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Antimicrobial activity and phytochemical screening of the fruit pulp of *Dialium guineense* (Velvet Tamarind) on some microbial isolates

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The antimicrobial activities of the aqueous, ethanol and n-hexane fruit pulp extracts of *Dialium guineense* were evaluated against clinical isolates of *Klebsiella pneumonia, Staphylococus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Candida albicans* using the agar well diffusion technique. The results reveal that the crude aqueous and ethanol extracts possess antimicrobial activities on the tested organisms with the exception of n-hexane extract which showed no zone of inhibition. The highest zone of inhibition diameter at 24.67 mm of the ethanol extract and 19.33 mm of the aqueous extract was recorded against *C. albicans* while *S. aureus* showed the lowest inhibition zone to the aqueous extract with 7.33 mm in diameter. However, statistical analysis indicates no significance as P>0.05. The minimum inhibitory concentration (MIC) of the aqueous and ethanol extracts to the isolates was between 100 - 200 mg/ml with only *C. albicans* at 50 mg/ml of the ethanol extract. Also, the minimum lethal concentration (MLC) of the aqueous and ethanol extracts on majority of the organisms was above 200 mg/ml but *P. aeruginosa* and *P. mirabilis* showed MLC at 200 mg/ml and, *C. albicans* at 100 mg/ml of the ethanol extract. Meanwhile, only *C. albicans* showed MLC to the aqueous extract at 100 mg/ml. In addition, the phytochemical screening revealed the presence of flavonoids, alkaloids, tannin, saponins, oxalates and glycosides. The results of this work suggest further exploitation of the fruit pulp of *D. guineense* to possibly unveil its potential use for the treatment of diseases.

Key words: *Dialium guineense*, antimicrobial activity, phytochemical screening, microbial isolates, fruit pulp.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compounds as antimicrobial agent. Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk medicines,

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pharmaceuticals, intermediate and chemical entitled for synthetic drugs (Hammer et al., 1999). It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno-medicinal use of the plants (Ncube et al., 2008). Also some plant parts have been used as antimicrobial agents, especially their extract as decoctions, infusions, or oral administration (Okemo et al., 2001). Importantly, plants have been known to exhibit medicinal properties on the internal organs of animals. If the toxic effect after administration is low, there is a possible chance of introduction of such drugs for therapeutic purpose (Ibeh, 1998). Plants have limitless ability to synthesize aromatic secondary metabolites, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. These groups of compounds show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganisms**.** Plants that contain substances which can be used for therapeutic purposes or which can be used as precursors for the synthesis of useful drugs is a medicinal plant (WHO, 1997; Sofowora, 1982). In spite of the millions of chemical structures currently available for screening for therapeutic value, natural products particularly of plant origin remain the most important source of new drugs (Odugbemi and Akinsulire, 2006). However, Scientists from divergent fields are investigating plants with a new age for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agent with general as well as specific activity (Nair and Chanda, 2007). Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects (Mukherjee and Wahile, 2006) and have an enormous therapeutic potential to treat many infectious diseases. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs (Iqbal and Arina, 2001).

Moreover, infectious disease is the number one among all causes of death, accounting approximately one-thirdly all deaths throughout the world. About 50-75% of hospital deaths are reported due to infections disease. These numbers are still increasing due to development of resistance in microorganisms to the existing first line drug (Akinpelu et al., 2008). *Staphylococcus aureus* is a bacterium found primarily on the skin and in the nose of humans. *S. aureus* is an important human pathogen which causes a range of diseases ranging from minor issues such as minor skin infections to severe toxin mediated diseases (Online Textbook of Bacteriology, 2008). One particular strain responsible for the increasing number of in-hospital infections is Methicillin Resistant *S. aureus* (MRSA), which has evolved multidrug resistance to strong antibiotics such as oxacillin, penicillin and amoxicillin (CDCP, 2006). *Klebsiella* spp*, Escherichia coli, Vibrio cholera, Shigella* spp*., Salmonella* spp*., Staphylococcus aureus* as well as *Pseudomonas* spp are primarily responsible for gastroenteritis and diarrhea infections (Thangjam et al., 2011). *Proteus* species have been reported to cause a series of clinical diseases. Also, the yeast *Candida albicans* which belongs to the class of fungi Ascomycetes and the family, Saccharomycetaceae is responsible for fungal diseases of mammals, mycoses, range from the common mild cutaneous or subcutaneous skin infections, such as athletes foot, to the potentially lethal acute or chronic infection of deep tissues as well as vaginal and urinary tract infections of females.

