recorded in millimetres (mm) using a transparent ruler.

Determination of the minimum inhibitory concentration (MIC)

The plant extracts that demonstrated significant antibacterial activity by the agar well diffusion method were subjected to MIC assay using the broth dilution method of Ogu et al. (2013). One ml of 24 h culture of test organisms, previously adjusted to a turbidity standard of 1.0 x 10^6 cfu/mL, was incubated in serial dilutions of 0.625, 1.25, 2.5, 5 and 10 mg/mL of plant extracts in physiological saline at 37°C for 24 h. The concentrations at which the lowest dilution had no detectable bacterial growth were considered as minimum inhibitory concentration (MIC).

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was determined by sub-culturing 10 µL from each MIC tube which did not display growth post 24 h incubation onto fresh sterile MHA plates. They were then incubated for 48 h and thereafter examined for colony counts. The lowest concentration where there was greater than or equal to 99.9 % cell death (no detectable cell growth) when compared with the initial viable counts, was interpreted as the MBC (Ogu et al., 2013; Igbinosa and Idemudia, 2016).

Determination of mode of action (Bactericidal or Bacteriostatic)

The mode of action of any antibacterial agent can be bactericidal or bacteriostatic. To determine this effect, the ratio of MBC/MIC of the extract was calculated. Thereafter, the effect was interpreted as bactericidal if the value obtained from the ratio of MBC/MIC was less than or equal to 4, and bacteriostatic if otherwise (Keepers et al., 2014).

Determination of time-kill assay

The time-kill assay of the MIC and 2 x MIC (twice MIC) was determined by adopting the techniques described by Igbinosa and Idemudia (2016). Briefly, the test bacteria were first cultured overnight at 37 ^{o}C before standardizing the turbidity to about 5.0 x 10^5 cfu/mL using MHB. Two different concentrations of the plant extracts, which correspond to MIC and two times the MIC (2 x MIC) were then prepared. From each bacterial suspension, 0.5 mL of the cell density was withdrawn and dispensed into sterile bottles containing 4.5 mL each of the MIC and 2 x MIC and incubated at 37 ^{o}C for 24 h. Aliquots of 0.5 mL of the medium were taken at time intervals of 0, 1, 3, 6, 12 and 24 h, serially diluted in recovery medium (containing 4.5 mL of MHB supplemented with 3 % Tween 80 to neutralize the effects of the antimicrobial agent on the test bacteria) before culturing 100 µL of the dilutions on fresh MHA at 37 ^{o}C for 24 h. Control set up, devoid of the extracts, was performed in triplicates along with the test experiments. The resulting colony forming units (cfu) were determined and converted to $\log_{10}$ cfu before plotting them against the time (h). The Time-kill end point was interpreted as bacteriostatic activity if a mean reduction of 0 to 3 Log_{10} cfu/mL occurs and bactericidal activity if a mean reduction of ≥ 3 Log_{10} cfu/mL when compared with the controls.

Phytochemical Analysis

The phytochemical content of the extract was analysed for the presence of alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids phenols and steroids using standard methods described earlier.

Determination of alkaloids

A 2.5 g measure of the root powder was extracted using 100 mL of 20 % acetic acid in ethanol for a period of 4 h. The contents were filtered and concentrated to about 25 mL. Thereafter, concentrated ammonium chloride was added in stepwise manner for precipitation to occur and settle. The precipitates were collected and washed with dilute ammonium hydroxide and filtered again. The final filtrates were discarded and pellets obtained were dried and weighed using electronic weighing balance Model B-218 (Harborne. 1973).

Determination of total flavonoids

The total flavonoid content was determined using the aluminium chloride colorimetric method (Sofowara, 1993). This method is based on the formation of a complex flavonoid–aluminium, having the absorbance maximum at 430 nm. 0.5 mL of the extract was mixed with 1.5 mL of methanol, 0.1 mL of 10 % $AlCl_3$, 0.1 mL of 1 M potassium acetate and 2.8

mL of distilled water. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm on UV–visible spectrophotometer [Shimadzu UVPC-1650 (Japan)]. Total flavonoid content of the extract was expressed as mg/100 g dry weight of extract through the calibration curve with rutin as standard.

Determination of saponins

A 5.0g weight of the powdered root sample was added to 100 cm^3 of 20 % aqueous ethanol and heated with constant stirring over a water bath (55 ^oC) for about 4 hrs. After filtering the content, the aqueous ethanol extraction was repeated for 4 hrs at 55 ^oC with continuous stirring. The pooled extracts were then evaporated using water bath (90 ^oC) to about 40 cm^3. The partially concentrated extract was placed in a separating funnel before adding 20 cm^3 of diethyl ether, mixed properly, and allowed to settle into layers. The aqueous layer was recovered while the ether layer was discarded before. Further purification was done using 60 cm^3 of n-butanol and 10 cm^3 of 5% sodium chloride. The sodium chloride layer was later discarded before concentrating the residues over water bath to dryness using oven (Jenway) and used to determine the saponins content (Harborne, 1973; Sofowara, 1993; Amadi et al., 2004; Ejikeme et al., 2014).

Determination of glycosides

A 1.0g measure of the root sample was weighed into 200 cm^3 distilled water, allowed to autolyse for 2 h before complete distillation in flask containing 2.5 % sodium hydroxide and tannic acid as an antifoaming agent. The distillates were mixed with 100 cm^3 of cyanogenic glycosides, 8 cm^3 of ammonium hydroxide and 2 cm^3 of potassium iodide, before titrating the content with 0.02 M silver nitrate against a dark background to a constant turbid end point. The cyanogenic contents of the sample were then calculated as described earlier (Amadi et al., 2004; Ejikeme et al., 2014).

Determination of total terpenoids

A 2.0g measure of the powdered root extract was weighed into 50 mL of absolute ethanol and allowed to stand for 24 h. The solution was filtered using Whatman's No 1 filter paper. The filtrate was again extracted using 50 ml of petroleum ether for another 24 h. The content was then concentrated to dryness on a water bath set at 70 ^oC. The concentrated ether extract was estimated as the total terpenoids (Ferguson, 1956).

Determination of steroids

1.0 g of the powdered root sample was weighed into conical flasks containing 20 cm^3 of ethanol and macerated for some hours before filtering using Whatman's No 1 filter paper. Equal volume of the filtrate (2 cm^3) was added to cholesterol colour reagent before taking the absorbance at 559 nm using Spectrum Lab23A spectrophotometer. The steroid content was then estimated from the standard curve (Harborne, 1973; Sofowara, 1993).

Determination of total phenols

The plant samples were initially defatted using ether. The total phenol contents were then extracted by boiling 0.5 g of each defatted sample in flask containing 50 cm^3 of ether reagent. Exactly, 5 cm^3 of the ether extract was added into a conical flask containing a mixture of 10 cm^3 of distilled water, 2 cm^3 of 0.1 N ammonium hydroxide and 5 cm^3 of amyl alcohol. The content was mixed thoroughly for 30 min (Ejikeme et al., 2014). The total phenol contents were then determined by comparing the optical density (505 nm) with the tannic acid standard solutions prepared along with the samples (Harborne, 1973; Ejikeme et al., 2014).