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc, that is, any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Parekh et al., 2006). Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors had reported about plant extract preparation from the fresh plant tissues. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. Although, as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Other researchers dry the plants in the oven at about 40°C for 72 h.

Dialium guineense is a tree (Figure 1) of an average height of 30 m with densely leafy crown, smooth greyish bark. Leaves are hairy and the flowers are usually whitish while the fruits are less circular and flattened. The pulp of the fruit is edible and sweet, fairly low levels of ascorbic acid and tannin are present. It is a fairly good source of protein, minerals and reported to possess antimicrobial activities in the cure of diarrhea, palpitations as well as fever (Arogba et al., 2006). Okwu and Ekeke (2003) reported in their studies that the plant contains saponins which are presumed to add to the cleaning effect of teeth and at the same time prevent caries and plaques on the teeth of the users. *D. guineense* leaves and stem bark are used as folklore remedies for the treatment of infections such as diarrhoea, severe cough, bronchitis, wound, stomachaches, malaria fever, jaundice, antiulcer

Figure 1. *D. guineense* fruits on the tree.

Figure 2. Plugged fruits of *D. guineense*

and haemorrhoids (Bero et al., 2009). Also, the molluscicidal activity of the fruits and leaves of D*. guineense* have been reported by Gideon and Ralphael, 2012. *D. guineense* can also be found in West African countries such as Ghana where it is known as Yoyi, Sierra Leone, Senegal, and Nigeria where it is known as "Awin" in Yoruba, "Icheku" in Igbo and "Tsamiyar kurm" in Hausa (Nwosu, 2000; Akinpelu, 2011). The fruit of *D*. *guineense* has been reported by Aline et al., 2008 to contain significant amount of phenolics and flavonoids. Also, the bark and leaves of the plant have medicinal properties and are used against several diseases. The fruits of the *D. guineense* are chewed among some women in southeast Nigeria to improve lactation and check genital infections (Nwosu, 2000). *D. guineense* leaf and bark extracts have been reported by Orji et al., (2012) to show antimicrobial properties against *Staphylococcus aureus* and *K. pneumoniae* at varying concentrations. The antibacterial activities of both the aqueous and ethanolic leaf and bark extracts of *D. guineense* were evaluated while the phytochemical analysis reveals the presence of flavonoids, alkaloids, tannin and saponin. Also, the methanolic crude leaf extract of *D. guineense* was found by Akinpelu et al., (2011) to possess bioactivity against fourteen out of eighteen environmental strains of *Vibrio* species. Phytochemical analysis of the plant extract revealed some phenolic compounds. These phenolic compounds include phenolic acids, flavonoids, tannins, saponins and cardiac glycosides among others. Phenolic compounds from medicinal herbs and dietary plants play important roles in health in addition to enhancing antimicrobial activities in these plants. Among the 85 medicinal plants investigated for their potency as anti-malaria, *D. guineense* was also found to inhibit the growth of *Plasmodium falciparum,* that is, the malaria parasite responsible for the illness (Hermans et al., 2010). It was reported in some literatures that *D. guineense* leaves and stem bark are used as folklore remedies for the treatment of infections such as diarrhoea, severe cough, bronchitis, wound, stomachaches, malaria fever, jaundice, antiulcer and haemorrhoids (Bero et al., 2009). Lawal et al. (2010) reported in their findings that *D. guineense* is used as antiulcer and as a vitamin supplement among some tribes in the southern part of Nigeria. *D. guineense* fruit pulp is edible and sweet, fairly low levels of ascorbic acid and tannin are present. It is a fairly good source of protein and minerals (Arogba et al., 2006). The fruits of the plant are chewed among some women in southeast Nigeria to improve lactation and check genital infection (Nwosu, 2000). *D. guineense* stem is used as chewing stick (indigenous tooth brush) among the Nigerian populace. Okwu and Ekeke (2003) reported in their studies that the plant contains saponin which is presumed to add to the cleaning effect of teeth and at the same time prevent caries and plaques on the teeth of the users. Significant antioxidant and molluscidal activities of *D*. *guineense* exhibited have also been reported (Lamien et al., 2008). The edible part (pulp) of ripe *D. guineense* fruits (Figure 2) have been reported (Arogba et al., 2006) as sweet but acidic and relatively poor in protein and oil with fairly low levels of ascorbic acid and tannin. The seed, however, is mildly acidic, poor in oil but a fairly good source of protein and minerals. Also, the molluscicidal activity of the fruit and leaves of *D. guineense* was found to be due to oleanolic acid saponins, three of which were isolated from the fruit and

a fourth from the leaves. *D. guineense* has been used traditionally as remedy for gastrointestinal disorder in South-Western Nigeria. Gideon and Ralphael (2012) reported the effect of the methonolic extract of *D. guineense* at oral doses on the castor oil-induced diarrhoea, gastrointestinal motility (charcoal meal) and castor oil-induced intestinal fluid accumulation (enteropooling) in rats to show a significant reduction in the disease levels. This study was aimed at determining the antimicrobial efficacy of the aqueous and ethanolic and n-hexane extracts of *D. guineense* as well as qualitative phytochemical screening for the bioactive components in each of the extracts.