Statistical Analysis

Data were analysed using SPSS version 16.0 and Microsoft Excel 2007 version and expressed as mean ± standard deviation. Comparison between the zones of inhibition diameters was done by analysis of variance (ANOVA) and differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Plants remain one of the major sources of natural bioactive molecules with promising potential for future exploitation as new antimicrobial agents. Several plants have been documented to possess significant antimicrobial properties and were recommended as complementary and or alternatives to chemotherapeutic agents. It was estimated that over 70 % of pharmacologically bioactive molecules of plants origin were discovered from information on their diverse ethno-medicinal applications by native users (Ncube et al., 2008). Hence, the need to continue the search for more botanicals with broad based antimicrobial properties. In this study, the *in vitro* antibacterial efficacy of ethanol extract of *D. guineense* root, commonly known as black velvet, was studied against three clinical isolates (*S. aureus, E. coli* and *K. pneumoniae*) of public health significance. Findings from the *in vitro* assay revealed that 10 mg/mL of the extract, yielded significant inhibitory effect against the three test bacteria. The zone inhibition diameters observed ranged from 15.2 ± 0.25 mm, against *K. pneumoniae* to 20.4 ± 1.02 mm, against *S. aureus* (Table 1). Statistically, the effects of the extracts were significant at p=0.05 when compared to those of the ciprofloxacin (0.002 mg/mL) with ranges of 18.8 - 24.5 mm. The observed results were expected based on the previous reports on the antimicrobial activities of the fruit pulps, leaves and stem barks extracts. For the fruit pulps, it was found that 10 mg/ml of the ethanol extracts demonstrated marked antimicrobial activities against *Klebsiella pneumoniae* (13.9 mm), *Staphylococus aureus* (14.5 mm), *Escherichia coli* (16.1 mm), *Pseudomonas aeruginosa* (13.33 mm), *Proteus mirabilis* (17.0 mm) and *Candida albicans* (24.67 mm) Ajiboye et al., 2015). Also, the methanol extracts of the leaf, at concentrations of 62.5 µg/mL - 250 µg/mL, was reported to inhibit *Staphylococcus aureus* (16.2 - 22.5 mm), *Streptococcus mutans* (17.8 - 25.9 mm), *Escherichia coli* (13.9 - 19.2 mm), *Bacillus cereus* (16.9 - 23.5 mm), *Pseudomonas aeruginosa* (11.0 - 18.9 mm), *Klebsiella pneumoniae* (10.2 - 17.0 mm), *Proteus mirabilis* (10.0 - 16.8 mm), *Salmonella typhi* (12.9 - 18.2 mm), *Candida albicans* (15.0 - 22.4 mm), *Microsporium gypseum* (13.2 - 16.9 mm), *Trichophyton mentagrophytes* (14.9 - 19.9 mm) and *Trichophyton rubrum* (11.0 - 15.8 mm) (Ogu et al., 2013). Similarly, the ethanol extracts of the stem bark (100 mg/mL), which produced inhibition zone diameters of 10.0 - 16.0 mm, was reported against some clinical isolates of bacteria and fungi in Nigeria (Olajubu et al., 2012). Thus, the findings from this study corroborate the earlier claims that the various parts of the studied plant possess medicinal values against human pathogens.

The MIC and MBC of the extract ranged from 0.625 – 1.25 mg/mL and 1.25 - 5 mg/mL respectively (Table 2). The MIC and MBC of *S. aureus* (0.625 – 1.25 mg/mL) were the lowest, followed by that for *E. coli* and *K. pneumoniae* (2.5 - 2.5 mg/mL). It shows that *S. aurues,* a Gram-positive bacterium, was the most sensitive test organism. The relatively lower sensitivity exhibited by *E. coli* and *K. pneumoniae* also suggest that these Gram-negative bacteria were relatively less sensitive than the Gram-positive strains, though not significantly different ($p<0.05$). A possible explanation for this finding could be attributed to the differences in cell wall composition of these groups of bacteria. Gram-negative bacteria possess an outer membrane consisting of lipoprotein and lipopolysaccharide, which is selectively permeable and thus regulates access to the underlying structures, and consequently renders them less susceptible to plant extracts than the Gram-positive bacteria (Pelczar et al., 1993; Chopra and Greenwood, 2001; Chan et al., 2007; Ogu et al., 2013). This

finding is however in disagreement with the report of Olajuyigbe and Afolayan (2012), where Gram-negative bacteria were more susceptible.

The mechanism of antibiosis (or MIC index) of the extract was found to be 2 for the three test bacteria (Table 2). According to previous report (Keepers et al., 2014), antimicrobials are usually considered bactericidal if the MBC/MIC or MFC/MIC ratio is ≤ 4, and bacteriostatic if >4. This suggests that the root extracts displayed significant bactericidal activities against the three test bacteria, and thus will be a potential antimicrobial agent against these pathogens. Similar findings were reported earlier (Olajuyigbe and Afolayan, 2012; Idris et al., 2013), and thus, in concordance with our report, but in disagreement with the reports of Appiah et al. (2017).

Table 1: *In vitro* antibacterial effects of *D. guineense* ethanolic root extract and reference drug

	Inhibition zone diameter (mm ± SD)*		
Bacterial isolates	Ethanol extracts (50 mg/mL)	Ciprofloxacin (0.002 mg/mL)	*p*-value
S. aureus	20.4 ± 1.02	23.0 ± 1.15	0.05
E. coli	18.5 ± 0.14	24.5 ± 0.05	0.05
K. pneumoniae	15.2 ± 0.25	18.8 ± 0.01	0.05

*Data are mean ± standard deviation (SD) (n=3), differences in mean values were considered significant at p<0.05 with reference to reference drugs

Table 2: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) and action of *D. guineense* root extract and reference drug

Bacterial isolates	Ciprofloxacin	Ethanol extracts (mg/mL)		
	MIC (mg/mL)	MIC	MBC	MBC/MIC*
S. aureus	0.032	0.625	1.25	2
E. coli	0.064	2.5	5	2
K. pneumonia	0.064	2.5	5	2

* Interpretation; Bactericidal ≤ 4; Bacteriostatic > 4

Furthermore, the bacterial time-kill kinetics was studied to understand the pharmacodynamics of each bacterium on exposure to the fixed MICs of extract. Generally, the standard measurement for bactericidal activity of any antimicrobial agent is a mean reduction of viable bacterial density by ≥ 99.9 % or ≥ 3 $\log_{10}$ cfu/mL. Results from the time-kill activity revealed that the 2 x MIC and MIC achieved bactericidal activities (≥ 3 $\log_{10}$) on all the isolates after 6 and 12 h interactions respectively (Figures 2 and 3). Each of the test bacterial population (viable counts) was almost completely eliminated after 24 h of incubation. This is an indication that the antibacterial efficacy of the extracts is dependent on the concentration and duration of exposure. The bactericidal effects achieved in this study at MIC and 2 x MIC within 6 h interaction were comparable to those reported for methanol leaf extract of *Lawsonia inermis* against *Bacillus subtilis* (NCIB 3610), *Enterococcus faecalis* (NCIB 775), *Micrococcus luteus* (NCIB 196), *Bacillus stearothermophilus* (NCIB 8222), *Bacillus cereus* (NCIB 6344), *Staphylococcus aureus* (NCIB 8588), *Bacillus anthracis* (LIO), *Streptococcus pneumoniae* (LIO), *Pseudomonas flourescens* (LIO), *Bacillus subtilis* (NCIB 3610) and *Trueperella pyogenes* (LIO), (*Escherichia coli* (NCIB 86), *Pseudomonas aeruginosa* (NCIB 950), *Klebsiella pneumoniae* (NCIB 418), *Proteus vulgaris* (LIO) and *Shigella* sp. (LIO)

(Olasunkanmi et al., 2017), acetone and aqueous leaf extracts of *Ocimum gratissimum* (Linn) against *Vibrio* species (Igbinosa and Idemudia, 2016); *Phyllanthus amarus* and *Diodia scandens* ethanol leaf extracts on *Staphylococcus species* (Ojo et al., 2013); organic and aqueous leaf extracts *Lawsonia inermis* against *Escherichia coli, Salmonella typhi, Klebsiella spp., Shigella sonnei, Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus epidermidis* (Gull et al., 2013), and methanol stem bark extract of *Acacia mearnsii* De Wild against bacteria in Shigellosis (Olajuyigbe and Afolayan, 2012) within 6-12 h interactions. Considering the efficacy of this extract in relation to other plant extracts, it therefore suggests that ethanol extracts of *D. guineense* roots will be a potential source of bioactive molecules for antibacterial chemotherapy against infections due to the test pathogens. *Staphylococcus aureus* and *Escherichia coli* are currently of public health concern and usually the leading pathogens commonly implicated in nosocomial infections (Odigie et al., 2017). They are responsible for various superficial, enteric and systemic infections in humans, which includes skin and soft tissues infections, surgical sites infections, bone and joint infections, urinary and respiratory infections, bacteremia, nosocomial pneumonia, cholecystitis, cholangitis, peritonitis, cellulitis, osteomyelitis, and infectious arthritis (Raho and Abouni, 2015). *K. pneumoniae* has been noted in recent times as an opportunistic pathogen of public health importance considering its roles in some life-threatening infections, such pneumonia, bacteremia, meningitis in immunocompromised individuals (Paczosa and Mecsas, 2016).