MATERIALS AND METHODS

Fruit pulp collection and processing

The fruits of *D. guinense* were plugged from the premises of the Ministry of Works and Transport, Kwara State, Ilorin on the 28th of February, 2013. The fruit were identified as UIH1064 at the Herbarium section of the Department of Plant Biology, University of Ilorin, Kwara State. The fruits were carefully de-capped and seeds were removed. The fruit pulps were dried at 60°C for two hours to gain a constant weight, grinded to powder using a mechanical grinder and stored in an enclosed sterile glass container.

Collection of Microbial Isolates

The test organisms which include *S. aureus, E. coli, K. pneumonia*, *P. aeruginosa* and *P. mirabilis* and *C. albicans* were obtained from stock cultures of the Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria, kept and labelled as pure isolates on nutrient agar slants (for bacteria) and yeast extract agar slant (for the yeast) respectively in McCartney bottles and taken for refrigeration at 4°C before use. However, further sub-culturing was done to keep the organisms viable. After then and before the antimicrobial sensitivity assay, these isolates were sub-cultured into Nutrient broth at 37°C (*C. albicans* in PDA broth) overnight to ensure that the organisms were at their exponential phase of growth before carrying out the sensitivity analysis (Abah and Egwari, 2011).

Preparation of extracts

The extraction was done using cold water, ethanol and n-hexane in the ratio 1 to 5 fruit pulp powder to solvent. 100 g of the powdered fruit pulp each was used with each solvent. Using Soxhelt apparatus with 500ml of 75% ethanol and n-hexane respectively, the extractions were done at boiling temperatures of both solvents separately for 8 hours (Adegoke et al., 2010; Lin et al., 1999; Orji et al., 2012). The filtrates were separately concentrated at 40°C by distillation (Orji et al., 2012). The semisolid concentrations of the extracts were collected in sterile pre-weighed screw capped bottles and labeled accordingly (Ogunjobi et al., 2007). The extracts were stored at refrigeration temperature until when needed. 100 g of the powder fruit pulp was dissolved in 500mls of distilled sterile water and rotated in an orbit shaker intermittently for 48 hours at 100 rpm. The mixture was filtered using Whatman No.1 filter paper (Hena et al., 2010). The filtrate was concentrated in water bath at 40°C while the semi-solid extract produced was kept in a sterile universal bottle, labeled and kept for storage at refrigeration temperature.

Sterility test for the extracts

Each of the extract was checked for sterility on Mueller-Hinton agar by streaking method after sterilization at pasteurization temperature for 30 min.

Antimicrobial sensitivity assay

Agar well diffusion technique as described by (Cheesbrough, 2006) was used to determine the antimicrobial activity of the extracts. The test organisms in the overnight incubated nutrient broth were respectively diluted to 0.5% McFarland equivalent standard by serial dilution using sterile distil water (Cheesbrough, 2006). An 18 ml of Mueller-Hinton Agar (MHA) plates (for bacteria) and Potato Dextrose Agar (PDA) plates (for *C. albicans*) that have been checked for sterility was then seeded with 1ml of the standardized inoculum of each of the bacterial and fungal isolates in sterile Petri-dishes. The crude extracts were tested on the isolates. The seeded plates were allowed to set after a uniform distribution of the inoculums following swirling of the Petri dish. A standard sterile cork borer of 6mm diameter was used to cut four uniform wells on the surface of the agar. Three wells on each isolate plate were filled with each of the three crude extracts with the aid of a sterile syringe. Sterile distilled water, 75% ethanol and n-hexane were used as controls. The plates were incubated at 37°C for 18-24 h and observed for zones of inhibition. A zone of clearance round each well signifies inhibition and the diameter of such zones were measured in millimeter (mm) with a transparent ruler (Abah and Egwari, 2011).

Determination of Minimum Inhibitory Concentration (MIC)

A three-fold double dilution of each of the crude extracts was made based on the results from the antimicrobial sensitivity assays with final concentrations of 200, 100 and 50 mg/ml respectively. Sterile tubes containing 5 ml of sterile Nutrient broth for the bacteria and PDA broth for C. *albicans* each were inoculated with 0.2 ml each of the test organism after adding 0.5 ml of each of the extract concentrations, thus making three tubes of the each of the extract for an organism. Also, the sterility of the broth was tested as well as the viability of the organisms. Equivalent volumes of the broth which was not added with the extracts but with the organisms were used to test for the viability of the organisms while a broth tube free of the test organism and the extract was used to test the sterility of the broths (Adegoke et al., 2010; Hena et al., 2010*).*

Determination of Minimum Lethal Concentration (MLC)

The MLCs were determined by first selecting tubes that showed no growth during MIC determination; a wire loop full of broth from each tube was sub-cultured onto extract-free Mueller-Hinton agar plates, incubated for another 24 h at 37°C. The minimum lethal concentration was considered as the lowest concentration that could not produce a single bacteria or fungi colony (Kambal and Hassan, 2010).

Qualitative phytochemical screening

Phytochemical examinations of the extract were carried out for

alkaloids, saponins, flavonoids and tannins, cardiac glycosides and oxalates using the standard methods as described by Akinpelu et al., 2011; Prashant et al., 2011; Essiett, Edet and Bala, 2011).

Test for alkaloids

0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. A millilitre of the filtrate was treated with drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

Test for saponins using "foam test"

0.5 g of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Test for flavonoids

0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colouration was indicative of the flavonoids.

Test for tannins

About 1 g of the extract was dissolved in 20 ml of distilled water and filtered. 2 to 3 drops of 10% of $FeCl_3$ was added to 2 ml of the filtrate. The production of a blackish-blue or blackish-green colouration was indicative of tannins.

Test for Cardiac glycosides

About 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% $FeCl_3$. This was under laid with concentrated H_2SO_4. A brown ring obtained at the interface indicated the presence of a deoxysugar, characteristic of cardiac glycosides. A violet ring may appear below the brown ring while in the acetic acid layer; a greenish ring may form just above ring and gradually spreads throughout this layer.

Test for oxalates

To 0.5 g of the extract was added two to three drops of 80% H_2SO_4 was added. The formation of Bright crystal disappeared on the addition of reagents confirms the presence of oxalate in calcium oxalate form.

Statistical analysis

Data presented were expressed as mean and standard deviation of triplicates and were statistically analyzed using Chi Square of SPSS statistical package of version 17.0. Values were considered significant at $p < 0.05$.

RESULTS

Antimicrobial Activity of the Extracts

The extracts of *D. guineense* fruit pulp showed antimicrobial activity against *S. aureus, E. coli, K. pneumonia, P. mirabilis*, *P. aeruginosa* and *C. albicans* with the exception of n-hexane extract showing no zone of inhibition to any of the isolates after the antimicrobial sensitivity assay was repeated twice. *C. albicans* showed the highest inhibition to the crude ethanol extract with 24.67 mm zone of inhibition. While *P. aeruginosa* shows the minimum zone of inhibition to the ethanol crude extract with 13.33 mm zone of inhibition. The ethanol extract however showed the highest zones of inhibition on the microbial isolates. Also, the aqueous crude extract had a varying range of inhibition on the organisms with *C. albicans* being the most sensitive with 19.33 mm diameter zone of inhibition while *S. aureus* showed the lowest zone of inhibition with 7.33 mm. Figure 3 illustrates the microbial growth inhibition of the extracts on Mueller-Hinton agar plates after incubation for 18-24 hours at 37°C. The Minimum Inhibitory Concentration (MIC) as well as the Minimum Lethal Concentrations (MLC) of the extracts was determined. Table 1 and 2 show the MIC and MLC results of the aqueous and ethanol extract respectively. Also, Minimum Lethal Concentration (MLC) of the aqueous and ethanol extracts was showed at concentrations above 200 mg/ml but *P. aeruginosa* and *P. mirabilis* both showed MLC at 200 mg/ml *while C. albicans* at 100 mg/ml of the ethanol extract. Also, C. *albicans* only showed MLC at 200 mg/ml of the aqueous extract.

Qualitative phytochemical analysis of *D. guineense* fruit pulp extracts

Results of the phytochemical screening for each of the extracts is shown in Table 3. The extracts were screened for the presence of alkaloids, flavonoids, saponins, oxalates and cardiac glycosides.