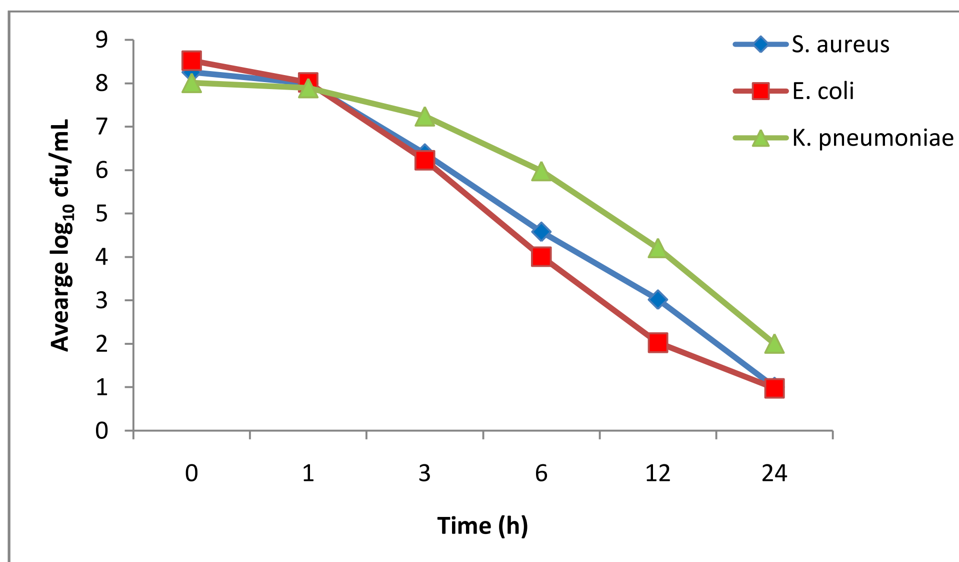

Figure 2: Average log_{10} reduction in viable count of test bacteria at MIC of extract

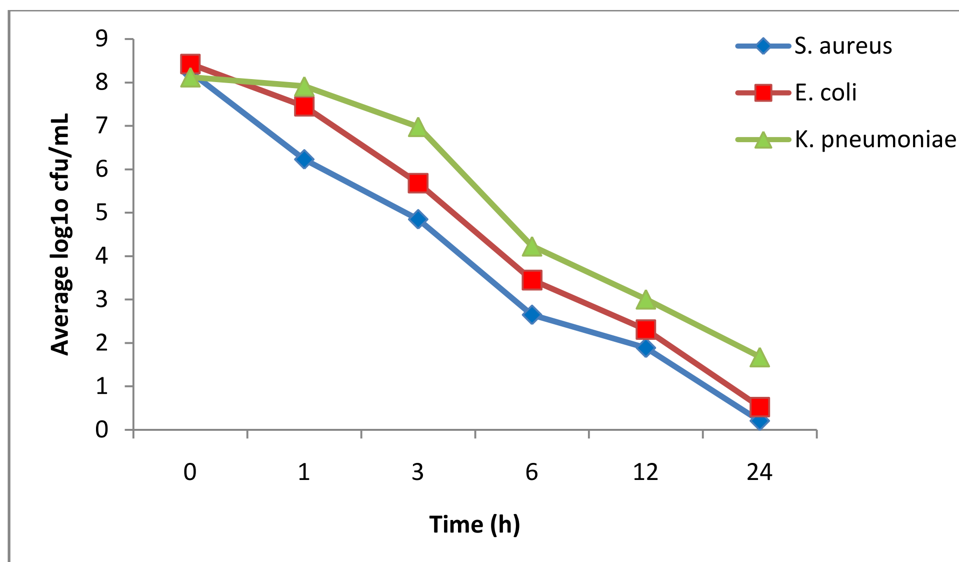

Figure 3: Average $\log_{10}$ reduction in viable count of test bacteria at 2 x MIC of extract

Analysis of the phytochemical contents of the root extract confirmed the presence of saponins, steroids, phenols, tannins, flavonoids, terpenoids, alkaloids, and glycosides at concentrations that ranged between 1.3 - 6.2 mg/100g (Figure 4). The phytochemicals that were relatively prominent in the extract included alkaloids (6.2 ± 0.09 mg/100g), phenols (4.6 ± 0.29 mg/100g), tannins (4.3 ± 0.50 mg/100g) and saponins (4.1 ± 0.41 mg/100g). Previous studies have reported that antimicrobial properties of plants depended on the amount and type of phytochemicals present in the extracts (Ehiobu and Ogu, 2018). Saponins have been reported to possess significant antimicrobial activities (Khanna and Kannabiran, 2008). Flavoniods and tannins extracts have also been reported to possess antioxidant and antimicrobial properties (Wafa et al., 2016). Apart from their antioxidants properties, phenolic compounds, glycosides and steroids have also been documented in most medicinal plants as active antimicrobial compounds against diverse pathogens of plants and animals (Iqbal et al., 2015). Alkaloids present in most stem barks, leaves and roots of plants have long been reported to possess potent antimicrobial properties (Cordell et al., 2001). Hence, the outstanding antibacterial efficacy demonstrated by the root extract of *D. guineense* in this study could be linked to the relatively high concentrations of saponins, tannins and alkaloids, and phenols, among others.