DISCUSSION

The multidrug resistance of microorganisms is a major medical concern; screening of natural products in a search for new antimicrobial agents that would be active against these microorganisms is the need of the hour (Prany and Rishabh, 2011). The results obtained from this study revealed that the fruit pulp of *D. guineense* contains bioactive agents that exhibit antimicrobial properties against both Gram positive and negative bacteria and also fungi. Hence, the fruit pulp had shown a broad spectrum activity for its effectiveness in the therapy of some of the acclaimed properties. However, due to the polarity of the hexane molecules, it could not extract some of the bioactive components compounds present in the fruit. Cowan (1999), explains that the herbal extracting solvents have varying polarity. Hence, the n-

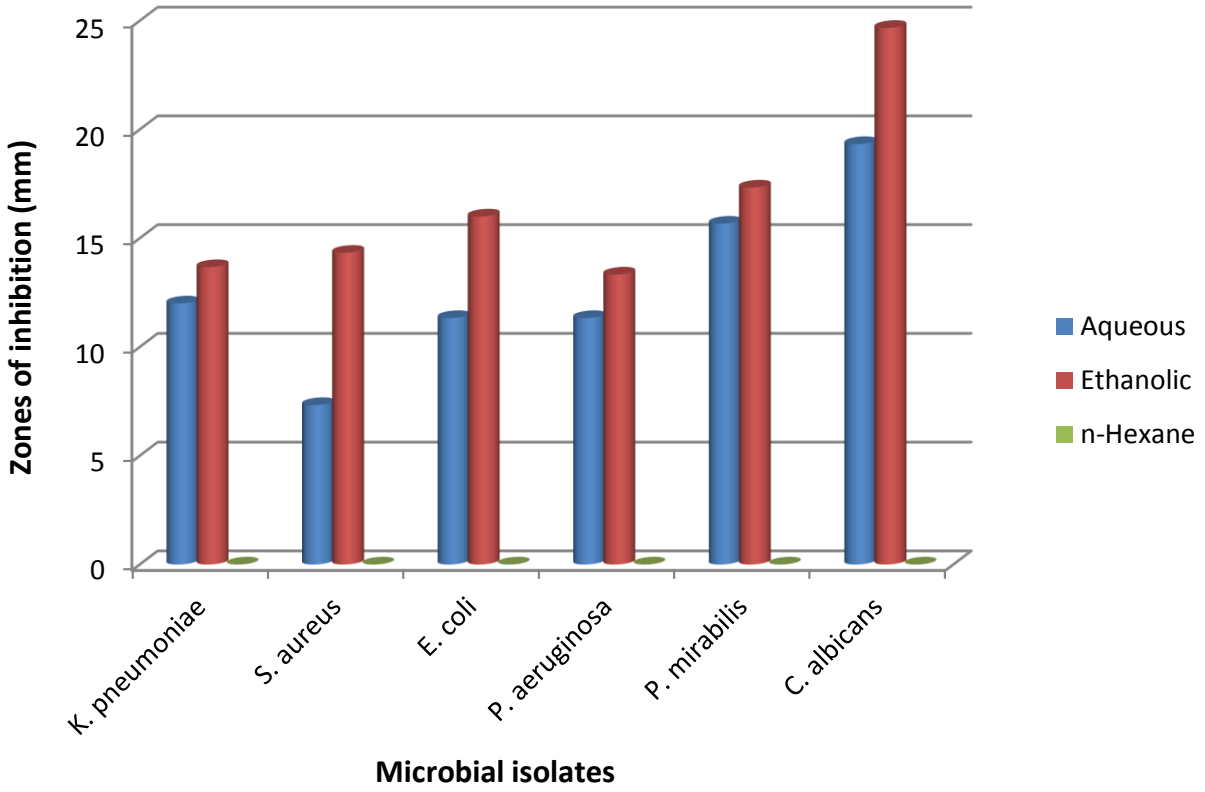

Figure 3. Average zones of inhibition (mm) of the crude extracts of *D. guineense* fruit pulp on microbial isolates

Table 1. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) determination of the Aqueous extract of *D. guineense* fruit pulp.

Isolates/concentration	200 mg/ml	100 mg/ml	50mg/ml	MLC mg/ml	Extract free
Klebsiella pneumonia	-	+	+	≥200	+
Staphylococcus aureus	-	+	+	>200	+
Escherichia coli	-	+	+	>200	+
Pseudomonas aeruginosa	-	+	+	200	+
Proteus mirabilis	-	+	+	>200	+
Candida albicans	-	+	+	200	+

Minus sign indicates No growth while plus indicates Growth.

Table 2. MIC (Minimum Inhibitory Concentration) and MLC (Minimum Lethal Concentration) determination of the ethanolic extract of *D. guineense* fruit pulp

Isolates/concentration	200 mg/ml	100 mg/ml	50 mg/ml	MLC mg/ml	Extract free
Klebsiella pneumonia	-	-	+	>200	+
Staphylococcus aureus	-	-	+	>200	+
Escherichia coli	-	-	+	>200	+
Pseudomonas aeruginosa	-	-	+	200	+
Proteus mirabilis	-	-	+	200	+
Candida albicans	-	-	-	100	+

Minus sign indicates No growth while plus sign indicates Growth.

Table 3. Qualitative phytochemical screening of the aqueous, ethanol and n-Hexane extracts of *D. guineense* fruit pulp.

Phytochemicals/ Extracts	Aqueous	Ethanol	n-Hexane
Saponins	++	+	-
Flavonoids	++	+	-
Tannins	++	+	-
Glycosides	-	-	+
Oxalate	++	++	+
Alkaloids	+	-	-

+ indicates present, ++ indicates more present while – indicate not present.

hexane extract could extract phytochemicals of equivalent polarity. Kubmarawa et al. (2012) also reported no sensitivity of n -hexane extract of the leaves, stem bark and roots of *Acacia tortilis* on *E.coli, Shigella* spp.*, P. Aeruginosa, S. aureus, Streptococcus pyogenes and Salmonella typhii.* In contrast, Anowi et al., 2013 reported a positive antimicrobial sensitivity of n-hexane leaves extract of *Synclisia scarbrida* on *S. aureus*, *P. aeruginosa, S. typhii*, *E. coli* and *Bacillus subtilis.* Besides, the aqueous and ethanol crude extracts showed greater range of inhibition to the microbial isolates with *C. albicans* showing the highest sensitivity and *Staphylococcus aureus* being the least sensitive. Orji et al., (2012) reported positive antimicrobial properties of the crude aqueous and ethanol leaf and bark extracts of *D. guineense* against *S. aureus* and *K. Pneumonia*. Akinpelu et al., (2011) also reported the bioactivity of the methanolic crude leaf extract of *D. guineense* on fourteen environmental strains of *Vibrio* species. The ethanolic extract of the leaves of three wild strains plant species of *D. guineense* has been reported by Osaugwu and Eme, (2012). Nevertheless, Orji et al., (2012) reported MIC values for the crude leaf and stem back aqueous and ethanol extract of *D. guineense* to *S. aureus* and *K. pneumonia*e also at high concentration of 200mg/ml. This variation could results from the variety of stains of microbial isolates used, extraction methods as well as varying phytochemical components of plant parts.

However, the n-hexane extract was the only one to test positive for cardiac glycosides while the aqueous extract only showed positive test for alkaloids and all the phytochemicals except for glycosides. The ethanol extract tested positive a number of the phytochemicals with the exception of alkaloids and glycosides. This however do not make the aqueous extract the most sensitive to the isolates as alkaloids only possess antihelminthic property by diminishing the support of glucose to the helminths and acts on cells causing paralysis (Prashant et al., 2011). However, this study reveals the presence of some of these phytochemicals in the three extracts of D. *guineese* fruit pulp with n-hexane showing the lowest constitution.

Although, medicinal plants are natural products of nature, as a result, they have little or no side effects when consumed for therapeutic purposes. Also, they are efficacious in combating many pathogens exhibiting multi-drug resistant traits to synthetic drugs. However, consuming medicinal plants could also results to adverse medical conditions among perpetual users, therefore, cautions and international standards should be employed while using them. The adverse conditions could arise via consuming herbs with toxic ingredients, unintentional substitution of herbs with toxic species, intentional addition of drugs, environmental contamination of toxic substances during preparation, and combination of herbs with synthetic drugs (Abas, 2001). For instance, Bernard and Clovis (2014), reported that red spinach (also known as Chinese spinach, *Amaranthus dubius*) which is used in treating high blood pressure, kidney infections and obesity could induce acute kidney injury and pulmonary injury and other complications. Also, South African geranium (*Pelargonium sidoides*) commonly used for the treatment of respiratory tracts infections and irritation and, gastrointestinal disorders could also cause gastrointestinal disorders, skin rashes, and allergic reactions. Nonetheless, this study has been able to strengthen the traditional uses of the fruit pulp of *D. guineense* and suggest that it contains bioactive that could be used as both antibacterial and antifungal agents.