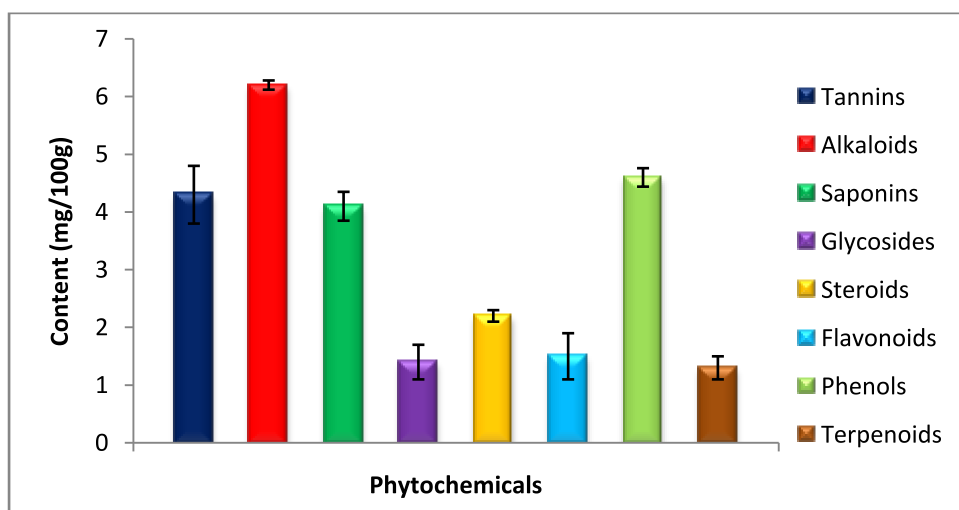

Figure 4: Phytochemical contents of ethanol extract of *D. guineense* root

Conclusion

This study has shown that the ethanol extract of *D. guineense* root possesses broad spectrum antibacterial properties against *S. aureus, E. coli* and *K. pneumoniae* and thus, provides additional scientific justification for its ethno-medicinal applications. The bactericidal activity of the extract, confirmed by $\geq 3 \log_{10}$ reduction of the bacterial populations, was found to be within 6 h of incubation, though dependent on minimum inhibitory concentrations and interaction periods. This *in vitro* bactericidal action could be linked to the presence of alkaloids, phenols, tannins, and saponins that were relatively high in the extract. Considering the degrees of the *in vitro* antibacterial activities of this extract, it is pertinent to suggest that the detected bioactive molecules could be beneficial in developing new potent antimicrobial agents against these pathogens, which are currently becoming resistant to available chemotherapeutic agents. However, further study is necessary to investigate their toxicities and *in vivo* antibacterial kinetics with a view to establishing their safety.

Reference

Ajiboye, A. E., Ameen, Tukur, M. and Adedayo, M. R. (2015). Antimicrobial activity and phytochemical screening of the fruit pulp of *Dialium guineense* (Velvet Tamarind) on some microbial isolates. *J. Microbiol. Antimcrob*. **7** (4): 33-41

Amadi, B. A., Agomuo, E. N., Ibegbulem, C.O. (2004). Research Methods in Biochemistry. Supreme Publishers, Owerri, pp. 90-115.

Amponsah, K., Crensil, O, R., odamtten, G. T. and Ofusohene-Djan, W. (2002). Manual for the propagation and cultivation of medicinal plants in Ghana. Aburi Botanic Garden; Ghana

Appiah, T., Boakye, Y. D. and Agyare, C. (2017). Antimicrobial activities and time-kill kinetics of extracts of selected Ghanaian mushrooms. *Evidence-Based Complemen. Alternat. Med.* 2017, e 4534350, 15 pp. https://doi.org/10.1155/2017/4534350.

Balogun, M. E., Oji, J. O., Besong, E. E. and Umahi, G. O. (2013). Evaluation of the anti-ulcer properties of aqueous leaf extracts of *Dialium guineense* (velvet tamarind) on experimentally induced ulcer models in rats. *Int. J. Dev. Res.* **3** (10): 106-110

Bero, J., Ganfon, H., Jonville, M. C., Frederich, M., Gbaguidi, F., De, M. P., Moudachirou, M, Quetin, L. J. (2009). *In vitro* antiplasmodial activity of plants used in Benin in Traditional medicine to treat malaria. *J. Ethnopharmacol*. **122**: 439-444.

Chan, E. W. C., Lim, Y. and Omar, M. (2007). Antioxidant and Antibacterial Activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular, Malaysia. *Food Chem*. **104** (4):1586- 1593.

Chopra, I. and Greenwood, D. (2001). Antibacterial Agents: Basis of Action, In: Encyclopedia of Life Sciences, Nature Publishing Group, John Wiley and Sons, Limited.

Cordell, G. A., Quinn-Beattie, M. L. and Farnsworth, N. R. (2001). The potential of alkaloids in drug discovery. *Phytother. Res.* **15**: 183-205.

Ehiobu, J. M. and **Ogu, G. I.** (2018). Phytochemical content and *in vitro* antimycelialefficacy of *Colocasia esculenta* (L), *Manihot esculenta (*Crantz*)* and *Dioscorea rotundata* (Poir) Leaf Extracts on *Aspergillus niger* and *Botryodiplodia theobromae. J.Horticult. Plant Res.* ***1****: 9-18.*

Ejikeme, C. M., Ezeonu, C. S., Eboatu, A. N. (2014). Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. *Europ. Sci. J.* **10** (18): 247–270.

Ezeja, M. I, Omeh, Y. S., Ezeigbo, I. I. and Ekechukwu, A. J. (2011). Evaluation of the analgesic activity of the methanolic stem bark extract of *Dialium guineense* (Wild). *Ann. Med. Health Sci. Res.***1** (1): 55-62.

Ferguson, N. M. (1956). *A Text Book of Pharmacognosy.* Mac Milan Company, New Delhi, p. 191.

Gull, I., Sohail, M., Aslam, M. S. and Athar, M. A. (2013). Phytochemical, toxicological and antimicrobial evaluation of *Lawsonia inermis* extracts against clinical isolates of pathogenic bacteria. *Ann. Clin. Microbiol. Antimicrob*. **12**: 36.

Harborne, J. B. (1973). Phytochemical Methods. A guide to modern techniques of plant analysis. London: Chapman and Hall, New York, pp. 6-466.

Hutchinson, J. and Daniel, J. M. (1958). Flora of West Tropical Africa: Vol. 1 Part 2, Crown Agent London, p. 444-728.

Idris, M. A., Muyibi, S. A., Karim, M. I. A. Jamal, P. and Jami, M. S. (2013). In-Vitro Antibacterial Activity of Crude Defatted *Moringa Oleifera* Seed Extract: Kill time study. *Austr. J. Basic Appl. Sci.* **7** (6): 149-153.

Idu, M., Umweni, A. A., Odaro, T. and Ojelede, L. (2009). Ethnobotanical plants used for oral health care among the Esan tribe of Edo State, Nigeria. *Ethnobot. Leafl.*1**3**: 548-563.

Igbinosa, E. O. and Idemudia, O. G. (2016). Anti-vibrio potentials of acetone and aqueous leaf extracts of *Ocimum gratissimum* (Linn). *Trop. J. Pharm. Res*. **15** (4): 743-750

Iqbal, E., Salim, K. A. and Lim, L.B. L. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam, *J. King Saud Univ. Sci.* **27**: 224–232.

Keepers, T. R., Gomez, M., Celeri, C., Nichols, W. W. and Krause, K. M. (2014). Bactericidal activity, absence of serumeffect, and timekill kinetics of ceftazidime-avibactam against β-lactamaseproducing enterobacteriaceae and *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemotherap.* **58** (9): 5297-5305.

Khanna, V. G. and Kannabiran, K. (2008). Antimicrobial activity of saponin fraction from root of *Hemidesmus indicus. Res. J. Med. Plant.* **2** (1): 39 - 42.

Moronkola, D. O. Kunle, O. F., Olaoluwa, O. and Ogukwe, C. (2017). Chemical Compositions of *Dialium guineense* Willd. leaf, stem-bark and fruit essential oils. *J. Complem. Alternat. Med. Res.* **3** (4): 1-8.