Conclusion

This study has revealed that the aqueous and ethanol fruit pulp extract of *D. guineense* have antimicrobial properties against *S. aureus, E. coli, K. pneumonia, P. mirabilis* and *C. albicans*. More so, the fruit pulp extracts were shown to possess significant amount of phytochemicals such as saponins, tannins, flavonoids, oxalate, alkaloids and glycosides. These findings are interesting essentially at the present time where the problems of emerging and re-emerging resistant strains of microorganism are becoming the order of the day. Another point which underscores the relevance of these findings is that medicinal plants are said to promote homeostatic balance in patients and are relatively less toxic than synthetic drugs (Obadori and Ochuko, 2001). Remarkably, *D. guineense* plant is readily available in Nigeria and could serve as alternative to curing of microbial infections at lower cost.

Owing to fact that the fruit pulp of *D. guineense* had shown promising antimicrobial properties, there ought to be a monitory strategy for the consumption of the fruit as many women and others do consume this fruit to an endless point. However, the following recommendations

should be noted;
(i) Further researches could be done to investigate the toxicity effect of the fruit of *D. guineense* as well as monitoring its effective dosages using laboratory animals as well as human clinical trials.
(ii) Health education for the populace on the effect of consuming microbial spoilt fruits of *D. guineense* should also be done. This has been evident in the course of this study that the fruits could easily be contaminated by fungi and bacteria due to its high sugar contents. Also, its sweetness could attract other toxic-producing spoilage pests and insects.
(iii) *D. guineense* tree varies in wild types, research should also be done to ascertain the most phytomedically important strain investigating its leaf, stem bark, roots as well as fruit pulp.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Abah SE, Egwari LO (2011). Methods of antimicrobial susceptibility testing of plant extract. Afr. J. Basic Appl. Sci. 3(5):205-20.

Abas HH (2001). Continuing pharmacy education: Adverse effects of herbs and drug-herbal interactions. Malaysian J. Pharm. 1(2):39-44.

Adegoke AA, Iberi PA, Akinpelu DA, Aiyegoro OA, Mboto CI (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. International J. Appl. Res. Nat. Prod. 3(3):6-12.

Akinpelu DA, Aiye-Gore OA, Okoh AL (2008). In vitro antimicrobial and phytochemical properties of crude extract of stem bark of *Afzelia africana* (smith). J. Biotech. 7(20):3665-3670.

Akinpelu AD, Awotorebo TO, Agunbiade OM, Aiyegoro AO, Okoh IA (2011). Anti-Vibrio and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of *Dialium guineense* (Wild). J. Med. Plants Res. 5(11):2398-2404.

Anowi FC, Onyekaba TU, Eze CC, Ike C, Ezenachi VC (2013). Preliminary Phytochemical investigations and evaluation of antimicrobial activity of n-hexane extract of the leaves of *Synclisia scabrida* family menispermaceae. Res. J. Pharmaceut. 2(3):1-51.

Arogba SS, Ajiboro AA, Odukwe IJ (2006). A physico-chemical study of Nigerian velvet tamarind *Dialium guineense* fruit. J. Sci. Food Agric. 66:533-534.

Bernard K, Clovis F (2014). Adverse drug reactions in some African herbal medicine: literature review and stakeholders' interview. Int. Med. Res. 3(3):126-132.

Bero J, Ganfon H, Jonville MC, Frederich M, Gbaguidi F, De MP, Moudachirou M, Quetin LJ (2009). In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J. Ethnopharmacol. 122(3):439-444.

Center for Disease Control and Prevention (CDCP) (2006). Healthcare-associated Methicillin Resistant Staphylococcus aureus (HA-MRSA).

Cheesbrough M (2006). District laboratory practice in tropical countries, Part 2. 137-150. Cambridge University Press, Cambridge, UK. ISBN: 9780521676311.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4):564-582.

Essiett UA., Edet NI, Bala DN (2011). Phytochemical and physicochemical analysis of the leaves of *Laportea aestuans* (Linn.) Chew and *Laportea ovalifolia (*Schumach) Chew (male and female). Asian J. Plant Sci. Res. 1(2):35-42.

Geissman TA (1963). Flavonoid compounds, tannins, lignins and related compounds, In: Florkin M, Stotz EH (eds), Pyrrole pigments, isoprenoid compounds and phenolic plant constituents: Elsevier, New York: 265.

Gideon IO, Ralphael A (2012). Phytochemical analysis and in vivo anti-diarrhoeal potentials of *Dialium guineense* (wild) stem bark extract. J. Intercult. Ethnopharmacol. 1(2):105-110.

Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86(6):985.

Hena JS, Adamu AK, Iortsuun DN, Olonitola OS (2010). Phytochemical screening and antimicrobial effects of the aqueous and methanolic extracts of roots of *Balanites aegyptiaca* (Del.) on some bacteria species. Sci. World J. 5(2):59-62.

Hermans M, Akpovi A, Vander MLG (2010). Medicinal plants used to treat malaria in Southern Benin. J. Ecol. Bot. 56:239-252.

Ibeh I.N (1998). General and reproductive toxicology. pp.68. Square One Publishers, Garden City Park, New York. ISBN-13: 9780757001444:

Iqbal A, Arina ZB (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol. 74:113-123.

Kamba AS, Hassan LG (2010). Phytochemical screening and antimicrobial activities of *Euphorbia balsamifera* leaves, stems and root against some pathogenic microorganisms. Afr. J. Pharm. Pharmacol. 4(9);645-652.

Kubmarawa D, Khan ME, Shuaibu A (2012). Comparative phytochemical screening and biological evaluation of n-hexane and water extracts of *Acacia tortilis*. Res. Pharmaceut. Biotech. 4(2):18-23.

Lamien MA, Lamien CE, Compaore MM, Meda RN, Kiendrebeogo M, Zeba BMJF, Nacoulma OG (2008). Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Mol. 13(3):581-594.

Lawal IO, Nzokwe NE, Igboanugo ABI, Adio AF, Awosan EA, Nwogwugwu JO, Faleye B, Olatunji BP, Adesoga AA (2010). Ethnomedicinal information on collation and identification of some medicinal plants in research institutes of South-west Nigeria. Afr. J. Biotech. 4(1):001-007.

Lin J, Opak W, Geheeb-Keller M (1999). Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. J. Ethnopharmacol. 68:267-274.

Mukherjee PK, Wahile A (2006). Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J. Ethnopharmacol. 103:25-35.

Nair R, Chanda S (2007). Antibacterial activities of some medicinal plants of the Western Region of India. Braz. J. Microbiol. 38:452-458.

Ncube N, Afolayan SAJ, Okoh AI (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr. J. Biotech. 7(12):1797-1806.

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

Obadori BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of the guide extracts of some home state plants in Edo and Delta State of Nigeria. Global J. Pure Appl. Sci. 81:203-208.

Odugbemi T, Akinsulire O (2006). Medical plants by species name. In: Odugbemi, T. (ed.) Outlines and Pictures of Medicinal Plants from Nigeria. University of Lagos Press, Lagos State. pp: 73-116.

Ogunjobi AA, Fagade OE, David OO (2007). Antimutagenic and potential anticarcinogenic activities of aloe-vera gel and aqueous garlic extract in the bacterial reverse mutation test (Ames assay). Afr. J. Biomed. Res. 10:275-278.

Okemo P, Nwanta W, Chabra S, Fabry W (2001). The kill kinetics of *Azadirachta indica*, *A. juss* (Maliaceae) extracts on *Stapylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. France J. Sci. Tech. (2):133-138.

Okwu DE, Okeke O (2003). Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. Global J. Pure Appl. Sci. 9(2):235-238.

Online Textbook of Bacteriology 2008. Retrieved March 30, 2010.http://www.textbookofbacteriology.net/staph.html

Orji JO, Alo MN, Anyim, C, Okonkwo EC (2012). Antibacterial activities of crude leaf and bark extracts of "Icheku" *Dialium guineense* on

bacterial isolates from bronchitis patients. J. Pharm. Biol. Sci. 1:2278-3008.
Parekh J, Karathia N, Chanda S (2006). Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata L*. (bark). Afr. J. Biomed. Res. 9:53-56.
Prashant T, Bimlesh K, Mandeep K, Gurpreet K, Harleen K (2011). Phytochemical screening and extraction: A review. Int. Pharmaceut. Sci.1(1): 98-106.
Sofowora EA (1982). Medicinal plants and traditional medicine in West Africa. 2nd Edition. John Willey and Sons Ltd, New York; ISBN-10: 0471103675. pp. 289.
Thangjam RC, Vasudha P, Rituparna C, Bangar R, Richard L, Mamatha B (2011). Screening for antidiarrheal activity of *Psidium Guajava*: A possible alternative in the treatment against diarrhea causing enteric pathogens. J. Chem. Pharmaceut. Res. 3(6):961-967.
World Health Organization WHO (1997). Resolution – Promotion and development of Training and Research in Traditional Medicine. WHO Document. NO. 30-49.