Ncube, N. S., Afolayan, A. J. and Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *Afr. J. Biotechnol*. **7** (12): 1797-806.

Nwosu, M. O. (2000). Plant resources used by traditional women as herbal medicine and cosmetics in Southwest Nigeria. *Arzte. fur Natur. Fahr.* **41**: 11.

Odigie, A. B., Ekhiase, F. O., Orjiakor, P. I. and Omozuwa, S. (2017). The Role of Door handles in the spread of microorganisms of public health consequences in

University of Benin Teaching Hospital (UBTH), Benin City, Edo State. *Pharm. Sci. Technol.* **2** (2): 15-21.

Odukoya, O. A., Houghton, P. J., Adelusi, A., Omogbai, E. K. I., Sanderson, L. and Whitfield, P. J. (1996). Molluscicidal triterpenoid glycosides of *Dialium guineense*. *J. Nat. Prod.* **59**: 632-634.

Ogu, G. I. and Amiebenomo, A. R. (2012). Phytochemical analysis and in vivo anti-diarrhoeal potentials of *Dialium guineense* (Wild) stem bark extract. *J. Intercult. Ethnopharmacol.* **1** (2): 105-110.

Ogu, G. I., Joachim, E. and Ehiobu, J. M. (2013). Antioxidant and antimicrobial activities of *Dialium guineense* (Wild) leaf extract. *Pharm. Pharmacol. Res.* **1** (1): 1-7.

Ojo, S. K. S., Ejims-Enukwe, O., Esumeh, F. I. (2013). In-vitro antibacterial time-kill assay of *Phyllanthus amarus* and *Diodia scandens* crude extracts on *Staphylococci* isolated from wounds and burns patients. *Int. J. Pharm. Sci. Inven.* **2** (8): 9-13.

Olajuyigbe, O. O. and Afolayan, A. J. (2012). *In vitro* antibacterial and time-kill evaluation of the *Erythrina caffra* Thunb. extract against bacteria associated with diarrhoea. *The Sci. World J.* 2012: Article ID 738314, 8pp.

Olajuyigbe, O. O. and Afolayan, A. J. (2012). *In vitro* antibacterial and time-kill assessment of crude methanolic stem bark extract of *Acacia mearnsii* De Wild against bacteria in shigellosis. *Mol.* **17**: 2103-2118.

Olasunkanmi, A., Olasunkanmi, O. O. and Akanbi, O. E. (2017). Studies on the antibacterial potential and time-kill assays of methanol leaf extract of *Lawsonia inermis* (Linn). *Asian J. Res. Med Pharm. Sci.* **1** (2): 1-9.

Orji, J. O., Alo, M. N., Anyim, C. and Okonkwo, E. C. (2012). Antibacterial activities of crude leaf and bark extracts of “Icheku” *Dialium guineense* on bacterial isolates from bronchitis patients. *J. Pharm. Biol. Sci.* **1**: 21-25.

Olajubu, F. A., Akpan, I., Ojo, D. A., Oluwalana, S. A.. (2012). Antimicrobial potential of *Dialium guineense* (Wild.) stem bark on some clinical isolates in Nigeria. *Int. J. Appl. Basic Med. Res.* **2** (1): 58-62.

Raho, G. B. and Abouni, B. (2015). *Escherichia coli* and *Staphylococcus aureus* most common source of infection. In: The Battle Against Microbial Pathogens: Basic Science, Technological Advances And Educational Programs. A. Mendez-Vilas Ed. FORMATEX microbiology series No. 5, Vol. 1. Pp. 637-648.

Paczosa, M. K. and Mecsas, J. (2016). *Klebsiella pneumoniae*: Going on the offense with a strong defense. *Microbiol. Mol. Bio. Rev.* **80** (3): 629-661.

Pelczar, M. J., Chan, E. C. S. and Kries, N. R. (1993). *Microbiology*, 5th Ed, Mac-Graw Hill Inc. pp. 221-224.

Sofowara, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books, Ibadan, Nigeria, pp. 1-20.

Wafa, N., Sofiane, G. and Mouhamed, K. (2016). The antioxidant and antimicrobial activities of flavonoids and tannins extracted from *Phlomis bovei* De Noé, *Europ. J. Exp. Bio*. **6** (3): 55-61.